





# Dear colleagues,

"Panta rhei" - everything flows. Heraklit was the first to state these very true words. For us in the lab this means that knowledge itself changes, the constant is the urge to have access to state of the art parameters and methods. Laboklin's mission is to provide you with the best lab work possible to meet the needs of the practice for the benefit of the animals. Thus our Directory of Tests is subject to constant change. In your hands, you are now holding the latest version – a Compendium, as we call it, because of the comprehensive information on lab parameters included. After all, we do not only want to let you know what we can do, but also when it makes sense to request these tests. And this is how the Directory of Tests became a Compendium. We hope you enjoy it as much as we do.

#### New:

The **microbiome** analysis for dogs, cats and now also for horses should be mentioned here. Especially in case of chronic diarrhoea, imbalances in the intestinal flora can be detected. A lot of new profiles and changes based on new findings have been included. Enjoy scrolling through the new Compendium!

#### Useful:

The new Laboklin app "**4Paws**" helps to ensure your treatment success: It reminds the animal owner of appointments such as the administration of medication, allergy treatment, vaccination, etc.

The Laboklin app "**My Lab**" keeps providing you with laboratory findings that can be accessed anytime, anywhere.

#### Interestina:

Our **Laboklin Academy** provides you with a large variety of interesting educational features. A new format is the expert panel, in which different specialists discuss a common topic. We think this is an attractive aspect of further education! In addition to the **Skin Day**, the **Dog Breeders' Day** and the **Horse Breeders' Day** have become established.

#### Proven:

Of course we are **accredited**, so you can rely on the quality of our laboratory services. There is always a large team of veterinarians, biologists and chemists involved in analysis and interpretation. On top of that, we spend quite some time and effort in scientific research interconnected with various research groups to improve the diagnostic panel. And it was with great pleasure that we accepted the awards for **Bavaria's Best 50** and **TOP 100 Innovator in Germany**.

#### Our mission:

Times are changing – and so are the demands the practices have on laboratories. What remains for us: We will continue to serve you with the best possible diagnostics to meet your needs.

With best regards from the laboratory, Your Laboklin team

hur/

Dr. Elisabeth Müller CEO LABOKLIN GmbH & Co. KG

#### 2022/23 Contents

# **LABOKLIN**

# LABOKLIN at a Glance

# LABOKLIN GmbH & Co. KG is accredited according to DIN EN ISO/IEC 17025:2018

## An overview of LABOKLINs range of services

## **Profiles and screenings**

- Small animals
- Small mammals
- Birds
- Reptiles
- Horse
- Ruminants
- New World camelids
- Pig
- Amphibians
- Fish

#### **Blood examinations**

- Allergy
- Endocrinology
- Function tests
- Haematology
- Immune status
- Clinical chemical parameters
- Serology/Infectious diseases
- Diagnosis of leukaemia/lymphoma
- Tumour markers

#### **Hereditary diseases**

- Dog
- Cat
- Horse
- Cattle
- Pig
- etc.

# Microbiology and parasitology

- Bacteriology
- Mycology
- Virology
- Parasitology
- Maldigestion/Malabsorption
- Dysbiosis/Microbiome analysis
- Autovaccines etc.

## Pathology

- Histopathology
- Immunohistology
- Cytology
- BRAF mutation
- Exsudate/Transudate
- Cerebrospinal fluid
- Synovia
- Other aspirates
- Tumour genetic tests

#### PCR detection

- Dog
- Cat
- Small mammals
- Birds
- Reptiles
- Horse
- Ruminants
- New World camelids
- Pig
- Amphibians
- Fish
- etc.

#### Other genetic examinations

- Sex determination in birds
- Breed analysis
- Identity and parentage
- DNA profile
- Species differentiation
- Coat colour/Coat structure

#### **Water Tests**

• Water of aquarium/ponds

#### Hygiene

- Hygiene examinations
- Profiles

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# Abbreviations/Additional Information Concerning the Test Descriptions

# Sample quantities

The indicated sample quantities are minimum quantities. Please note that larger minimum quantities may be required, depending on the type of test tube used (see Chapter 1.1.4, p. 17).

#### Sample material

You will also find these abbreviations on our submission forms. The required materials for the individual tests are indicated in this compendium but may (for lack of space) not all be listed on the submission forms.

Abbreviations written in smaller type are only used on submission forms.

, (comma)	Information connected with comma: you can choose	K KM L	scab bone marrow liver
+	which sample material you want to submit (see p. 22) Information connected with "+": you must submit all of	Ln LQ LSP	lymph node CSF pulmonary lavage
٨	these sample materials	M MH	spleen morning urine
A AM AP	swab without medium abortion material contact plates	Mi MSP	milk gastric lavage
AS	ascites	<sub>N</sub> NaFB	kidney sodium fluoride blood
B BAI	bees bronchoalveolar lavage	NSP	nasal lavage
BI-D	bioindicator steam steriliser	OT	specimen slide
BI-H BL	bioindicator dry heat steriliser bee larvae	PSP	preputial lavage
BS	blood smear	S	serum
CB CP	citrate blood citrate plasma	Sp SV	sperm synovia
CSF	cerebrospinal fluid	TaM	tank milk
EB EP	EDTA blood EDTA plasma	TBS TM TSP	tracheobronchial secretion swab with medium tracheal lavage
F	feather	V	vomit
FA FL	faeces fleas	Z	tick
FNA	fine-needle aspiration	Test metho	ods
GW	tissue	AAS	atom absorption spectrometry
H HA	urine hairs	CEDIA	cloned enzyme donor immuno assay
HB HP HS HT	heparin blood heparin plasma urolith skin	cELISA CLIA CFT	competitive ELISA chemiluminescence assay complement fixation test

11

droplet digital PCR

sorbent assav

neutralisation

spectrometry

spectrometry

chromatography

mass spectrometry

technique

spectrometry

MALDI-TOF matrix-assisted laser

ments

corresponds to ELISA

enzyme linked immuno-

fluorescent antibody virus

Fourier transform infrared

gas chromatography-mass

haemagglutination inhibition

high performance liquid

immunochromatography

inductively coupled plasma

indirect fluorescent antibody

liquid chromatography-mass

desorption/ionisation - time of

microscopic agglutination test

polymerase chain reaction for

flight mass spectrometry

near-infrared spectroscopy

antigen receptor rearrange-

polymerase chain reaction

acid-formalin concentration

short tandem repeats (micro-

radioimmunoassav

satellite analysis)

sodium acetate-acetic

virus neutralisation test

ion-selective electrodes

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# Pre-analytics

# **Blood, Plasma, Serum Samples**

The first step in the process of examining a sample is the pre-analysis. Pre-analysis includes all steps from patient preparation, specimen collection and transport of the sample to the lab to the preparation of the sample for analysis.

# 1.1.1 Preparation of the Patient

Before taking a blood sample, the patient should normally fast for 10 – 12 hours, provided the physiology of the species concerned permits this. Otherwise, faulty results are to be expected, especially for cholesterol, glucose and TLI. In addition, parameters such as α-amylase. ALT, AST, bilirubin, total protein, triglycerides, serum bile acids. leukocytes and calcium can be affected.

Fasted blood samples are not unproblematic in horses and only indicated for special tests (e.g., insulin and glucose for the diagnosis of the Equine Metabolic Syndrome). It is advisable to inform the owner about the influence of physical activity or stress on the results of a blood examination. Particularly muscular enzymes such as CK, LDH and AST can show increased levels in the serum after physical exertion. Additionally, glucose and lactate can also show elevated serum levels.

Before doing any allergy tests, including feedstuff tests, any administration of corticosteroids should be stopped. To do so, the following withdrawal times are recommended:

- local/topical corticosteroids: 2 4 weeks
- oral corticosteroids (e.g. Prednisolone): up to 8 weeks
- depot cortisone preparations (e.g., Voren®); up to 3 months

If these times cannot be observed, false negative results are possible. In the case of a positive result, the reaction class must be assessed taking into account the previous administration of cortisone.

Please note that other itch-suppressing medication may also have a negative impact on the allergy test. Our allergy team will be pleased to advise you.

Allergy tests should be performed during the season or at the end of the season and not earlier than one month after the onset of the symptoms, as the test may be false negative if performed out of season.

# 1.1.2 Which Sample?

Details on the recommended material (blood, serum, plasma) for the requested test can be taken from our test descriptions or the submission form. For labelling the sample, it is also necessary to indicate the sample type (see Chapters 1.9, p. 22, and 1.10, p. 25).

- Specifications apply to testing with method (1)
- Specifications apply to testing (2)
  - with method (2)

Numbers in the test descriptions

Specifications apply to testing fragment length polymorphism

# with method (3)

#### The specified standard testing Duration

times apply from the date of arrival of the samples at

Laboklin.

"Days" means "working days". Due to delays in transport. test duration may take longer for tests which are carried out by a partner laboratory. The specified test durations are supplied without liability.

# **Species**

Large Horses and farm animals animals

Small mammals

Rabbit, guinea pig, rat, mouse, hamster, ferret and other small mammals which

are kept as pets.

In individual cases, tests for small mammals may also be applicable for small wild mammals (e.g. hedgehogs).

Small

animals Dog and cat

New World

llama, alpaca; camelids

> Being polygastric, New World camelids may appear under the heading "ruminants".

Farm

Ruminants and pigs animals

# Other abbreviations

ddPCR

EΙΑ

**ELISA** 

FAVN

FLP

**FTIR** 

**GCMS** 

HAH

**HPLC** 

ICA

**IFAT** 

ISE

**LCMS** 

MAT

**NIRS** 

**PARR** 

PCR

RIA

SAFC

**STRs** 

VNT

**ICPMS** 

AR antibodies bdw body weight

revolutions per minute rpm partner laboratory

#### **Further notes**

The obligation to notify the authorities upon suspicion of a disease applies to Germany.

The obligation to notify the authorities upon diagnosis of a disease applies to Germany.

> greater than less than

# Whole blood samples

## EDTA blood (EB)

- For doing a blood count, EDTA blood is the most suitable material in mammals (however, for birds and reptiles it is heparin blood, see below).
- For the serological examination of the blood type, EDTA whole blood is needed as well.
- As the cells in the sample are not stable, EDTA samples for haematological tests should not be older than 48 hours.
- For most PCR analyses and genetic tests, EDTA blood is required.
- To determine certain parameters such as ACTH or pro-BNP, only EDTA plasma which was promptly centrifuged and cooled can be used to obtain reliable results.

#### Heparin blood (HB)

- To collect heparin samples, lithium heparin (LiHep) tubes are available.
- For doing a blood count in reptiles and birds, lithium heparin blood should generally be used.
- Since the amount of blood is often very low in small mammals, lithium heparin tubes are particularly suitable, as they cannot only be used for doing a blood count but also for determining a wide range of blood-chemical parameters.
- For the PCR, lithium heparin whole blood should only be used under exceptional circumstances, as lithium heparin can inhibit the PCR and might thus lead to false negative results.

#### Citrate blood (CB)

- To determine the coagulation parameters, only the appropriate citrate tubes should be used. For getting a correct evaluation, their shelf life may not be exceeded. It is also necessary to have an exact mix ratio of 1:10 (1 part citrate + 9 parts blood).
- For correctly performing platelet function tests, citrate whole blood is required.

# Sodium fluoride blood (NaFB)

Sodium fluoride inhibits enzyme activities which lead to a reduction of some parameters. It should be used for the correct determination of glucose and lactate.

#### Plasma

- Samples are drawn into tubes with anticoagulants (heparin, EDTA, citrate).
- Can be centrifuged immediately after collection (10 min, 2000 g).
- Remove the supernatant by pipette and transfer it into an uncoated test tube, then indicate the sample materials on the test tube or use the appropriate bar code label (see Chapter 1.10, p. 25)
- Please note: The additives limit the number of analyses!
- <u>Heparin plasma (HP)</u> is needed for many clinical-chemical examinations. HP cannot be used for agglutination tests.
- The collection of <u>EDTA plasma (EP)</u> for clinical-chemical and/or serological parameters should only take place in exceptional cases, as EDTA disturbs through various mechanisms the measurement of individual parameters such as calcium.

magnesium and AP. Likewise, potassium cannot be determined when using EDTA plasma, since EDTA is added as K-EDTA.

- Some coagulation parameters can only be analysed using <u>citrate plasma (CP).</u>

Performing platelet function tests using centrifuged citrate plasma is not possible.

#### Serum

- Samples are drawn into tubes without anticoagulants.
- Allow to stand for 30 60 min.
- Centrifuge for 10 min at 2000 g.
- Remove the supernatant by pipette and transfer it into an uncoated test tube, then label the test tube.
- For the correct determination of individual parameters, only serum should be used (see detailed descriptions provided for the individual parameters).
- Sending non-centrifuged samples should only be done exceptionally (e.g. in case of a very low sample quantity), as the transport might result in cell damage and thus lead to haemolytic serum.

An overview of the different tubes can be found in Chapter 1.9, p. 22.

# 1.1.3 Factors Interfering with Analysis

#### Haemolysis

Haemolysis is caused by leakage of intracellular components of the erythrocytes such as phosphate, iron, potassium and especially haemoglobin due to a damage of the cell membrane. Haemoglobin causes a red colouration of serum/plasma which primarily interferes with the photometric testing done in clinical chemistry.

#### Lipaemia

Lipaemia refers to the milky/turbid discolouration of serum/plasma due to triglycerides. It is mostly caused by diet- or stress-related factors. Lipaemia can also occur as a result of endocrinological diseases like Cushing's syndrome or hypothyroidism. Lipaemic samples often complicate the measurement of certain clinical parameters,

# Icterus

e. g. bilirubin.

Icterus is a yellowish discolouration of serum/plasma. Excess amounts of bilirubin, which is the reason for the yellow colouring, are normally caused by a medical condition and cannot be influenced. Very severe icterus may sporadically affect certain parameters. The yellow colouration is physiological in horses.

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Interfering factor	Parameter	Level
Haemolysis	LDH, HBDH, CK, AST, bilirubin, creatinine, PO4, K, Mg, Fe, fructosamine	•
Haemolysis	Ca, glucose	•
Lipaemia	ALT, AST, GLDH, γ-GT, AP, bilirubin, creatinine, haemoglobin	•
Lipaemia	amylase, Na, CI, K	1

Medicine	Parameter	Level
Penicillin G	K	•
Tetracyclines	PO4	1
Tetracyclines	К	•
Salicylates	CK, AP, glucose, Na, total protein	1
Salicylates	K, Ca	•
Corticosteroids	CK, AP, glucose, Na, total protein	1
Corticosteroids	K, Ca	•
Phenylbutazone	Ca, Na	1
Barbiturates	СК	1
Halothane anaesthesia	CK, PO4	1
Glucose infusion	glucose	•
Glucose infusion	PO4	•

# 1.1.4 Specific Features

#### Blood counts

- EDTA or lithium heparin blood
- When collecting the sample, discard the first 0.5 ml of blood, if possible, as they contain an increased amount of coagulation factors, or first obtain a serum sample.
- Let the blood run down slowly on the side of the sample tube.

- Pay attention to the fill volume! Preferably fill up to the mark, since an insufficient volume can result in changes in cell morphology. Do not overfill the tube in any case, as the sample might clot.
- After drawing the sample, tilt the test tube carefully several times. Do not shake it.
- Do not store blood smears in the refrigerator and not close to formalin.
- Pack the samples frost-proof in winter; possibly cool them in summer.
- Reliable results can only be obtained from samples not older than 48 hours.

#### Clinical chemistry of serum or heparin plasma

Prompt centrifugation of the samples will lead to better test results, as it reduces the risk of haemolysis caused by transportation. However, serum should be allowed to stand for a minimum of 30 min to ensure a complete clotting of the sample.

Serum samples can also be shipped frozen; they will then reach the laboratory cooled. Repeated freezing/thawing, though, should absolutely be avoided.

#### Determination of glucose and lactate

- Requires sodium fluoride blood or sodium oxalate blood or serum which was promptly centrifuged.
- **Fill volume:** Fill the sample tubes **exactly up to the mark**. If the quantity is too small or too large, results may be incorrect.

## Coagulation parameters

- Determination is carried out using sodium citrate plasma which is obtained from citrate blood with the mix ratio being 10:1 (9 parts blood + 1 part sodium citrate).
   Centrifugation should take place at the practice. When testing for the von Willebrand antigen, it is imperative to do this promptly after collection.
- If commercial citrate-treated tubes are used, the expiry date needs to be checked before collecting the sample. Expired tubes may no longer be used, as skewed results are to be expected. When drawing the sample, special attention must be paid to the **exact fill level** (marking on the tube).
- If no commercial tubes are available, sodium citrate 3.13% can be drawn into a syringe.
- No heparinised needles or catheters may be used.

# Sample collection for bone marrow cytology

- Sample material from the first aspirate should be used to prepare the smears for cytological examination (avoid contamination with peripheral blood).
- The syringe used for aspiration should be preloaded with an anticoagulant. The aspirate should be placed into an EDTA, lithium heparin or citrate tube at the latest immediately after the puncture and then inverted well to avoid clots.
- To prepare a smear, the aspirate is put in a Petri dish and gently inverted in order to find the bone marrow spicules.
- The spicules are each placed on a slide and carefully spread to form a monolayer.
- The remaining aspirate is then put back into the tube (wetted with the same anticoagulant) and sent in as well.
- In addition, peripheral blood is collected in a tube, a blood smear is prepared and both are also sent to the laboratory.

# 1.2 Microbiology

- It is important to collect samples as sterile as possible to avoid contamination with physiological flora.
- Swabs for bacteriology (aerobic and anaerobic microbes) and for mycology should be sent with a transport medium ("swab with medium" = TM) to protect the microbes during shipment.
- Swabs for PCR tests should be sent without a transport medium ("swab without medium" = A, "dry swab") or with a special medium.
- Urine should be sent in a sterile tube using a swab with transport medium or using Uricult, always in combination with a urine sample. If cystocentesis urine is sent in, please make sure to remove the needle.
- Hairs and/or skin scrapings (without the scalpel!) for the diagnosis of dermatophytes are best sent in a sterile container, a paper bag or in aluminium foil.
- For sending faeces/excrement, special transport tubes should be used, no bags or gloves tied with a knot; also avoid the use of glass containers.
- Blood culture flasks can be ordered with prior written notice (subject to a charge). Information on the different blood culture bottles available can be found in Chapter 14.1, p. 266 under the service Blood Culture.

# 1.3 Hygiene

The **test materials** and the instructions will be sent to you after we have received your submission form. Samples can be collected up to the expiration date of the test kit, as long as the kit is stored according to the specifications in the accompanying documents. For monitoring the **surface disinfection** you will be provided with contact plates. The surfaces to be sampled have to be cleaned and disinfected and must be well dried before sampling. The contact plates, which need to be labelled on the bottom, must be returned to the laboratory within 24 hours together with the filled-in submission form. The bioindicators necessary for evaluating the proper functioning of the **sterilisers** can be applied right in the device together with the regular sterilisation material. After testing, these bioindicators have to be sent directly to the laboratory together with the filled-in submission form.

For testing the disinfection of **endoscopes**, two contact plates and two rinse samples are necessary for each endoscope. It is required to return the collected samples to the laboratory within 24 hours as described in the instructions.

# 1.4 Water Examination

To take water samples from **aquariums/ponds** for testing chemical water parameters, you need a non-sterile glass or plastic container (e.g. a 500 ml bottle which previously contained water).

To obtain a representative sample without any air included, collect the sample approximately in the middle of the aquarium and close the container under water. Samples from ponds should be taken in an area of the pond with little water flow, not close to the filter and not directly underneath the surface.

If possible, avoid larger pieces of dirt in the sample.

When shipping the samples, cooling is recommended.

# 1.5 Histology and Immunohistochemistry

When submitting tissue samples for histopathological and immunohistochemical examinations, the following points must be observed:

- artefact-free extraction of a typical lesion sufficient in size (diameter > 0.5 cm)
- preparation of an anamnesis including diagnostic task and clinical picture
- shipment in a suitable container (available from us free of charge)
- Immunohistochemistry can always be done after histopathology with the material supplied.

#### Detailed explanations:

As a sample, a representative piece of tissue free of preparation artefacts (e.g. disruption, squashing, electrocoagulation) should be taken. The diameter of the sample should not be less than 0.5 cm. An exception to this are samples which, for technical reasons, cannot be obtained otherwise (such as endoscopically taken stomach biopsies). Furthermore, it should be borne in mind that samples which are too small only provide little information, whereas samples that are too large cannot be fixed properly. Pieces of tissue with an edge length of 1 cm are recommended. However, this might vary depending on the lesion to be examined, the sampling site and the objective.

Small lesions should be placed centrally so they are not overlooked and thus truncated during preparation. If in doubt, several samples should be collected.

#### 1.5.1 Skin Punches

As skin samples, punch biopsies of all dermal layers with a diameter  $\geq 0.6$  cm are to be submitted. Primary lesions from several locations should be selected. The biopsied area should not be pre-treated by scraping or shaving. The anamnesis should contain all relevant data which might be important for the diagnosis. It is recommended to use our submission form Pathology, which especially focuses on skin and tumour diagnostics, but also leaves room for any other type of anamnesis.

# 1.5.2 Cytology

Samples can primarily be taken by wipe test, scraping or puncture (with or without aspiration). The most common technique is the fine needle aspiration, using a thin hollow needle (G 22 – G 27) attached to a syringe. A vacuum is created and, if possible,



the tissue should be punctured several times in different directions. Before detaching the needle, the vacuum must be released to avoid the material receding into the syringe. The material obtained is then pressed out of the needle onto the side of a glass slide. A second slide is placed flat at a right angle on top of the first one and is then carefully pulled away across the slide. If the sample is more liquid, a steeper angle (45°) – like in a blood smear – should be applied.

For the cytological examination of aspirates, excretions or secretions, the fluids obtained are centrifuged at 2500 – 3000 rpm for three to five minutes. The supernatant is decanted and the sediment is carefully spread like a blood smear and shipped air-dried. Please indicate on the submission form whether it is a sediment smear or a native smear. If the aspirates are sent directly, EDTA tubes should be used as test vessels.

For bronchial, conjunctival and vaginal cytology, the swab obtained (cytobrush) should be rolled onto a glass slide, not smeared.

All smears should generally be sent in air-dried, but unfixed. If desired, the smears can already be stained at the practice (please note: do not use a cover glass). The most important point is to create a thin smear consisting of only one layer (monolayer). The most common reason for getting a limited quality up to not being able to assess at all are smears that are too thick.

# 1.6 Polymerase Chain Reaction (PCR)

PCR is a very sensitive and specific method for the **direct detection** of infectious agents. Via PCR, gene sequences characteristic for the respective pathogen are reproduced and detected – if necessary, even of pathogens which are no longer viable.

The sample material that must be supplied for the PCR highly depends on the pathogen to be detected and the present signs or the diagnostic task. Depending on how the pathogen has spread in the body and its excretion, different sample materials are suitable.

At this stage of infection, pathogens causing viraemia, parasitaemia or bacteraemia can be detected directly in an **EDTA blood sample** (EB). Lithium heparin is less suitable as an anticoagulant, as it can inhibit the PCR. For blood samples or other liquid samples, an amount of at least 0.2 ml is required.

In contrast to cultural bacteriological/mycological examinations, for PCR tests it is recommended to use sterile **swabs without transport medium** ("swab without medium" = A, "dry swab"). If the concentration of the pathogen is low, swabs in a medium can lead to false negative results. For collecting the sample, the swabs can be moistened with physiological saline solution. For PCR tests, so-called cytobrushes (brush swabs) are also suitable, which can be shipped in an uncoated sterile tube.

For the detection of pathogens in faeces, a sample of approximately the size of a hazelnut is needed. For some agents (e.g. coronavirus, Tritrichomonas foetus) we recommend collecting faecal samples for 3 days, since these pathogens are excreted intermittently in the faeces.

Further sample materials, e.g. skin biopsies, organ material, urine, synovial fluid, CSF, bone marrow aspirates and lymph node aspirates, for PCR tests are best sent in sterile, uncoated test vessels. Fixation solutions such as formalin or the like can lead to DNA degradation, PCR inhibition and thus to false negative results.

Samples do not normally need to be sent cooled. Until it is dispatched, the sample material can be stored in the refrigerator at  $2-8\,^{\circ}$ C. Repeated freezing/thawing should absolutely be avoided.

Please note: Creating an antibiogram is not possible after doing a PCR test.

# 1.7 Genetic Testing

As sample material for the molecular genetic detection of hereditary diseases, for parentage analysis as well as for the genetic determination of coat colours and blood types, **EDTA whole blood samples (approx. 1 ml)** are suitable. Alternatively, in dogs and cats, buccal swabs, so-called cheek swabs, can be used. For each animal, **2 buccal swabs** (without transport medium) should be supplied. To create DNA profiles and parentage reports in dogs and cats, we recommend to always send in a blood sample. For all genetic testing in horses, it is sufficient to supply about **20 hair roots** from mane or tail for DNA isolation.

**EDTA blood** is the most suitable sample material. It is absolutely essential to use EDTA as anticoagulant. Lithium heparin or citrate are unsuitable as anticoagulants, as they may inhibit the subsequent PCR. In very rare cases, haemolysis induced by transport or extreme stress during sample collection might lead to the situation that no result can be obtained. However, the percentage of blood samples which cannot be evaluated is extremely low, being < 1%.

**Buccal swabs,** often incorrectly called saliva samples, are very suitable sample materials for genetic testing in dogs and cats, as long as the sampling procedure is performed correctly observing the following rules:

- 1. The animal should not have eaten anything for about 1 hour prior to the sample collection. It should be ensured that puppies and kittens have not been nursed for a minimum of 2 hours, as otherwise maternal cells might skew the results.
- 2. When taking the sample, it should be scrubbed strongly at the inside of the cheek to make sure that enough cells of the oral mucosa and thus genetic material is attached to the swab. Genetic testing can only be conducted if enough genetic material adheres to the swab. Generally, saliva alone is not sufficient. However, there should not be any blood on the swabs!

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3. In order to prevent the growth of bacteria and mould, the swabs should be dried for about 2 – 4 hours after collecting the sample. This is done best by keeping the test tubes a little open for a while.

As there is considerably less cell material available from mucosal swabs compared to blood samples, it is not always possible to isolate enough DNA from buccal swabs for a genetic test. This applies to about 5% of the submitted buccal swabs. We recommend sending two buccal samples per animal, so there is more material available for testing

For horses, hair roots can be used to perform genetic examinations. To do so, about 20 pulled mane or tail hairs are needed. If samples are taken from various animals, hands must be cleaned thoroughly after each sampling - even a single hair of a different animal can skew the result.

Hairs can, for instance, be shipped in little plastic bags or in envelopes. It is, however, absolutely necessary to make sure that the hairs are put in a closed envelope, separate from the submission form, when sent in.

There should not be any blood samples sent in for cattle from multiple births because of a possible blood chimerism, but if the test allows it, hair roots, sperm or tissue samples can be used. One exception to this is the freemartin test, for which a blood sample is mandatory.

If you wish to supply sample materials different from those listed above for performing genetic tests, please contact us before sending the samples.

# **Immune Status**

EDTA blood samples must not be older than 48 hours!

# Sample Material/Shipping Material

Note regarding the sample materials listed in the test descriptions from Chapter 2 onwards:

If the abbreviations are separated by a comma, you can choose the material which is easiest for you to collect from the given list. When collecting sample material for the detection of a pathogen using PCR, you should preferably collect that material from the listed alternatives which is likely to have the highest concentration of pathogens. If the specifications are connected by one or more "+", both or all the materials joined with "+" need to be provided for determining all the parameters of the selected testing block. The materials required for the individual tests are also indicated on the submission forms, however, for lack of space, not always completely.

Sample and shipping containers that are available for the collection and transport of the samples include:

## (1) EDTA tube#

EB = EDTA blood: It can be shipped in this tube (+ No. 8). EP = EDTA plasma: EDTA blood has to be centrifuged and the supernatant needs to be transferred into a neutral tube (e.g. Eppendorf tube). It must then be marked accordingly as EP or labelled with the appropriate bar code.

#### (2) Heparin tube#

HB = Heparin blood: It can be shipped in this tube (+ No. 8). HP = Heparin plasma: Heparin blood has to be centrifuged and the supernatant needs to be transferred into a neutral tube (e.g. Eppendorf tube). It must then be marked accordingly as HP.

(3) NaFB = Sodium fluoride blood With NaFB samples, too, please pay attention to the labelling.

#### (4) S = Serum#

To collect serum, the coagulated blood should be centrifuged at 2000 g 30 minutes after being collected. The supernatant should then be transferred into a neutral tube or another serum tube (remove beads before!) and marked accordingly as serum or labelled with the appropriate bar code.

## (5) Citrate tube#

CB = Citrate blood: It can be shipped in this tube (+ No. 8). CP = Citrate plasma: The sample should be centrifuged and the supernatant needs to be transferred into a neutral tube (e.g. Eppendorf tube). It must then be marked accordingly as CP.

#### (6) Salivette®

for collecting saliva samples



#### (7) Blood smear

Blood smears should always be sent in air-dried, unfixed and unstained. For transportation, the depicted transport covers (shipping containers) are suitable. Before transport, store at room temperature (25 °C) (may not be cooled).

(8) Shipping containers for blood tubes or urine tubes

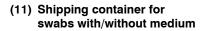


(9) TM = Swab with transport medium

(orange: thin swab. Amies medium clear: black: thick swab. Amies with charcoal)



(10) A = Swab without transport medium, (dry swab)





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(12) Urine tube (suitable shipping container see No. 8)



(15) Blood culture flask set (aerobic and anaerobic)



(13) Container for histology (formalin tube with shipping container)



(16) Blood culture bottle Peds Plus™



(14) Faeces tube with shipping container

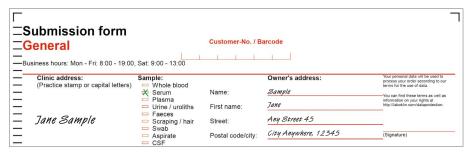


#On special request, **small test tubes** (see each of the tubes shown on the left) are provided for collecting small amounts of EDTA blood, EDTA plasma, heparin blood, heparin plasma, citrate blood, citrate plasma and serum, e.g. from small mammals. If required, please **order** these **small test tubes** by **e-mail** or **telephone only.** At your express request (information by telephone or e-mail), we can also send you vacuum tubes for the examination of blood samples at Laboklin.

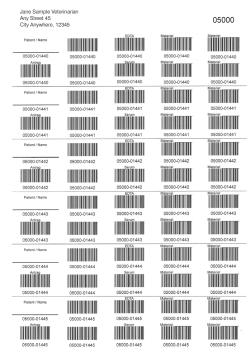
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# 1.10 Labelling

- The name of the animal or the owner and, for farm animals, the ear tag number(s) should be clearly marked on the submission form and the sample. Alternatively, bar code labels can be used to unmistakably identify the sample and the form they will automatically be sent along when submission forms are ordered. For farm animals, there are special sample lists for the submission of several samples, even from different animals in a livestock.
- For function tests, also indicate the respective time of sampling.



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#### Bar code labels:

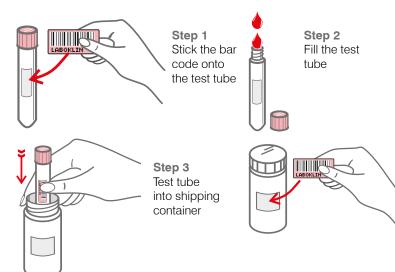
On one sheet, you will find the labels for 6 patients one below the other. For each patient, there are labels for your laboratory journal, the submission form and to mark the sample tubes/vessels to be sent.

There is a pre-printed label for serum and EDTA; for any other label, the material must be added in handwriting.

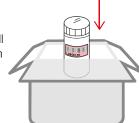
Please stick the bar code exactly over the tube label so that the content is still visible through the uncovered areas



# Labelling of samples and shipping materials



Step 5
Completely fill in submission form and, if necessary, sample list



Step 6
Put sample and submission form and, if necessary, sample list, with sufficient cushioning material into the cardboard box

Step 4

Additional

bar code

onto the

shippina

container



\_\_\_\_

\_\_\_\_

\_\_\_\_

Step 7
Please choose
the correct label
and stick it on
the box

**Exempt Animal Specimen** 



Step 8
Pay attention to correct shipping information

# 1.11 Packaging and Transport

# 1.11.1 Packaging Requirements

Please remember to pack your shipment according to EU regulations (European Agreement concerning the International Carriage of Dangerous Goods by Road, ADR, and International Air Transport Association, IATA):

Generally, transport containers that are transparent, break-proof and contain absorbent material for leakage protection should be used and then packed, together with the submission form and cushioning material (not provided by the lab), in the transport (courier) box (min. dimension of 100x100x100 mm). Volume restriction: sample of 1000 ml (applies to liquid samples) or a total weight of 4 kg (applies to illiquid samples). LABOKLIN provides such protective outer packaging free of charge.

There are 2 possible **categories of samples.** The outer package needs to be tagged according to the respective category with the labels shown in the illustration above. An **exempt animal specimen** is a patient sample for which there is minimal likelihood that pathogens are present (e.g. blood, serum or formalin-fixed tissue samples). Classification must depend on professional judgement which is based on the anamnesis, the signs, the patient's individual circumstances and local endemic conditions. In case of doubt, it is recommended to ship as infectious substance of Category B.

**Infectious category B samples** (swabs, urine, faeces etc.) must be marked as "Biological substance, Category B" and "UN 3373"; while the specification "UN 3373" needs to be in a rhomb of at least 5 cm x 5 cm of size. The edge of the rhomb must be at least 2 mm wide and the letter height of both specifications must be at least 6 mm.

## Important:

The sender is liable for the goods to be transported (i.e. sender is liable to recourse in case of damage/costs caused by samples that are not properly packed). If requirements are not met, there is a risk of your shipment being returned to you by the courier company.

Please adhere to your local national regulations as well as to the EU regulations concerning the transport of the biological samples no matter whether you send them per post or courier. In different countries different rules can apply.

Please do not to leave any needles in the sample tubes! Do not seal the tubes!

If protective covers/transport containers are used, the lids will remain securely closed.

For shipment from a non-EU country, please contact LABOKLIN in advance.

Should you have any other questions, please do not hesitate to contact your local LABOKLIN representative or contact us directly: service@laboklin.com.

# 1.11.2 Transport of Cooled or Frozen Samples

For certain tests, it is necessary to cool or freeze the samples after collection. The cold chain must not be interrupted **until the sample arrives at the laboratory.** 

# How necessary cooling of the sample is indicated:

• On the **submission forms**, samples that need to be refrigerated are marked with "!" behind the name of the service.

If freezing is required, it is explicitly stated.

If there is a mark for refrigeration in services that require the submission of several materials, often only one of them needs to be cooled. In this case, there is an additional "!" behind the material that must be cooled. If all of the materials which need to be submitted must be refrigerated, you will find the "!" mark only behind the name of the service.

Examples:

"Pre-OP Screening! CP!+S+EB/1ml": only citrate plasma must be sent in

refrigerated

"PPID Profile! EP!+S!+NaFB/2ml": EDTA plasma and serum must be sent in

refrigerated

"Large Vitamin Profile! S+EB/3ml": serum and EDTA blood must be sent in

refrigerated

• In this **compendium**, the need for cooling/freezing is always indicated in text form.

The samples are neither cooled during transport by post nor by courier. Therefore, send samples which need to be cooled/frozen with a **cold/ice pack** and, if necessary, in an additional **polystyrene box**.

It should be noted that not only the cold/ice packs but also the samples must be brought to the right temperature before transport, as the cooling/freezing capacity of the packs and the box alone is not sufficient to adequately refrigerate or freeze samples.

You can purchase a special box from Laboklin; it consists of a polystyrene box and a special sample cooling/freezing pack, which can be used to cool 2 sample tubes all around. When you purchase this box, it will be personalised for your practice/clinic and will be returned to you free of charge each time we receive your samples.

# 1.12 Reordering Tests

You can request additional tests for sample material that has already been sent in if

- reordering is done within the sample storage period (see below)
- the sample contains sufficient material
- the maximum sample age that may be indicated for the newly requested test is not exceeded (e.g. for morphology, flow cytometry). Generally, parameters with special requirements for pre-analytics (cooled or frozen) cannot be reordered.

Reordering can be done

- by e-mail to befund@laboklin.com or to info@laboklin.com
- by MyLab
- by telephone +49 971 7 20 20 via the switchboard or as part of our specialist counselling
- by fax on +49 971 6 85 46
- by post

For reorders that shall be invoiced to the animal owner, please read the notes in Chapter 26.

**Storage periods** depend on the type of sample material and the purpose of the submission, i.e. the type of test that was originally requested. The periods specified below apply to samples tested in Bad Kissingen (as of December 2021).

Storage after clinical-chemical examinations, allergy tests, serological examinations (antibody detection; antigen detection, except those from faeces):

- serum, heparin plasma, citrate plasma, EDTA plasma: 14 days
- EDTA blood, heparin blood: 7 days
- urine: 7 days
- uroliths: 7 days (in most cases, however, the material is needed completely for analysis)
- punctures and CSF: 7 daysblood smears: 14 days

Storage after bacteriological, mycological and parasitological examination (detection by culture, all types of faecal analysis incl. antigen detection from

faeces), tests for maldigestion:

• faeces: 7 days

skin/hair, swabs, urine, milk: 14 days

• punctures: 4 weeks

• isolated pathogens: 7 days

# Storage after pathogen detection using PCR:

- independent of the sample material (blood, CSF, urine, swabs, tissue, feather, etc.): 3 weeks
- extracted DNA/RNA: 1 year
- Please note that extracted DNA/RNA is only suitable for reorders of further tests using PCR/genetic methods, but not for tests that require microbial growth. It is therefore not possible to request an additional resistance test if the sample had been sent in for PCR pathogen detection.

# Storage after histopathological examination:

- wet material (tissue samples): 3 weeks
- cytology object slides/cytology samples: 3 weeks
- paraffin blocks and sections: 5 years

# Storage after testing for hereditary diseases or coat colours/determination of breed, parentage:

extracted DNA: at least 5 years

## Storage after sex determination in birds:

extracted DNA: 1 year

# 2 Profiles and Screenings

Our profiles contain well-combined, complimentary parameters which offer a clear price advantage compared to individual requests. Results are usually transmitted on the day the sample is received – on Saturdays, only partial findings might be transmitted.

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

# 2.1 Profiles/Screenings – Small Animals

#### 2.1.1 Clinical Chemical Profiles

(in alphabetical order)

### **Adrenal Profile**

Material S 0.7 ml

Parameter 17-OH-progesterone, androstenedione, oestradiol Note Profile for dog and ferret (Chapter 2.2.1, p. 47)

Allergy Profiles > see Chapter 2.1.2, p. 38

# Anaemia Screening Basic

Material S 1 ml + EB 1 ml

Parameter Iron, protein, bilirubin total, complete blood count, (incl. reticulocytes)

# Anaemia Screening Cat

Material S 1 ml + EB 1 ml

Parameter Anaemia Screening Basic + Coombs test + PCR: haemotropic

mycoplasma (incl. species differentiation)

#### **Anaemia Screening Dog**

Material S 1 ml + EB 1 ml

Parameter Anaemia Screening Basic + Coombs test + PCR: babesia, Ehrlichia

canis, Anaplasma phagocytophilum, haemotropic mycoplasma

(incl. species differentiation)

#### **BARF Profile**

Material Fasting required: S 3ml (cooled, centrifuged) + EB 1 ml

Parameter ALT, creatinine, protein, albumin, calcium, phosphate, copper, zinc,

vitamin A, vitamin D, vitamin E, T4, small blood count

2022/23 Profiles – Small Animals

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Note

• For an additional assessment of iodine status, it is recommended to also determine the iodine/creatinine ratio (see Chapter 4.3, p. 96).

See also BARF Faecal Profile (Chapter 2.1.7, p. 45).
See also Feed Ration Calculation in this chapter.

# **Behaviour Profile (dog)**

Material S 1 ml (fasting required; cooled, protected from light – test tube in

aluminium foil)

Parameter Serotonin, T4

# **Cardiac Screening**

Material S 1 ml (cooled, centrifuged)

Parameter CK, LDH, α-HBDH, AST, calcium, magnesium, potassium, troponin I

# Coagulation

Material CP (1 part citrate + 9 parts blood) 1 ml (cooled)

Parameter PT, PTT, thrombin time

#### **CSF Basic Test**

Material CSF 0.5 ml

Parameter Cell count, protein

# **CSF Profile Small (dog)**

Material CSF 0.5 ml
Parameter Protein, IqA, CRP

Note The parameters listed above may also be ordered in combination

with antibody and PCR detection as Neurology Profile dog (see

Chapter 2.1.6, p. 44).

# **Diabetes Monitoring**

Material S 1 ml + NaFB 1 ml

Parameter Glucose, fructosamines, creatinine, protein, β-HBS, lipase, ALT, AST,

sodium, potassium

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

#### **DIC Profile**

Material EB 1 ml + CP 2 ml (CP cooled)

Parameter PT. PTT. thrombin time. D-dimers, complete blood count

**Feed Ration Calculation** 

Note Compilation of an individual diet, please contact us.

Feline Profile Large

Material S 1 ml + NaFB 1 ml

Parameter FCoV (FIP), FeLV, FIV + Screening Large

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

**Feline Profile Small** 

Material S 1 ml

Parameter FCoV (FIP), FeLV, FIV, protein, albumin, albumin/globulin ratio

FIP Screening (cat)

Material S 1 ml

Parameter AST, bilirubin total, protein and serum electrophoresis, FCoV antibodies

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

**FIV Monitoring (cat)** 

Material S 2 ml + EB 2 ml

Parameter ALT, GLDH, AP, urea, creatinine, CD4/CD8 ratio (sample EB < 48h

old), FIV PCR (quantitative)\*

Note A complete blood count can be ordered in addition to this profile.

Geriatric Profile + SDMA

Material S 1 ml + EB 1 ml + NaFB 1 ml

Parameter ALT, GLDH, AP, bilirubin, urea, creatinine, glucose, fructosamines,

protein, albumin, globulin, albumin/globulin ratio, CK, potassium, calcium, sodium, phosphate, iron, lipase, T4, SDMA + complete

blood count

Note For male dogs, this profile can also be combined with CPSE.

In this case, please send the serum cooled and definitely centrifuged.

#### Geriatric Profile + U-P/C

Material S 1 ml + EB 1 ml + NaFB 1 ml + urine 1 ml

Parameter ALT, GLDH, AP, bilirubin, urea, creatinine, glucose, fructosamines,

protein, albumin, globulin, albumin/globulin ratio, CK, potassium, calcium, sodium, phosphate, iron, lipase, T4, urine protein/creatinine

ratio + complete blood count

Note For male dogs, this profile can also be combined with CPSE.

In this case, please send the serum cooled and definitely centrifuged.

## **Heavy Metall Toxicity Screening**

Material S 2 ml + EB 2 ml + urine 5 ml

Parameter Arsenic, cadmium, chromium, copper, lead, manganese, mercury,

thallium, zinc

Note Arsenic and thallium are determined from serum and urine. The

analysis of the other parameters is done from serum or EB.

#### Iodine/Creatinine Ratio > see Chapter 4.3, p. 96

#### **Juvenile Profile**

Material S 1 ml + EB 1 ml + faeces (fasting required)

Parameter ALT, AP, GLDH, calcium, protein, urea, creatinine, bile acids, phos-

phate, small blood count, endoparasites

#### Kidney - Initial Screening

Material S 0.5 ml

Parameter Urea, creatinine

#### **Kidney Screening Extended**

Material S 1 ml

Parameter Urea, creatinine, protein, albumin, sodium, potassium, calcium,

phosphate, SDMA, indoxyl sulphate

## Leishmania Profile Large

Material S 1 ml + EB 1 ml

Parameter Leishmania (ELISA), protein, serum protein electrophoresis (incl.

graph with protein fractions), creatinine, urea, ALT, AST, AP, GLDH,

blood count

Species Dog

Note This profile can also be ordered in combination with the determination

of the urine protein/creatinine ratio (U-P/C) and microalbumin. In this

case, please send 1 ml of urine extra.

Leishmania Profile Small

Material S 1 ml + EB 1 ml

Parameter Leishmania (ELISA), protein, serum protein electrophoresis (incl. graph

with protein fractions), creatinine, urea, ALT, small blood count

Species Dog

Note

# Leukaemia/Lymphoma Profile

Material Lymph node puncture (in NaCl plus a few drops of serum),

peripheral blood (EB, HB 3 ml) + cytology/blood smear

Parameter Complete blood count (if peripheral blood is supplied), cytology/

cytological blood smear, leukaemia immunophenotyping (by flow cytometry; myeloid and lymphoid cells), progenitor cells (depending

on species), sample < 48h old, clonality (by PARR)

It is recommended to send in more blood, if possible, as immuno-

phenotyping requires up to 5 ml of sample volume if the total

leukocyte count is low.

 For a complete evaluation in case of suspected leukaemia, the leukaemia profile is always recommended and should be interpreted in correlation with the clinical picture and history.

• See also Leukaemia Immunophenotyping (Chapter 7, p. 117)

• See also Lymphocytes Clonality (Chapter 18.4, p. 297).

# Liver - Initial Screening

Material S 0.5 ml

Parameter ALT, GLDH, AP

# **Liver Screening 1**

Material S 1 ml (fasting required)

Parameter ALT, GLDH, AP, γ-GT, bilirubin (total + direct), protein, albumin, bile

acids

# Liver Screening 2

Material S 1 ml (fasting required)

Parameter ALT, AP, AST, protein, albumin, bile acids, manganese, copper

Species Dog (can also be requested for cats)

Note Recommended in case of **shunt/cirrhosis/hepatitis**.

# **Muscular Screening**

Material S 1 m

Parameter CK, α-HBDH, AST, LDH, sodium, potassium, sodium/potassium ratio.

calcium, phosphate, magnesium, iron

# **Muscular Screening Extended**

Material S 3 ml (cooled, centrifuged)

Parameter CK,  $\alpha$ -HBDH, AST, LDH, sodium, potassium, sodium/potassium ratio,

calcium, phosphate, magnesium, iron, vitamin E, selenium

Pancreas Insufficiency (EPI) Profile

Material S 1 ml + NaFB 1 ml (fasting required)

Parameter AST, ALT, albumin, sodium, potassium, calcium, chloride, glucose,

TLI, vitamin B12

**Pancreatitis Profile** 

Material S 1 ml + NaFB 1 ml

Parameter PLI, cholesterol, triglycerides, amylase, lipase, ALT, AST, protein,

sodium, potassium, chloride, calcium, glucose

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

Polydipsia Polyuria Screening

Material S 1 ml + EB 1 ml + NaFB 1 ml + urine 1 ml

Parameter Glucose, fructosamines, ALT, GLDH, AP, sodium, potassium,

calcium, protein, urea, creatinine, small blood count, urinalysis

**Pre-OP Screening** 

Material S 1 ml + EB 1 ml + CP 1 ml (CP cooled)

Parameter ALT, protein, urea, creatinine, small blood count (incl. platelets), PT

Screening Large

Material S 1 ml + NaFB 1 ml

Parameter α-amylase, lipase, glucose, fructosamines, triglycerides, cholesterol,

bilirubin total, AP, GLDH,  $\gamma$ -GT, ALT, AST, CK, protein, albumin, globulins, albumin/globulin ratio, urea, creatinine, phosphate, magnesium,

calcium, potassium, sodium, sodium/potassium ratio, iron

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

**Screening Small** 

Material S 1 ml

Parameter ALT, GLDH, lipase, urea, creatinine

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

Seizures Screening Cat

Material S 1 ml + EB 1 ml + NaFB 1 ml (fasting required)

Parameter Glucose, urea, calcium, phosphate, bile acids + toxoplasma anti-

bodies, small blood count

**Seizures Screening Dog** 

Material S 1 ml + EB 1 ml + NaFB 1 ml (fasting required)

Parameter Glucose, urea, calcium, phosphate, bile acids + neospora antibodies,

toxoplasma antibodies, small blood count

T4 + TSH

Material S 1 ml Parameter T4, TSH

T4 + TSH + Thyroglobulin Antibodies (dog)

Material S 1 ml

Parameter T4, TSH, thyroglobulin antibodies

fT4 + TSH

Material S 1 ml Parameter fT4. TSH

fT4 + TSH + Thyroglobulin Antibodies (dog)

Material S 1 ml

Parameter fT4, TSH, thyroglobulin antibodies

Thrombocytopenia Profile > see Travel Profiles (Chapter 2.1.4, p. 41)

Thyroid Profile Cat

Material S 1.5 ml

Parameter T4, fT4, T3, fT3, TSH, cholesterol

Thyroid Profile Dog

Material S 1.5 ml

Parameter T4, fT4, T3, fT3, TSH, thyroglobulin antibodies, T4 AB\*, T3 AB\*

TLI, B12, Folic Acid (small intestine profile)

Material S 1 ml (fasting required)
Parameter TLI. vitamin B12, folic acid

# **Tumour Diagnostics**

Material S 1 ml (cooled)

Parameter Thymidine kinase, haptoglobin, CRP (dog), SAA (cat)

# **Vitamin Profile Large**

Material S 3 ml + EB 3 ml (cooled!)

Parameter Vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin D

(25 OH), vitamin E, folic acid

Note An additional order is possible up to a maximum of 1 day.

#### Vitamin Profile Small

Material S, EP, HP 3 ml (cooled!)

Parameter Vitamin A, vitamin B12, vitamin D (25 OH), vitamin E, folic acid

#### **Vomitus Profile**

Material S 0.5 ml + EB 1 ml

Parameter Lipase, AST, bile acids, protein, albumin, urea, sodium, potassium,

chloride, large blood count

# 2.1.2 Allergy Profiles Dog/Cat

# Food Allergen Profile (dog, cat)

Material S 1.5 ml

Parameter Food Allergens Basic, Extended and Exotic

## Pruritus Profile – Large (dog)

Material S 3.5 ml

Parameter Seasonal and Perennial Panel, Food Allergens Basic and Extended,

sarcoptes antibodies, flea saliva

#### Pruritus Profile - Medium (dog, cat)

Material S 2.5 ml

Parameter Seasonal and Perennial Panel, Food Allergens Basic and Extended

# Pruritus Profile - Small (dog)

Material S 2.5 ml

Parameter Allergy Screening Test, sarcoptes antibodies

# 2.1.3 Serological Profiles

## FeLV, FCoV, FIV

Material S, EP, HP 0.4 ml Parameter Antibodies: FCoV, FIV

Antigen: FeLV

# Herpes-/Calicivirus (cat)

Material S, EP, HP 0.5 ml Parameter Antibodies: FHV, FCV

#### **Vaccination Control**

Material S, EP, HP 0.5 ml

Parameter Antibodies dog: distemper virus, parvovirus, adenovirus

Antibodies cat: calicivirus, herpesvirus, parvovirus (panleukopenia)

# 2.1.4 Travel Profiles Dog and Cat/Thrombocytopenia Profile Dog/Tick-borne Diseases

# Anaemia Small (dog)

Material El

Parameter PCR: Anaplasma phagocytophilum, babesia

#### Anaemia Vector-borne (dog)

Material Ef

Parameter PCR: babesia, Ehrlichia canis, Anaplasma phagocytophilum,

haemotropic mycoplasma (incl. species differenciation)

# **Anaplasma Screening (dog)**

Material EB 1 ml + S, HP 1 ml Parameter CRP, small blood count

Antibodies: Anaplasma phagocytophilum

#### Canine Travel Profile 1

Material S 1 ml

Parameter Antibodies: leishmania, Ehrlichia canis, babesia

**Canine Travel Profile 2** 

Material S 1 ml + EB 1 ml

Parameter Antibodies: babesia and leishmania, Ehrlichia canis, Rickettsia sp.

Antigen: dirofilaria

PCR: hepatozoon, microfilaria, Anaplasma platys

Note Travel-related diseases Southern Spain, Balearic/Canary Islands,

Portugal, Greece, Southern Italy, Croatia, Turkey, Albania, Southern

Romania, Bulgaria, Serbia, Bosnia

**Canine Travel Profile 3** 

Material S 1 ml + EB 1 ml

Parameter Antibodies: leishmania, Ehrlichia canis, babesia, Anaplasma

phagocytophilum Antigen: dirofilaria PCR: microfilaria

Note Travel-related diseases France, Northern Spain, Northern Italy,

Slovenia

**Canine Travel Profile 4** 

Material S 1 ml + EB 1 ml

Parameter Antibodies: babesia, Anaplasma phagocytophilum, Rickettsia sp.

Antigen: dirofilaria PCR: microfilaria

Note Travel-related diseases Poland, Czech Republic, Hungary, Ukraine,

Slovakia, Northern Romania, Russia

**Canine Travel Profile Acute** 

Material EB 1 ml

Parameter Small blood count

PCR: Anaplasma phagocytophilum, Anaplasma platys, babesia,

hepatozoon, Ehrlichia canis, haemotropic mycoplasma

**Canine Travel Profile USA** 

Material S 1 ml + EB 1 ml

Parameter Antibodies: leishmania, ehrlichia, babesia, Rickettsia rickettsii

Antigen: dirofilaria

PCR: hepatozoon, microfilaria, Anaplasma platys

Feline Travel Profile

Material S 1 ml + EB 1 ml

Parameter Antibodies: leishmania, ehrlichia, Rickettsia felis

PCR: hepatozoon, microfilaria, from 1st July 2022 onwards:

+ cytauxzoon

Leishmania Profiles ➤ see Clinical Chemical Profiles (Chapter 2.1.1, p. 34)

Thrombocytopenia Profile (dog)

Material EB 1 ml

Parameter Thrombocyte antibodies (sample < 72 hours old)

PCR: Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia canis

Tick I - PCR

Material Tick

Parameter PCR: Borrelia, TBE virus

Tick II - PCR

Material Tick

Parameter PCR: Anaplasma phagocytophilum, babesia, borrelia, TBE virus

Tick III - PCR

Material Tick

Parameter PCR: Anaplasma phagocytophilum, Anaplasma platys, babesia,

borrelia, Ehrlichia canis, Hepatozoon

Tick IV - PCR

Material Tic

Parameter PCR: Anaplasma phagocytophilum, babesia, borrelia, TBE virus,

rickettsia

**Vector-borne Diseases** 

Material S. EP. HP 1 ml

Parameter Antibodies: borrelia, babesia, Anaplasma phagocytophilum

# 2.1.5 Blood Donor Profiles - Small Animals

The profiles for testing donated blood are based on the (German) guidelines for the collection, storage, transport and use of blood and blood products in the veterinary sector as published by the German Federal Office of Consumer Protection and Food Safety.

## **Blood Donor Profile (cat)**

Material EB + S + NaFB + urine 2 ml each

Parameter Blood group (serological)

Complete blood count

Urea, creatinine, sodium, potassium, calcium, phosphate, bilirubin,

ALT, AP, AST, GLDH, protein, albumin, glucose

Antibodies: FIV; antigen: FeLV

PCR: haemotropic mycoplasma (incl. species differentiation)

Urinalysis

#### Blood Donor Profile (cat) - Follow Up

Material EB + S + NaFB + urine 2 ml each

Parameter The same as Blood Donation Profile (cat) without blood typing.

# **Blood Donor Profile (dog)**

Material EB + S + NaFB + urine 2 ml each

Parameter Blood group (serological)

Complete blood count

Urea, creatinine, sodium, potassium, calcium, phosphate, bilirubin,

ALT, AP, AST, GLDH, protein, albumin, glucose PCR: Anaplasma phagocytophilum, babesia

Urinalysis

#### Blood Donor Profile (dog) - Follow Up

Material EB + S + NaFB + urine 2 ml each

Parameter The same as Blood Donation Profile (dog) without blood typing.

# 2.1.6 PCR Profiles - Dog/Cat

Anaemia Small (dog) > see Travel Profiles (Chapter 2.1.4, p. 39)

Anaemia Vector-borne (dog) > see Travel Profiles (Chapter 2.1.4, p. 39)

BAL Profile > see Cytology (Chapter 18.1, p. 296)

#### Diarrhoea Pathogens Cat

Material Faeces

Parameter Coronavirus, Tritrichomonas foetus, giardia, parvovirus, cryptosporidia

#### **Diarrhoea Pathogens Dog**

Material Faeces

Parameter Coronavirus, parvovirus, circovirus, giardia, cryptosporidia

## Diarrhoea, Human Pathogenic Causes

Material Faeces

Parameter Salmonella, Yersinia enterocolitica, Campylobacter jejuni

## **Dysbiosis Profile**

Material Faeces

Parameters Key bacteria intestinal microbiome quantitative (PCR), mycology,

calprotectin, endoparasites

until 30th June 2022:  $+ \alpha$ -1 antitrypsin,

from 1st July 2022 onwards: + pancreatic elastase (dog) and micro-

scopic nutritive digestion (cat)

Note For information on microbiome analysis see Chapter 16.5, p. 292

#### Eye (cat)

Material Swab without medium (eye)
Parameter FHV, chlamydia, Mycoplasma felis

#### Eye (dog)

Material Swab without medium (eye)
Parameter CHV, chlamydia, mycoplasma

#### Flea (cat)

Material Flea, EB

Parameter Haemotropic mycoplasma (incl. species differentiation), rickettsia,

Bartonella henselae

# **Neurology (cat)**

Material CSF 0.2 ml

Parameter Coronavirus, Toxoplasma gondii, Bartonella henselae, bornavirus

# Neurology (dog)

Material CSF 0.5 ml

Parameter Protein, IgA, CRP, TBE antibodies (IgG)

PCR: distemper virus, Neospora caninum, Toxoplasma gondii,

Anaplasma phagocytophilum

Note The clinical chemical parameters can also be ordered via the CSF

Profile Small.

# **Neurology Small (dog)**

Material CSF 0.5 ml Parameter Protein

PCR: distemper virus, Neospora caninum, Toxoplasma gondii

## Reproduction (cat)

Material Swab without medium (vagina, prepuce), abortion material

Parameter FHV, chlamydia, Mycoplasma felis

# Reproduction (dog)

Material Swab without medium (vagina, prepuce), abortion material

Parameter CHV, chlamydia, mycoplasma, Brucella canis

# Respiratory I (cat)

Material Swab without medium (pharynx, nose, eye)

Parameter FCV, FHV, chlamydia, Mycoplasma felis, Bordetella bronchiseptica

#### Respiratory II (cat)

Material Swab without medium (pharynx, nose, eye)
Parameter FHV, FCV, chlamydia, Mycoplasma felis

# Respiratory III (cat)

Material Swab without medium (pharynx, nose, eye)

Parameter FCV, FHV, chlamydia

# Respiratory IV (cat)

Material Swab without medium (pharynx, nose, eye)

Parameter FCV. FHV

## Respiratory Large (dog)

Material Swab without medium (pharynx, nose, eye)

Parameter CHV, CAV-2, CPiV, CRCoV, influenza A virus, distemper virus,

Bordetella bronchiseptica, mycoplasma

## **Respiratory Small (dog)**

Material Swab without medium (pharynx, nose, eye)

Parameter CAV-2, CPiV, mycoplasma

Tick-borne Pathogen Detection ➤ see Travel Profiles (Chapter 2.1.4, p. 41)

# 2.1.7 Faecal Profiles - Dog/Cat

#### PCR

Diarrhoea Profiles > see Chapter 2.1.6, p. 43

Dysbiosis Profile > see Chapter 2.1.6, p. 43

#### Culture

If possible, please submit a faeces tube that is  $\frac{3}{4}$  full. An aerobic bacteriological and possibly mycological examination, including enrichment for salmonella, is performed. **Pathogen differentiation** is done by **MALDI-TOF**. Unless otherwise stated, the test duration is 2-3 days.

If required, **serological pathogen differentiation** and an **antibiogram**, which are subject to a charge, will be performed additionally.

#### **BARF Faecal Profile**

Salmonella including enrichment, yersinia including enrichment, campylobacter, listeria, endoparasites

Duration 3 days; yersinia: up to 28 days

Note See also clinical chemical BARF Profile (Chapter 2.1.1, p. 31)

#### Combined Faecal Profile

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, gas producers, endoparasites as well as Giardia sp. antigen EIA and cryptosporidium antigen EIA



## Faecal Profile Pathogenic Bacteria

Salmonella including enrichment, yersinia including enrichment, campylobacter, enteropathogenic E. coli including virulence factors (STa, stx1, stx2, eae)

Duration 2 – 3 days; yersinia: up to 28 days

# Faecal Profile Puppy/Kitten

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, gas producers, parvovirus, endoparasites, Giardia sp. antigen EIA

# **Large Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, Clostridium perfringens enterotoxin and Clostridioides difficile toxin A and B, gas producers, endoparasites, Giardia sp. antigen EIA

#### **Small Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella and gas producers

Faecal Profiles PCR > see Profiles "Diarrhoea" (Chapter 2.1.6, PCR Profiles – Dog/Cat, p. 43)

# **Parasitological**

## **Canine Endoparasite Profile**

Endoparasites, Giardia sp. antigen EIA

Note This profile can only be ordered for dogs. For other animal species,

the parameters can be ordered individually.

# Endoparasites + IFAT (from 1st July 2022 onwards)

Helminths, protozoa, giardia (IFAT), cryptosporidia (IFAT)

Note 3-day pooled faecal sample

# Feline Endoparasite Profile

Endoparasites, Giardia sp. antigen EIA, Tritrichomonas foetus (PCR)

Note This profile can only be ordered for cats. For other animal species,

the parameters can be ordered individually.

Endoparasites are still part of most faecal profiles (see above).

# Virological

# Virological Faecal Profile (EIA)

Parvovirus, rotavirus, coronavirus

# 2.2 Profiles/Screenings – Small Mammals, Birds and Reptiles

#### 2.2.1 Clinical Chemical Profiles

#### Small Mammals

#### E. cuniculi Profile

Material S 0.5 ml

Parameter Encephalitozoon cuniculi IgG and IgM, serum protein electrophoresis

Note From 1<sup>st</sup> July 2022 onwards, this profile can also be ordered in

combination with a complete blood count. In this case, please send

0.5 ml HB or 0.5 ml EB extra.

#### Ferret - Adrenal Profile

Material S 0.7 ml

Parameter 17-OH-progesterone, androstenedione, oestradiol

#### Ferret Screening

Material S. HP 0.5 ml + NaFB 0.5 ml

Parameter Glucose, triglycerides, AST, CK, protein, albumin, globulins, urea,

creatinine

until 30<sup>th</sup> June 2022: + γ-GT, LDH, calcium

from 1<sup>st</sup> July 2022 onwards: + GLDH, ALT, fructosamines

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Note A complete blood count can be ordered in addition to this profile.

In this case, please send 0.5 ml HB or 0.5 ml EB extra.

Hedgehog Screening (from 1st July 2022 onwards: Wild Hedgehog Profile)

Material S, HP 0.4 ml, from 1st July 2022 onwards: + faeces

Parameter Urea, creatinine, ALT, protein

from 1st July 2022 onwards: + GLDH and Baermann test (lung worm

larvae)

Species Hedgehog

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 0.5 ml HB or 0.5 ml EB extra.

**Rodent Screening** 

(from 1st July 2022 onwards: Small Mammals - Large Screening)

Material S, HP 0.7 ml

Parameter Urea, creatinine, protein, fructosamines, AST, ALT, GLDH, γ-GT, AP,

CK, potassium, sodium, calcium, bile acids, phosphate;

until 30th June 2022: + magnesium,

from 1<sup>st</sup> July 2022 onwards: + glucose, triglycerides, bilirubin

Species Rabbit, guinea pig, rat, mouse, hamster, ferret, hedgehog, other

species on request

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 0.5 ml HB or 0.5 ml EB extra.

Rodent Screening + T4

(from 1st July 2022 onwards: Small Mammals - Large Screening + T4)

Material S. HP 0.7 ml

Parameter Urea, creatinine, protein, fructosamines, AST, ALT, GLDH, γ-GT, AP,

CK, potassium, sodium, calcium, bile acids, phosphate, T4;

until 30th June 2022: + magnesium.

from 1st July 2022 onwards: + glucose, triglycerides, bilirubin

Species Rabbit, guinea pig

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 0.5 ml HB or 0.5 ml EB extra.

**Small Screening** 

(from 1st July 2022 onwards: Small Mammals - Small Screening)

Material HP. S 0.4 ml

Parameter Urea, creatinine, ALT, GLDH; from 1st July 2022 onwards: + protein

Species Rabbit, guinea pig, rat, mouse, hamster, ferret, hedgehog

Birds

**Avian Screening** 

Material S. HP 0.4 ml

Parameter LDH, AST, amylase, uric acid, bile acids, cholinesterase, CK, protein,

triglycerides, cholesterol, GLDH, sodium, potassium, calcium,

phosphate

Reptiles

Brumation ➤ see PCR Profiles (Chapter 2.2.2, p. 51)

Reptile Screening (large)

Material S, HP 0.4 ml

Parameter AP, GLDH, ALT, AST, bile acids, CK, protein, triglycerides, cholesterol,

urea, uric acid, phosphate, calcium, potassium, sodium

Reptile Screening (small)

Material S. HP 0.2 ml

Parameter AP, GLDH, protein, uric acid, phosphate, calcium

2.2.2 PCR Profiles - Small Mammals, Birds and Reptiles

**Small Mammals** 

Ferret: Respiratory Profile

Material Swab without medium, bronchoalveolar lavage, tissue

Parameter Distemper virus, Influenza A virus, SARS-CoV-2

Rabbit: Respiratory Profile

Material Swab without medium

Parameter Bordetella bronchiseptica, toxigenic Pasteurella multocida, chlamydia

**Birds** 

Avian Profile I

Material EB. feather

Parameter Sex determination, PBFD

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## Avian Profile II

Material EB, feather

Parameter PBFD, polyomavirus

#### **Avian Profile III**

Material EB, feather

Parameter Sex determination, PBFD, polyomavirus

#### **Avian Profile IV**

Material EB, feather

Parameter Sex determination, PBFD, polyomavirus, herpesviruses

(e.g. Pacheco)

#### **Avian Profile V**

Material EB, feather + swab without medium (eye, pharynx, cloaca; preferably

1 swab from all 3 sites)

Parameter PBFD, polyomavirus, herpesviruses (e.g. Pacheco), chlamydia,

bornavirus

#### Avian Profile VI

Material EB, feather + swab without medium (eye, pharynx, cloaca; preferably

1 swab from all 3 sites)

Parameter PBFD, polyomavirus, chlamydia

# Reptiles/Amphibians

#### **Amphibian Profile**

Material Swab without medium (skin), tissue (skin, organs)

Batrachochytrium dendrobatidis, Batrachochytrium

salamandrivorans, ranaviruses

## **Aquatic Turtle**

Parameter

Material Swab without medium (pharynx, cloaca; preferably 1 swab from

both sites), nasal lavage

Parameter Herpesviruses, mycoplasma, ranaviruses

#### **Brumation Check Large (tortoise)**

Material S, HP 0.5 ml + HB 0.5 ml + blood smear + swab without medium

(pharynx) + faeces

Parameter Large reptile profile, blood count

Antibodies: herpesvirus (TeHV-1 and TeHV-3)

PCR: herpesviruses, mycoplasma

Endoparasites

# **Brumation Check Small (tortoise)**

Material S, HP 0,5 ml + swab without medium (pharynx)

Parameter Large reptile profile

Antibodies: herpesvirus (TeHV-1 and TeHV-3)

PCR: herpesviruses, mycoplasma

# Quarantine (boa/python)

Material Swab without medium (pharynx, cloaca; preferably 1 swab from

both sites), tracheal lavage + EB

Parameter Adenoviruses, arenaviruses, paramyxoviruses/ferlaviruses, reoviruses,

mycoplasma

# Quarantine (colubrid/viper)

Material Swab without medium (pharynx, cloaca; preferably 1 swab from

both sites), tracheal lavage + skin (swab without medium or tissue)

Parameter Adenoviruses, paramyxoviruses/ferlaviruses, reoviruses, Ophidio-

myces ophidiicola

Note For pharvnx + cloaca. 1 swab can be used. Please take a separate

swab for the skin.

#### Quarantine (lizard)

Material Swab without medium (pharynx, cloaca; preferably 1 swab from

both sites)

Parameter Adenoviruses, ranaviruses, reoviruses

# Quarantine (tortoise)

Material Swab without medium (pharynx), nasal flush + S, HP 0.4 ml

Parameter Anitibodies: herpesvirus (TeHV-1, TeHV-3)

PCR: herpesviruses, mycoplasma, picornavirus, ranaviruses

# Respiratory/Neurology (boa)

Material Swab without medium, tracheal lavage + EB

Parameter Adenoviruses, arenaviruses, paramyxoviruses/ferlaviruses, reoviruses

# Respiratory/Neurology (pythons)

Material Swab without medium (pharynx, cloaca; preferably 1 swab from

both sites), tracheal lavage + EB

Parameter Adenoviruses, arenaviruses, nidoviruses, paramyxoviruses/

ferlaviruses, reoviruses, mycoplasma

#### **Respiratory Profile Large (tortoise)**

Material Swab without medium (pharynx), nasal flush Parameter Herpesviruses, mycoplasma, picornavirus

# Respiratory Profile Small (tortoise, turtle)

Material Swab without medium (pharynx), nasal flush

Parameter Herpesviruses, mycoplasma

#### Skin Profile (lizard)

Material Skin + swab without medium (skin)

Parameter Mycology

PCR: Adenoviruses, iridovirus, ranaviruses

# 2.2.3 Faecal Profiles – Small Mammals, Birds and Reptiles

#### Culture

If possible, please submit a faeces tube that is  $\frac{3}{4}$  full. An aerobic bacteriological and possibly mycological examination, including enrichment for salmonella, is performed. **Pathogen differentiation** is done by **MALDI-TOF**. Unless otherwise stated, the test duration is 2-3 days.

If required, **serological pathogen differentiation** and an **antibiogram**, which are subject to a charge, will be performed additionally.

#### **Ferret Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites, Giardia sp. antigen EIA

#### Rabbit and Rodent Faecal Profile

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites

#### **Avian Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites

# **Pigeon Faecal Profile**

Salmonella incl. enrichment, endoparasites (incl. coccidia)

#### **Reptile Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella

# **Parasitological**

# Ferret and Chinchilla Parasite Profile (from 1st July 2022 onwards)

Endoparasites, Giardia sp antigen (EIA)

# Hedgehog Parasite Profile (from 1st July 2022 onwards)

Endoparasites, lung worm larvae

# **Endoparasites (reptiles)**

Flotation and SAFC, Ziehl-Neelsen stain, native smear

In small mammals and also in birds, endoparasites are part of the mainly cultural faecal profiles (see above).

# 2.3 Profiles/Screenings – Horse

## 2.3.1 Clinical Chemical Profiles

# **Blood Donor Profile**

Material EB + S + NaFB + CP + urine 2 ml each

Parameter Complete blood count, fibrinogen, urea, creatinine, sodium, potassium, calcium, phosphate, bilirubin, ALT, AP, AST, GLDH,

protein, albumin, glucose

Antibodies: equine infectious anaemia virus (Coggins test), equine

arteritis virus (VNT), babesia (c-ELISA)

Urinalysis

Note The profiles for testing donated blood are based on the (German)

guidelines for the collection, storage, transport and use of blood and blood products in the veterinary sector as published by the German

Federal Office of Consumer Protection and Food Safety.

#### **EMS Profile (Equine Metabolic Syndrome)**

Material S 2 ml (cooled) + NaFB 2 ml

Parameter Insulin, glucose, fructosamines, RISQI (reciprocal inverse square

of insulin), MIRG (modified insulin to glucose ratio), I/G ratio

(insulin:glucose)

A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

• MIRG is always calculated. If the value is not plausible, no result

will be specified in the findings.

#### **Equine Cushing Profile - PPID Profile**

Material S 2 ml (cooled) + EP 2 ml (cooled) + NaFB 2 ml

Parameter Insulin, ACTH, glucose, fructosamines, triglycerides, γ-GT, RISQI,

MIRG, IG ratio (for full text descriptions see EMS profile)

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

#### **Foal Profile**

Material S 1 ml

Parameter Triglycerides, urea, creatinine, protein, γ-GT, sodium, calcium, mag-

nesium, phosphate, serum protein electrophoresis

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Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

# Heavy Metall Toxicity Screening

Material S 2 ml + EB 2 ml + urine 5 ml

Parameter Arsenic, lead, cadmium, chromium, copper, manganese, mercury,

thallium, zinc

Note Arsenic and thallium are determined from serum and urine. The

analysis of the other parameters is done from serum or EB.

#### Kidney - Initial Screening

Material S 0.5 ml

Parameter Urea, creatinine

# Liver - Initial Screening Horse

Material S 0.5 ml

Parameter AST, GLDH, γ-GT

#### **Liver Screening Horse**

Material S 1 ml

Parameter AST, GLDH, AP, γ-GT, bilirubin total and direct (II), protein, albumin,

globulins, bile acids

#### Mineral Profile II

Material S, HP 3 ml

Parameter Manganese, zinc, selenium, copper, sodium, potassium, calcium,

magnesium, phosphate, chloride, iron

#### Muscular Screening

Material S 1 ml

Parameter CK, α-HBDH, AST, LDH, sodium, potassium, calcium, phosphate,

magnesium, iron

## **Muscular Screening Extended**

Material S 3 ml (cooled!)

Parameter CK, α-HBDH, AST, LDH, sodium, potassium, sodium/potassium

ratio, calcium, phosphate, magnesium, iron, vitamin E, selenium

**Performance Profile Horse** 

Material S 1 ml + NaFB 1 ml

Parameter AP, γ-GT, GLDH, bilirubin total, triglycerides, cholesterol, glucose,

lactate, AST, LDH, CK, protein, albumin, globulins, urea, creatinine,

phosphate, calcium, magnesium, potassium, sodium, iron

A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

**Screening Large** 

Note

Note

Material S. HP 1 ml + NaFB 1 ml

Parameter AP, γ-GT, GLDH, bilirubin total, triglycerides, cholesterol, glucose, AST,

LDH, CK, protein, albumin, globulins, urea, creatinine, phosphate, calcium, magnesium, potassium, sodium, iron, copper, zinc, selenium

A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

Screening Large + SAA

Material S 1 ml + NaFB 1 ml

Parameter AP, y-GT, GLDH, bilirubin total, triglycerides, cholesterol, glucose,

AST, LDH, CK, protein, albumin, globulins, urea, creatinine,

phosphate, calcium, magnesium, potassium, sodium, iron, copper,

zinc. selenium. SAA

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

**Screening Small** 

Material S 1 ml

Parameter GLDH, y-GT, AST, LDH, CK, urea, creatinine, protein, triglycerides

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

**Senior Profile** 

Material S 1 ml + NaFB 1 ml

Parameter Urea, creatinine, SDMA, phosphate, calcium, bilirubin total, γ-GT,

GLDH, protein, albumin, globulins, glucose, lipase (DGGR),

triglycerides, zinc, selenium

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

Tumour Diagnostics

Material S 1 ml (cooled)

Parameter Thymidine kinase, SAA, haptoglobin

Vitamin Profile Small

Material S, EP, HP 3 ml (cooled!)

Parameter Vitamin A, vitamin D (25 OH), vitamin E, vitamin B12, folic acid

2.3.2 Allergy Profiles Horse

**Allergy Profile Respiratory** 

Material S 1 ml

Parameter Seasonal, Perennial Panel

Allergy Profile Skin

Material S 3 m

Parameter Seasonal, Perennial, Insect, Food Panel

2.3.3 Serological Profiles Horse

Blood Donation Profile Horse > see Chapter 2.3.1, p. 54

Neurology Large

Material S 1 ml + EB, CSF 0.5 ml, swab without medium

Parameter SAA

Antibodies: West Nile virus IgG and IgM, TBE virus IgG and IgM PCR: bornavirus, equine herpesvirus 1 and 4 (EHV-1, EHV-4)

**Neurology Small** 

Material S 1 ml

Parameter Antibodies: West Nile virus IgG and IgM, TBE virus IgG and IgM

# 2.3.4 PCR Profiles - Horse

**Abortion Profile** 

Material Swab without medium, abortion material

Parameter EHV1, EHV4, EVA, leptospira

Anaemia Small

Material EE

Parameter Anaplasma phagocytophilum, babesia

**CEM Profile Mare 1** 

Material 2 swabs with medium with charcoal, e.g. Amies (fossa clitoridis,

sinus clitoridis)

Parameter Taylorella equigentialis from the 2 sites listed above

• The sites meet the requirements of the EU Council Directive 92/65/EEC (cf. Chapter 13.2.33, p. 237).

• Samples must be analysed within 48 hours after collection.

**CEM Profile Mare 2** 

Material 3 swabs with medium with charcoal, e.g. Amies (fossa clitoridis,

sinus clitoridis, cervix)

Parameter Taylorella equigenitalis from the 3 sites listed above

**CEM Profile Stallion 1** 

Material 3 swabs with medium with charcoal, e.g. Amies (penile shaft,

urethra, fossa glandis)

Parameter Taylorella equigenitalis from the 3 sites listed above

Note • The sites meet the requirements of the EU Council

Directive 92/65/EEC (cf. Chapter 13.2.33, p. 237).

• Samples must be analysed within 48 hours after collection.

**CEM Profile Stallion 2** 

Material 3 swabs with medium with charcoal, e.g. Amies (penile shaft,

urethra, fossa glandis) + sperm

Parameter Taylorella equigenitalis from the 3 sites listed above and in sperm

Eye Profile

Material

Swab without medium (eye)

Parameter EHV-2, EHV-5

Foal Diarrhoea Pathogens

Material

Faeces

Parameter Coronavirus, Lawsonia intracellularis, Rhodococcus hoagii (formerly

Rhodococcus equi) (incl. vapA)

Neurology Large > see Chapter 2.3.3, p. 57

**Respiratory Profile (foal)** 

Material Swab without medium (nose), bronchoalveolar lavage, TBS

Parameter EHV-1, EHV-4, Influenza A virus, Rhodococcus hoagii (formerly

Rhodococcus equi) (incl. vapA)

Respiratory I (horse)

Material Swab without medium (nose), bronchoalveolar lavage, TBS

Parameter EHV-1, EHV-5, Influenza A virus, Streptococcus equi equi/

zooepidemicus

Respiratory II (horse)

Material Swab without medium (nose) + faeces

Parameter EHV-1, EHV-4, Influenza A virus, Streptococcus equi equi, equine

coronavirus

Respiratory III (horse)

Material Swab without medium (nose), bronchoalveolar lavage, TBS

Parameter EHV-1, EHV-4, Influenza A virus

Respiratory IV (horse)

Material Swab without medium (nose or pharynx), bronchoalveolar lavage,

EB 0.2 ml (viraemia) (detection from buffy coat is possible on re-

quest; we need at least 5 ml EB for this)

Parameter EHV-1, EHV-4

Note Herpesviruses usually have only a short viraemic phase. Detection

from EB is therefore often only useful at the beginning of the disease.

#### **Uveitis Profile**

Material Intraocular fluid 0.5 ml Parameter Antibodies: leptospira

PCR: leptospira, EHV-1

#### 2.3.5 Faecal Profiles - Horse

#### Culture

If possible, please submit a faeces tube that is  $\frac{3}{4}$  full. An aerobic bacteriological and possibly mycological examination, including enrichment for salmonella, is performed. **Pathogen differentiation** is done by **MALDI-TOF**. Unless otherwise stated, the test duration is 2-3 days.

If required, **serological pathogen differentiation** and an **antibiogram**, which are subject to a charge, will be performed additionally.

#### Faecal Profile Foal

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, gas producers, rotavirus, Clostridium perfringens enterotoxin, endoparasites (incl. protozoa, strongyloides)

# **Large Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, Clostridium perfringens enterotoxin, Clostridioides difficile toxin A and B, gas producers, endoparasites, PCR: equine coronavirus

### **Small Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites

# **Parasitological**

# **Equine Endoparasite Profile**

Flotation, SAFC, and modified McMaster

#### **PCR**

Dysbiosis Analysis > see Chapter 16.5, p. 292

# 2.4 Profiles/Screenings – Ruminants

## 2.4.1 Clinical Chemical Profiles

## **Bovine Fertility Profile**

Material S, HP 1 ml

Parameter Calcium, phosphate, sodium, magnesium, AST, β-HBS, NEFA

#### **Downer Cow Syndrome**

Material S, HP 1 ml

Parameter Calcium, phosphate, magnesium, AST, CK, urea, protein

### **Heavy Metall Toxicity Screening**

Material S 2 ml + EB 2 ml + urine 5 ml

Parameter Arsenic, lead, cadmium, chromium, copper, manganese, mercury,

thallium, zinc

Note Arsenic and thallium are determined from serum and urine. The

analysis of the other parameters is done from serum or EB.

#### **Ketosis Profile**

Material S, HP 1 ml

Parameter GLDH, γ-GT, bilirubin, protein, β-HBS, NEFA, cholesterol, urea

## Kidney - Initial Screening

Material S, HP 0.5 ml Parameter Urea, creatinine

# Llama/Alpaca Profile

Material S. HP 1 ml + NaFB 1 ml

Parameter AP, γ-GT, GLDH, bilirubin, cholesterol, triglycerides, glucose, AST,

LDH, CK, protein, albumin, globulins, urea, creatinine, calcium, phosphate, sodium, magnesium, potassium, iron, copper, zinc,

selenium

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

# Liver - Initial Screening

Material S, HP 0.5 ml Parameter AST, GLDH, γ-GT

#### Mineral Profile I

Material S, HP 1 ml

Parameter Calcium, sodium, phosphate, magnesium, selenium, zinc, copper

# Mineral Profile II

Material S, HP 3 ml

Parameter Manganese, zinc, selenium, copper, sodium, potassium, calcium,

magnesium, phosphate, chloride, iron

# **Muscular Screening**

Material S. HP 1 ml

Parameter CK, α-HBDH, AST, LDH, sodium, potassium, calcium, phosphate,

magnesium, iron

#### **Ruminant Performance Profile**

Material EB 1 ml + S, HP 1 ml

Parameter Protein, urea, cholesterol, GLDH, NEFA, CK, γ-GT, AST, bilirubin,

calcium, phosphate, magnesium, chloride, β-HBS, selenium

Note A complete blood count can be ordered in addition to this profile.

#### Screening Large

Material S. HP 1 ml + NaFB 1 ml

Parameter AP, γ-GT, GLDH, bilirubin total, triglycerides, cholesterol, glucose, AST,

LDH, CK, protein, albumin, globulins, urea, creatinine, phosphate, calcium, magnesium, potassium, chloride, sodium, iron, copper, zinc,

selenium

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

#### **Supply Profile Calf**

Material S. HP 1 ml + NaFB 1 ml

Parameter Glucose, protein, calcium, sodium, phosphate, magnesium, iron,

immunoglobulin G

Note The determination of vitamin E/selenium can be ordered in addition

to this profile. In this case, please send 2 ml NaFB + 2 ml S cooled

extra.

Vitamin Profile Large

Material S 3 ml + EB 3 ml (cooled)

Parameter Vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin D

(25-OH), vitamin E, folic acid

Note An additional order is possible up to a maximum of 1 day.

Vitamin Profile Small

Material S, EP, HP 3 ml (cooled)

Parameter Vitamin A, vitamin B12, vitamin D (25-OH), vitamin E, folic acid

**Weak Calf Profile** 

Material S. HP 1 ml

Parameter Protein, calcium, phosphate, zinc, iron

Note A parasitological faecal examination can be ordered in addition to

this profile.

# 2.4.2 Serological Profiles - Ruminants

**Bovine Abortion Profile** 

Material S, HP 3 ml

Parameter Neospora caninum, Coxiella burnetii, leptospira, chlamydia, BVDV\*

**Bovine Respiratory Profile** 

Material S. HP 1 ml

Parameter Bovine respiratory syncytial virus (BRSV), bovine parainfluenzavirus

3 (BPIV-3), Mycoplasma bovis

Note Be careful with vaccinated animals! In acute cases, it is more useful

to determine pathogens by PCR using the "Bovine Respiratory

Profile 1" (see next chapter).

**Camelid Abortion Profile** 

Material S, HP 2 ml

Parameter Leptospira, chlamydia, Toxoplasma gondii

**Small Ruminant Abortion Profile** 

Material S. HP 3 ml

Parameter Coxiella burnetii, chlamvdia, leptospira, listeria

# 2.4.3 PCR Profiles - Ruminants (Infectious Disease Profiles)

#### **Bovine Abortion Profile**

Material Abortion material, swab without medium + swab with medium

Parameter Bacteriology

PCR: Neospora caninum, Coxiella burnetii, chlamydia, BVDV

## **Bovine Respiratory Profile 1**

Material Nasal lavage, swab without medium + swab with medium

Parameter Bacteriology

PCR: bovine respiratory syncytial virus (BRSV), bovine parainfluenza-

virus 3 (BPIV-3), Mycoplasma bovis

# **Bovine Respiratory Profile 2**

Material Swab without medium, nasal lavage

Parameter PCR: Mannheimia haemolytica, Histophilus somni, Mycoplasma

bovis, Pasteurella multocida (toxin producing)

#### **Camelid Abortion Profile**

Material Abortion material, swab without medium Parameter Leptospira, Toxoplasma gondii, chlamydia

#### Mastitis PCR Profile\*

Material Milk

Parameter PCR test of 16 mastitis pathogens (incl. mycoplasma, yeasts,

Prototheca sp.) and  $\beta$ -lactamase gene (no antibiogram)

#### **Small Ruminant Abortion Profile**

Material Abortion material, swab without medium + swab with medium

Parameter Bacteriology

PCR: chlamydia, Coxiella burnetii

#### 2.4.4 Faecal Profiles - Ruminants

#### Culture

If possible, please submit a faeces tube that is  $\frac{3}{4}$  full. An aerobic bacteriological and possibly mycological examination, including enrichment for salmonella, is performed. **Pathogen differentiation** is done by **MALDI-TOF**. Unless otherwise stated, the test duration is 2-3 days.

If required, **serological pathogen differentiation** (e.g. salmonella, E. coli) and an **antibiogram**, which are subject to a charge, will be performed additionally. A different regulation applies for the detection of E. coli in the Calf Faecal Profile Large.

#### **Bovine Faecal Profile**

In addition to an aerobic bacteriological, mycological examination and the test for obligate and facultative pathogenic bacteria, including enrichment for salmonella, this Bovine Faecal Profile also includes the detection of Mycobacterium avium ssp. paratuberculosis by means of PCR as well as testing for endoparasites.

## **Calf Faecal Profile Large**

The Calf Faecal Profile Large includes the general aerobic bacteriological and mycological examination, including enrichment for salmonella and, if E. coli is present, its serological typing (K99). Furthermore, this profile includes testing for endoparasites, cryptosporidia and coccidia as well as virological testing for rotavirus and coronavirus. If salmonella are detected, they will be serologically typed as an additional service (subject to a charge).

#### Camelid Faecal Profile (Alpaka, Llama)

This profile includes the general aerobic bacteriological and mycological examination, incl. salmonella, the examination for endoparasites, incl. coccidia, cryptosporidia, as well as virological testing for rotavirus and coronavirus.

# **Immunological**

# Calf Faecal Profile (EIA)

The Calf Faecal Profile includes testing for rotavirus and coronavirus, E. coli K99 as well as cryptosporidia. The advantage of ELISA is its short test duration (1 day).

# **Parasitological**

# **Ruminant Endoparasites**

Flotation, SAFC and Baermann test

Endoparasites are also part of the mainly cultural faecal profiles (see above).

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# 2.5 Profiles/Screenings – Pig

#### 2.5.1 Clinical Chemical Profiles

Kidney - Initial Screening

Material S 0.5 ml

Parameter Urea, creatinine

Liver - Initial Screening

Material S, HP 0.5 ml Parameter AST, GLDH, AP

**Liver Screening** 

Material S. HP 1 ml

Parameter ALT, GLDH, AP, γ-GT, bilirubin total and direct (II), protein, albumin,

bile acids

Mineral Profile II

Material S. HP 3 ml

Parameter Manganese, zinc, selenium, copper, sodium, potassium, calcium,

magnesium, phosphate, chloride, iron

**Muscular Screening** 

Material S. HP 1 ml

Parameter CK, α-HBDH, AST, LDH, sodium, potassium, calcium, phosphate,

magnesium, iron

**Porcine Profile Large** 

Material S 1 m

Parameter y-GT, GLDH, bilirubin, ALT, AST, protein, CK, urea, creatinine,

calcium, phosphate, sodium, magnesium, potassium, selenium,

zinc, copper

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

**Porcine Profile Small** 

Material S 0.5 ml

Parameter y-GT, GLDH, bilirubin, urea, creatinine

Note A complete blood count can be ordered in addition to this profile.

In this case, please send 1 ml EB extra.

# 2.5.2 Serological Profiles - Pig

**Porcine Reproduction Profile** 

Material S, HP 2 ml

Parameter Leptospira, PRRSV\*, chlamydia

**Porcine Respiratory Profile** 

Material S, HP 3 ml

Parameter APP\*, Mycoplasma hyopneumoniae\*, PRRSV\*

# 2.5.3 PCR Profiles – Pig (Infectious Diseases Profiles)

**Porcine Reproduction Profile** 

Material Abortion material, swab without medium Parameter PPV, PRRSV, PCV-2, leptospira, chlamydia

**Porcine Respiratory Profile** 

Material Nasal flush, swab without medium + swab with medium

Parameter Bacteriology

PCR: Mycoplasma hyopneumoniae, APP\*, PRRSV, Influenza A,

Pasteurella multocida (toxin producing)

# 2.5.4 Faecal Profiles - Pig

If possible, please submit a faeces tube that is  $\frac{3}{4}$  full. An aerobic bacteriological and possibly mycological examination, including enrichment for salmonella, is performed. **Pathogen differentiation** is done by **MALDI-TOF**. Unless otherwise stated, the test duration is 2-3 days.

If required, **serological pathogen differentiation** (e.g. salmonella and E. coli K88) and an **antibiogram**, which are subject to a charge, will be performed additionally.

#### **Piglet Faecal Profile**

The Piglet Faecal Profile includes the general aerobic bacteriological and mycological examination, including examination for salmonella, testing for endoparasites, virological testing for rotavirus and coronavirus as well es the test for Clostridium perfringens enterotoxin. If salmonella or E. coli are detected, they will be serologically typed as an additional service (see above).



#### **Porcine Faecal Profile**

The Porcine Faecal Profile includes the general aerobic bacteriological and mycological examination, including salmonella, as well as the detection of Lawsonia intracellularis by means of PCR.

# 2.6 Profiles - Fish

**Bacteriology Fish + Fish Tuberculosis** 

Material Swab with medium, tissue

Parameter Bacteriology, Ziehl-Neelsen staining

Fish Screening

Material S. HP 0.4 ml

Parameter AP, ALT, γ-GT, AST, bile acids, CK, protein, cholesterol, urea,

phosphate, calcium, potassium, sodium

**Koi Carp Profile** 

Material Tissue (gills)

Parameter PCR: Koi herpesvirus, carp edema virus

Combi Water Profile + Bacteriology (aerobic)

Material 500 ml water (cooled, if possible) + swab with medium (e.g. gills,

wounds), tissue

Parameter pH, total hardness, carbonate hardness, nitrate, nitrite, phosphate,

copper, ammonium, bacteriology (aerobic)

Note See Large Water Profile.

Large Water Profile

Note

Material 500 ml water (cooled, if possible)

Parameter pH, total hardness, carbonate hardness, nitrate, nitrite, phosphate.

copper, ammonium

Please indicate whether the sample is freshwater or saltwater.

• Determination of the essential chemical water parameters of an

aguarium/pond.

 For clarification of intoxications, differential diagnosis if infectious fish diseases are suspected or for setting ideal husbandry/bree-

ding conditions.

hardness), the organic load of the water (e.g. ammonium, nitrite, nitrate) and the purification capacity of the filter.

• Determination of phosphate and copper is indicated in case of

algae problems or suspected copper intoxication, especially in

• Assessment of the acid-base balance (e.g. pH, carbonate

invertebrates.

Small Water Profile

Material 500 ml water (cooled, if possible)

Parameter pH, total hardness, carbonate hardness, nitrate, nitrite, ammonium

Note See bullet points 1 – 4 of the Large Water Profile.

# 2.7 Profiles - Hygiene

# Hygiene Monitoring – Steriliser + Surface Disinfection Efficacy Testing

Hygiene monitoring includes testing of an autoclave OR a dry heat steriliser by means of bioindicators AND the examination of 3 surfaces (using contact plates) after disinfection. The test kit will be sent to you after you have ordered the test.

Culture examination of your samples will take 7 days. If you participate regularly (2x per year), you will get a certificate stating the successful annual monitoring of the disinfection performance of your autoclave/dry heat steriliser and the surface disinfection test.

This test is not available to third countries.

2022/23 Haematology

# **LABOKLIN**

# 3 Haematology

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

# 3.1 Blood Cells

Complete Blood Count

Material EB, HB 1 ml (+ blood smear)

Small mammals: EB, HB 0.5 ml (+ blood smear)

Birds, reptiles, amphibians, fish: HB 0.5 ml (+ blood smear)

Method Mammals: flow cytometry

Birds, reptiles, amphibians, fish: flow cytometry, leucocyte count and

leucogram: microscopy

Species Mammals, birds, reptiles (fish, amphibians on request)

Duration 1 day

• A leucogram is done in addition to a small blood count; and in dogs and cats, reticulocytes and their haemoglobine concentra-

tion (CHr) are determined.

• For reliable results, the sample should not be older than 48 hours.

• If possible, an air-dried, unstained and unfixed blood smear should be submitted additionally in case further examinations are

necessary.

 In turtles, tortoises and some bird species (corvids, hornbills, ostriches, cranes, some duck species), determination from EB is

not possible due to the cell lysis effect.

**Small Blood Count** 

Material EB. HB 1 ml

Small mammals: EB, HB 0.5 ml

Method Flow cytometry
Species Mammals
Duration 1 day

For reliable results, the sample should not be older than 48 hours.

• Small blood count includes erythrocytes, leucocytes, haemoglobin

and haematocrit.

# Blood Smear, Cytological

Material Blood smear + EB, HB 1 ml

Small mammals: EB, HB 0.5 ml + blood smear

Birds, reptiles: HB 0.5 ml + blood smear

Method Microscopic

Species Mammals, birds, reptiles

Fish, amphibians on request

Duration 1 day

Note

 Cell morphology is assessed for special diagnostic tasks such as haematopoietic neoplasms (lymphoid/myeloid), anaemia, leukopenia, thrombocytopenia, leukocytosis, thrombocytosis and blood-specific infectious agents.

An additional CBC is needed for full information.

• Please add anamnesis, diagnostic task and previous fin-

dings.

# **Bone Marrow Cytology**

Material Bone marrow smear + bone marrow aspirate (see Chapter 1.1.4, p. 17)

+ peripheral blood: blood smear + EB, HB 1 ml

Method Blood count: flow cytometry

Cytological assessment of bone marrow: microscopy

Species Mammals, others on request

Duration 2 days

Note • For diagnostically conclusive findings, a **detailed clinical history** 

with diagnostic task must be supplied!

 Cellularity as well as cell morphology in the bone marrow are assessed for special diagnostic tasks such as cytopenia (anaemia, leukopenia, thrombocytopenia) of unspecified cause

or haematopoietic neoplasms.

• In addition, a complete blood count is performed.

Leucogram

Material EB, HB 1 ml (+ blood smear)

Small mammals: EB, HB 0.5 ml (+ blood smear)

Birds, reptiles, amphibians, fish: HB 0.5 ml (+ blood smear)

Method Mammals: flow cytometry

Birds, reptiles, amphibians, fish: microscopic

Species Mammals, birds, reptiles (fish, amphibians on request)

Duration 1 day

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• Platelet aggregates in the sample can cause pseudothrombocvtopenia.

- Validity check by microscope for platelet concentrations <90 G/l or 60 G/I (equidae).
- No microscopic platelet count.
- Detection of thrombocyte antibodies: see Chapter 7 (p. 120) and Thrombocytopenia Profile (Chapter 2.1.4, p. 41).

# Coagulation

Valid results can only be obtained if the **shelf life** of the **citrate tube** has not been exceeded and the fill level has been correctly observed after collection (also see Chapter 1.1.2, p. 14 and Chapter 1.1.4, p. 17).

Important: The centrifuged citrate plasma must be put in an uncoated tube without anticoagulant (also see Chapter 1.1.2, p. 14).

### Activated **Clotting Time**

D Dimoro

This test can be performed by veterinarians in their own practice. The appropriate tubes (ACT tubes) are subject to a fee and can be ordered from us. Please pay attention to the detailed instructions for use included in the delivery.

# Coagulation Status > see Chapter 2.1.1, p. 32

ט-טוmers	
Material	CP (1 part citrate + 9 parts blood) 0.5 ml (cooled). Please note the introduction to this chapter!
Method	Photometry
Species	Dog, cat, horse
Duration	1 day
Note	D-dimers are generated by lysis of cross-linked fibrin. D-dimers are, for example, detectable if there are internal bleedings as well as in surgical interventions and neoplasia. Particularly high amounts of D-dimers are generated in case of thromboembolism and disseminated intravascular coagulation (DIC). In diagnostic work, D-dimers are mostly used for DIC. D-dimers are a parameter of the DIC Profile (see Chapter 2.1.1, p. 32).

DIC Profile > see Chapter 2.1.1, p. 32

Note

- Determination of a differential blood count is only useful if the total leukocyte count is known.
- In turtles, tortoises and some bird species (corvids, hornbills, ostriches, cranes, some duck species), a determination from EB is not possible due to the cell lysis effect.

### MCHC, MCH, MCV

Material

EB 1 ml

Method Flow cytometry Species

Dog, cat, horse, ruminants, pig, others on request

(not birds, reptiles, amphibians, fish)

Duration

Note

1 day

 The calculated ervthrocyte indices help to differentiate between causes of anaemia.

• Since the cell volume of erythrocytes varies with the ageing of the blood, the indices have to be interpreted with caution in shipped samples.

# Reticulocytes

Material Method

EB. HB 0.5 ml Flow cytometry

Species

Dog, cat, small mammals, small ruminants, pig, others

Duration

1 day

Note

- Reticulocytes are iuvenile erythrocytes determining their number is necessary to be able to differentiate between regenerative and non-regenerative anaemia.
- For reliable results the sample should not be older than 48 hours.
- In dogs and cats, the haemoglobin concentration of reticulocytes (CHr) is measured additionally.

Thrombocytes/Platelets

EB, if applicable HB 1 ml

Flow cytometry Method Mammals Species Duration 1 day

Note

Material

- The most common coagulation disorders in dogs are caused by thrombocytopenia. A platelet count is recommended prior to planned surgery.
- Low counts are also seen in cases of tick-borne infections and travel-related diseases.



Note

Duration

Note

Note

This test comprises the coagulation factors of the extrinsic system. It has to be taken into account, however, that levels may be normal in chronic coagulation. Prothrombin time is used as diagnostic aid in suspected poisoning with vitamin K antagonists (coumarin and warfarin derivatives) and for therapy monitoring while vitamin K is administered.

#### Partial Thromboplastin Time (PTT)

Material CP (1 part citrate + 9 parts blood) 1 ml (cooled). Please note the

introduction to this chapter!

Method Chronometric

Species Dog, cat, horse, cattle, others on request

1 day

 PTT is used to monitor coagulation factors of the intrinsic system and can be used as global test to identify coagulopathies.

 An isolated prolongation of PTT without changes in prothrombin time may indicate a factor deficiency (factor VIII, IX, XI and XII).
 Haemophilia A and B can be identified by determining the concen-

tration of single factors (VIII and IX).

# **Thrombin Time**

Material CP (1 part citrate + 9 parts blood) 1 ml (cooled). Please note the

introduction to this chapter!

Method Chronometric

Species Dog, cat, horse, cattle, others on request

Duration 1 da

This is a second of the second

• This test covers the third phase of coagulation, the change from fibrinogen to fibrin.

 It is recommended for monitoring the treatment with heparin or streptokinase as well as in cases of suspected disseminated intravascular coagulation (DIC) or intoxication with vitamin K antagonists. Temporarily lowered concentrations of fibrinogen are seen after intensive surgery as well as in cases of DIC.

• In case of suspected DIC, the DIC profile may be used to confirm or disprove the diagnosis (see Chapter 2.1.1, p. 32).

Thrombocyte Antibodies > see Chapter 7, p. 120

Thrombocytopenia Profile > see Chapter 2.1.4, p. 41

Factor VIII	
Material	CP (1 part citrate + 9 parts blood) 1 ml (immediately centrifuged, cooled). Please note the introduction to this chapter!
Method	Chronometric
Species	Dog
Duration	1 day
Note	• Factor VIII deficiency is the most common single factor deficiency and the cause of haemophilia A.
	<ul> <li>The determination of single factors is only useful if there are chan- ges in partial thromboplastin time.</li> </ul>

Factor IX	
Material	CP (1 part citrate + 9 parts blood) 1 ml (immediately centrifuged, cooled). Please note the introduction to this chapter!
Method	Chronometric
Species	Dog

Dog
Duration

1 day

Note

• Haemophilia B is a congenital deficiency in factor IX activity, which occurs less frequently than haemophilia A.

• The determination of single factors is only useful if there are changes in partial thromboplastin time.

# Fibrinogen Material CP (1 part citrate + 9 parts blood) 1 ml (cooled). Please note the introduction to this chapter!

introduction to this chapter!

Method Chronometric

Species Dog, cat, horse, cattle

Duration 1 day

• Determination is recommended in case of possible disseminated intravascular coagulation or hypofibrinogenaemia.

 As fibrinogen is an acute-phase protein, the concentration will rise in case of acute inflammation.

# **Prothrombin Time (PT)**

Note

Material CP (1 part citrate + 9 parts blood) 1 ml (cooled). Please note the

introduction to this chapter!

Method Chronometric

Species Dog, cat, horse, cattle, others on request

Duration 1 day

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# **Thromboelastography**

CP (1 part citrate + 9 parts blood) minimum 2 ml Material

(here, the fill level required for each citrate tube must exactly be

adhered to - if necessary, send in several tubes)

Method Thromboelastography Dog, cat, horse, cattle Species

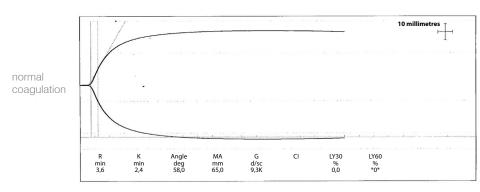
Duration 1 day

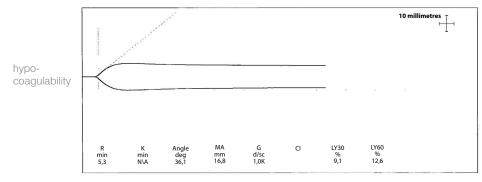
Note

• The citrate blood sample should not be older than 48 hours.

• Global test to determine coagulation disorders, including DIC and thrombocytopathies.

• If DIC is suspected, the DIC profile is also available for diagnostic assessment (Chapter 2.1.1, p. 32).





#### Thromboelastography

Tracing of clot formation and lysis (y axis: amplitude, x axis: time).

R indicates the time until the initiation of clot formation. K and angle are measures for clot kinetics. MA and G for clot strength and LY30 and LY60 for clot lysis.

Hypocoagulability results in a reduction of the maximum amplitude (see above), whereas hypercoagulability (no picture) causes the maximum amplitude to be greater than in normal coagulation.

# **Von Willebrand Antigen**

CP (1 part citrate + 9 parts blood) 1 ml (immediately centrifuged. Material

cooled or frozen). Please note the introduction to this chapter 3.2!

Method Photometry

Species Dog Duration 1 day

Note

 Determination of the von Willebrand antigen is used for further evaluation of coagulation disorders.

• The von Willebrand disease (vWD) has been described in many dog breeds: only genetic testing can detect whether the disease is

carried.

# **Blood Grouping**

#### **Blood Group**

Material EB 1 ml

Agalutination test for determining the serological blood group Method

Species Dog, cat

Horse (only Ca)

1 day, it is possible to send out rapid blood typing tests for dogs and Duration

cats for use in the practice

Note Dog:

DEA 1 positive/negative

 Prior to blood transfusions, it is advisable to test donor and recipient animals for blood group compatibility.

#### Cat:

• A, B, C (formerly AB)

• Do not use umbilical cord blood.

• In cat breeds, the blood groups of the parent animals should be determined before mating in order to avoid neonatal iso-immune haemolytic anaemia.

Genetic testing in A animals is indicated to detect carriers of the recessive B gene.

#### Horse:

Factor Ca

#### **Profiles:**

 Serological blood grouping is also part of the Blood Donation Profiles dog and cat (see Chapter 2.1.5, p. 42).

### Genetic Blood Groups (cat)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Species Cat Breed All

In European Shorthair, there may be discrepancies between serology

and genetics!

Duration 3 – 5 days

Note The AB system is the major blood group system in domestic cats.

The most common blood types are A and B. Cats with blood type B usually have high anti-A antibody titres and cats with blood type A usually have low anti-B antibody titres. Some breeds have the rather rare blood type C (also called blood type "AB"). Cats with blood type C do not have anti-A or anti-B antibodies and are thus universal receivers in case of blood transfusions. Phenotype C ("AB") is not

caused by a co-dominance of A and B.

Genetic blood grouping in cats allows for genetic differentiation of the serologically determined blood group before breeding.

Blood type A is dominant to B. With the genetic test, it is possible to identify the recessive b allele which is associated with the B serotype. Cats with two copies of the b allele have blood type B. Cats with blood type A can carry the genotype AA or Ab. To clarify the genetic basis with blood type A or C ("AB"), the genetic test is recommended. (See also Chapter 20.3.1, p. 372)

#### **Crossmatch Test**

Material Dog, cat: EB 1.5 ml (not older than 48 h)

Horse: EB 3 ml + S 3 ml (not older than 48 h)

Method Dog, cat: immunochromatography

Horse: flow cytometry

Species Dog, cat, horse

Duration 1 day

Note

Please contact us before taking samples.

• Testing for possible negative effects between donor and recipient

blood.

• Dog and cat: To ensure a safe whole blood transfusion, the test includes the major and minor crossmatch test.

(Major crossmatch test: donor erythrocytes + recipient plasma;

Minor crossmatch test: recipient erythrocytes + donor plasma)
• Crossmatch tests for dogs and cats can also be sent out for use in

the practice.

# 3.4 Blood Parasites

Babesia – Microscopic > see Chapter 13.4.3, p. 246

#### **Blood Parasites - Microscopical**

Material EB 1 ml + blood smear

Birds: EB, HB 0.5 ml + blood smear Reptiles: HB 0.5 ml + blood smear

Method Microscopic

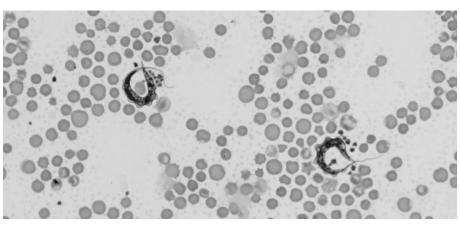
Species Mammals, birds, reptiles

Duration 1 day

Note Please note that smears from HB may not always be suitable due to

the formation of artefacts.

#### Microfilaria - Knott Test ➤ see Chapter 13.4.8, p. 255



Trypanosoma theileri in cattle

1 day

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# 4 Clinical Chemistry

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

# 4.1 Enzymes

#### ALT (GPT)

### Alanine Aminotransferase (Glutamate Pyruvate Transaminase)

Material S, EP, HP 0.5 ml Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, mouse, ferret, birds, reptiles, cattle,

goat, sheep, pig

Duration 1 day

Note In contrast to horses, cattle and pigs, this parameter is liver-specific

in dogs and cats. ALT is found only in the cytoplasm, therefore even minor cell damage may cause elevated levels. Isolated elevations

also occur in case of portosystemic shunt.

### α-Amylase

Material S, EP, HP 0.5 ml Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, horse, cattle, sheep, pig

Duration 1 day

Note • The enzyme is elevated in the acute phase of pancreatitis for 3 – 5

days. Slight elevations also occur due to diseases of other organs and in case of renal dysfunction. As the enzyme is also produced in the liver and small intestine, it is not pancreas-specific.

Therefore, its suitability for diagnosing pancreatitis is limited.

• In order to confirm the diagnosis of pancreatitis, determination of

PLI is recommended (see PLI).

# AP (Alkaline Phosphatase)

Material S 0.5 ml Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration

Note

 The enzyme is found in almost all organs. AP is diagnostically especially significant in diseases of the skeletal and the hepatobiliary system. In dogs, there is also steroid-induced AP, which particularly plays a role in the diagnosis of hyperadrenocorticism (Cushing's disease).

- In the context of bone diseases, high levels are present in case of ostitis deformans, which allows to differentiate from osteoporosis.
   In bone tumours, increases in activity are measured whose extent correlates to the osteoblast activity (very high levels in osteosarcoma, hardly any increases in benign tumours). Rachitis and osteomalacia have elevated levels with decreased calcium levels.
- Increased levels may indicate cholestasis.
- Young animals: physiological concentration up to 2.5-fold.
- Dog: Diagnosis of corticosteroid-induced AP is possible by determining the heat-stable isofraction.
- Cattle: The AP level ante partum allows to assess the risk of parturient paresis.

#### AP (heat-stable 65 °C)

#### (heat-stable Alkaline Phosphatase)

Material S 0.5 ml Method Photometry

Species Dog; not relevant for other species

Duration 1 day

Note

- The heat-stable isoenzyme of AP is induced by endogenous steroid hormones or by cortisone therapy and can be used to diagnose overtreatment with steroids.
- Only useful in combination with the determination of the "total AP" if it is elevated. With the sum of all isoenzymes of AP ("total AP") and the heat-stable AP, the residual activity of AP can be determined in percentage.

#### AST (GOT)

#### Aspartate Aminotransferase (Glutamate Oxaloacetate Transaminase)

Material S, EP, HP 0.5 ml Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration 1 day

Note

- Elevated levels can be caused by diseases of various parenchymatous organs but also by muscle damage. If the latter, it cannot be distinguished between damage of skeletal muscles and cardiac muscles. A simultaneous increase of CK indicates a myogenic origin.
- Cat: sensitive marker of hepatopathies; to differentiate muscle damage, CK levels should be determined additionally.
- Horse: indicates lesions of the skeletal muscles (in combination with other parameters, for example LDH, CK) or the liver.

#### Cholinesterase

S, EP, HP 0.5 ml Material Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse, others

on request

Duration 1 day

Note

Note

- In case of an intoxication with organophosphoric acid esters, the enzyme is blocked and its activity in the blood plasma decreases.
- Birds: liver-specific, decreased in hepatic diseases

### **CK (Creatine Kinase)**

S. EP. HP 0.5 ml Material Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration 1 day

The highest enzyme activity by far is found in the skeletal muscles. followed by brain tissue and cardiac muscles. All conditions that cause destruction of muscle cell membranes result in elevated serum concentrations (e.g. myopathies, traumata due to i.m. injections and after intensive training). Preanalytically, haemolysis also leads to elevated levels. Brain tissue damage does not cause increased serum levels because of the blood-brain barrier.

# **GLDH** (Glutamate Dehydrogenase)

S, EP, HP 0.5 ml Material Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration 1 day



Note

- The enzyme is liver-specific and located in the mitochondrion. Thus, elevations are indicative of massive destruction of liver cells and necrobiotic processes, especially in the centrilobular area. If levels of GLDH are increased, but at the same time ALT levels are changed only slightly, it indicates chronic inflammation of the liver.
- Dog: Single values have no diagnostic significance. Slight elevation of GLDH and stronger elevation of transaminases indicate acute disease of the liver. Opposite enzyme activity indicates chronic processes.
- Cattle: Levels depend on stage of lactation.

#### Glutathione Peroxidase (GPx)

Material EB. HB 0.5 ml Method Photometry

Species Horse, ruminants, pig

Duration 1 – 2 days

Note

- GPx can only be determined in unclotted EDTA or heparin whole blood samples, because the value is linked to haemoglobin.
- GPx is an antioxidant.
- As a selenium-containing enzyme, the GPx level indicates the supply of selenium to the animal within the last 2 – 3 weeks. Thus, GPx cannot indiate an acute undersupply of selenium. An oversupply cannot be diagnosed by determining the GPx level.

### y-GT (y-Glutamyl Transferase)

Material S, EP, HP 0.5 ml Method Photometry

Dog, cat, rabbit, guinea pig, rat, ferret, horse, ruminants, pig Species Duration 1 day

Note

- Although this membrane-bound enzyme is not liver-specific. elevations occur almost exclusively in diseases of the liver and bile ducts.
- Horse: Elevated concentrations are indicative of cholestasis. Increased levels may also be seen in other diseases with liver involvement, such as colic, enteritis and the like.
- Cattle: The y-GT level strongly correlates with the degree of hepatic fatty degeneration and the degree of swelling of the liver and the edge of the liver. Decreased levels of this enzyme indicate insufficient intake of colostrum in calves up to an age of approximately 1 week.

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α-HBDH (α-Hydroxybutyrate Dehydrogenase)

Material S. EP. HP 0.5 ml Method Photometry

Species Dog. cat. horse, ruminants, pig

Duration 1 day

Note

Note

• This isoenzyme of LDH is found in many kinds of tissue, especially in cardiac and skeletal muscles and in the liver; the activity of  $\alpha$ -HBDH varies depending on the species.

• If  $\alpha$ -HBDH in the LDH/ $\alpha$ -HBDH ratio is disproportionately increased, it indicates possible damage of the cardiac muscle. Determination of c-Troponin I concentration has replaced the

analysis of  $\alpha$ -HBDH in case of this indication, though.

• Proportional or slight elevations of the enzyme point to other causes (e.g. liver damage, damage of skeletal muscles, haemolysis and others). In this case, CK and AST levels should be considered.

# LDH (Lactate Dehydrogenase)

Material S. EP. HP 0.5 ml Method Photometry

Dog, cat, rabbit, guinea pig, rat, ferret, birds, horse, ruminants, pig Species

Duration

• LDH is composed of 5 isoenzymes. It is found in many organs. mainly in the liver, in cardiac and skeletal muscles. High concentrations of LDH are also present in erythrocytes, so that even slight haemolysis in serum or plasma may cause falsely elevated values.

 Elevations occur in case of myopathies, cardiomyopathies and liver diseases.

• The ratio of  $\alpha$ -HBDH to LDH can indicate problems of the cardiac or skeletal muscles.

# Lipase (DGGR)

S (EP, HP) 0.5 ml Material

Method Photometry (using DGGR reagent)

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, others on

request

Duration 1 day

Note

 Measurement mainly covers the activity of pancreatic lipase, but also the lipase activity in other tissues (stomach, small intestine). A threefold increase in value indicates acute pancreatitis.

- In serum, lipase activity remains elevated much longer than amylase activity. Therefore, if pancreatitis is suspected, both enzymes should always be determined. What applies to both enzymes is the fact that the enzyme concentration is not proportional to the severity of illness. Especially in cats, unaffected levels of lipase can be seen despite pancreatitis.
- In order to confirm the diagnosis of pancreatitis, pancreatic lipase immunoreactivity (PLI) should be determined.

### PLI (Pancreatic Lipase Immunoreactivity)

S 0.5 ml Material Method **ELISA** Species Dog, cat Duration 1 day

Note

- Detection of specific pancreatic lipase in suspected pancreatitis. The determination of pancreatic lipase in the serum of dogs and cats is considered to be the most sensitive non-invasive marker for the diagnosis of pancreatitis. As part of an inflammatory reaction, the pancreatic acinar cells are destroyed leading to an increase in the pancreatic lipase concentration in the serum.
- If possible, 12 hours of fasting is recommended.

# TLI Test (Trypsin-like Immunoreactivity)

Material S 0.5 ml

Method CLIA (dog), ELISA (cat)

Species Dog, cat

Duration Dog: 1 day, cat: 2 – 3 days

Note · Most sensitive test for the detection of exocrine pancreatic insufficiency.

- Elevated levels can indicate pancreatitis in dogs and cats.
- Renal insufficiencies may also lead to increased TLI values.
- Dogs and cats must fast for 12 hours prior to sampling.



TLI results:

With 8.9%, exocrine pancreatic insufficiency (EPI) is not uncommon in cats of all ages.

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# 4.2 Substrates

Albumin	
Material Method Species Duration	S, EP, HP 0.5 ml Photometry Dog, cat, rabbit, guinea pig, rat, ferret, horse, ruminants, pig 1 day
Note	<ul> <li>Hypoalbuminaemia results from losses of albumin (kidney, intestine, haemorrhage), albumin synthesis disorder (liver) and inflammation. Albumin is a negative acute-phase protein. Cattle: hypoalbuminaemia, particularly in hepatic diseases, reduced feed intake and inflammation</li> <li>Increased levels are mainly found as relative hyperalbuminaemia in case of dehydration.</li> <li>Due to species-specific peculiarities, protein electrophoresis should be preferred in birds and reptiles.</li> </ul>

Bile Acids	
Material Method Species Duration	S 0.5 ml Photometry Dog, cat, rabbit, guinea pig, birds, reptiles, horse, cattle 1 day
Note	<ul> <li>Serum bile acid concentration correlates with liver function. In contrast to the determination of ammonia, which is very susceptible to faults and needs to be performed immediately after sampling, this parameter is very stable.</li> <li>Elevation of bile acids can also indicate the existence of a portosystemic shunt.</li> <li>Single determinations can lead to false normal results, therefore, the performance of bile acid stimulation tests is preferable – except for horses.</li> <li>Please note that animals must fast for 12 hours before sampling (except horses).</li> </ul>

Bilirubin (	(total)
Dilliubili (	(iOiai)

Material	S, EP, HP 0.5 ml
Method	Photometry
	D

Species Dog, cat, rabbit, guinea pig, rat, ferret, horse, ruminants, pig

86

Duration 1 day

#### Note

- Prehepatic icterus: Excessive haemoglobin concentration due to erythrocyte destruction causes increased levels of indirect bilirubin.
- Intrahepatic icterus: Damage of liver cells causes increase in both direct and indirect bilirubin.
- Posthepatic icterus (rare): Increase in direct bilirubin caused by retention of bile.
- Cattle: Total bilirubin has a strong negative correlation with blood glucose levels and hence is a sensitive indicator for imbalances in the composition of food rations. Strong increase occurs due to microhaemolysis as part of septicaemia, e.g. in case of mastitis, endometritis or salmonellosis, and is prognostically unfavourable.

# Bilirubin II (direct)

Material	S, EP, HP 0.5 ml
Method	Photometry

Species Dog, cat, rat, ferret, horse, others on request

Duration 1 da

Note In the liver cells, bilirubin II is formed from bilirubin I by glucuroni-

dation. Determination is only useful in case of elevated levels of total bilirubin. Measurement can be strongly affected by lipidaemia.

# Cholesterol

Material	S, EP, HP 0.5 ml
√lethod	Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

87

ruminants, pig

Duration 1 day

Note

- Cholesterol is formed mainly in the liver and in the mucosa of the small intestine and serves as starting material for many compounds which are synthesised in the liver (e.g. bile acids and steroid compounds).
- In cattle, cholesterol levels correlate with feed intake and milk yield.
- Please note: Fasting is needed 12 hours prior to sampling!



Cholesterol: HDL (High Density Lipoproteins)

Material S, EP, HP 0.5 ml Method Photometry

Species All Duration 1 day

# **Cholesterol: LDL (Low Density Lipoproteins)**

Material S, EP, HP 0.5 ml Method Photometry

Species All Duration 1 day

#### Creatinine

Material Method S, EP, HP 0.5 ml Photometry

Species

Dog, cat, rabbit, guinea pig, rat, ferret, horse, ruminants, pig

Duration 1 day

Note

- In addition to SDMA, creatinine is the most specific indicator for renal function. However, due to the reserve capacity of the kidneys, elevated levels only occur if the kidney damage exceeds 70%. Lipaemia and haemolysis can cause false elevation of values. In well-muscled or trained dogs, creatinine may be increased physiologically, without there being a renal dysfunction.
- Protein/creatinine ratio in the urine (midstream urine or urine from cystocentesis) and SDMA serve for an early detection of renal dvsfunction.
- Cattle: An increase in creatinine is an important indicator for insufficient feed intake/body weight loss.

#### Cystatin C

Material Method Species

Duration

S 0.5 ml Photometry Dog, cat

1 day

Note

Parameter for early renal diagnosis in dogs and cats. Most
of the nucleated cells produce cystatin C at a constant rate;
the production also seems to remain unaffected in cases of
inflammation or other pathological conditions. Levels can be
increased in animals suffering from manifest hyperthyroidism or
after administration of glucocorticoids.

 Cystatin C is filtered in the kidney, probably also tubularly secreted, 99% reabsorbed in the proximal tubule and then degraded.

#### **Fructosamines**

Material Method S 1 ml

Photometry

Species Duration Dog, cat, rabbit, guinea pig, rat, ferret, horse

1 da

Note

- This glycoprotein reflects the average blood glucose level of the past 3 weeks, determination therefore serves the diagnosis and the long-term monitoring of patients with diabetes mellitus. A distinction between spontaneous, stress-induced hyperglycaemia and diabetes mellitus is possible as well. Increased levels can also be found in case of hyperproteinaemia.
- Low fructosamine levels are associated with protein deficiency or increased protein metabolism.
- Horse: often increased in case of EMS.

#### Glucose

Material Method Species NaFB or S promptly centrifuged after collection 1 ml, CSF

Photometry

Dog, cat, rabbit, guinea pig, rat, mouse, ferret, birds, reptiles, horse,

ruminants, pig

Duration

Note

1 day

 Increased blood glucose levels occur in case of diabetes mellitus but also due to brain diseases, pancreatitis and Cushing's disease. Levels particularly increase physiologically due to stress and after administration of glucocorticoids.

- Diseases of the liver, Addison's disease and insulinoma cause hypoglycaemia.
- Drugs which can lead to hypoglycaemia are (among others): antihistamines, beta blockers, anabolic steroids.
- **Dog:** Starving young dogs of toy breeds tend to develop lifethreatening hypoglycaemia in stress situations.
- **Horse:** The determination of glucose levels is a necessary part of the diagnosis of Equine Metabolic Syndrome (EMS). For further information, please see "Insulin" (Chapter 8, p. 124).
- In cattle, hypoglycaemia indicates ketosis due to energy deficiency. hyperglycaemia is caused by stress and endotoxaemia.
- The detection of glucose in urine is part of the Urinalysis (see Chapter 5, p. 102).

**β-Hydroxybutyrate (β-HBA)** 

Material S. HP 0.5 ml Method Photometry

Species Dog. cat. farm animals

Duration 1 day

Note

• Ketone bodies are formed in the organism during the degradation of fatty acids.

• Ruminants: The determination of β-HBA provides an indication of energy supply and can be used for diagnosing ketosis.

**Cattle:** increased ketone body levels in ketosis resulting from an energy deficit, in alimentary ketosis (too much concentrated feed) or secondary (e.g. in case of abomasal displacement).

Small ruminants: for the diagnostic assessment of gestational

toxicosis

### see Cholesterol, p. 88

#### **Indoxyl Sulfate**

Material S 0.5 ml (cooled)

Method HPI C Species Dog, cat Duration 5 days

Note • When breaking down tryptophan, intestinal bacteria form indole,

which is metabolised in the liver to indoxyl sulphate.

 Indoxyl sulphate is a uraemic toxin which is physiologically excreted by the kidneys. In renal dysfunction, indoxyl sulphate accumulates in the body and leads to increased concentrations in serum. Increased indoxyl sulphate levels then lead to further damage of the renal parenchyma, in addition to numerous other damages in the organism, and thus result in the progression of renal dysfunction.

#### Lactate

NaFB 0.5 ml Material Method Photometry

Species Dog, cat, horse, cattle, pig

Duration 1 day

Note Anaerobic degradation of glucose leads to the formation of

lactate. Elevated lactate concentrations may be caused by increased formation of lactate due to a higher glucose uptake or increased glycogenolysis (e.g. diabetes mellitus), impaired metabolisation (hypovolemic, cardiovascular or neurogenic shock) or enhanced formation due to oxygen deficiency in the tissue (fitness level, stress due to blood sampling, increase of lactate in immature neonates).

• Cattle: Elevation occurs in case of ruminal acidosis, circulatory disorders, severe pneumonia.

#### LDL > see Cholesterol, p. 88

# **NEFA (Non-Esterised Free Fatty Acids)**

Material S. HP 0.5 ml Method Photometry Species Ruminants, pig

Duration 1 day

Note The degradation of adipose tissue releases NEFA (non-esterised

> free fatty acids). They are a quick and sensitive marker of nutritional deficiency or of reduced feed intake in case of stress situations or disease, and serve to estimate e.g. the fat mobilisation in metabolic

states.

#### **Protein (Total Protein)**

Material S, EP, HP, CSF 0.5 ml, H

Method Photometry

Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse, Species

ruminants, pig

Duration 1 day

Note

• The main tasks of plasma proteins are water retention, transport, coagulation and defence.

• Absolute hyperproteinaemia usually occurs due to chronic infection, relative rises are common in case of fluid loss. Absolute hypoproteinaemia occurs due to nephropathies, blood loss or intestinal loss of protein into the third space, relative reductions in total protein only in case of increased hydration. In cerebrospinal fluid, elevated levels are seen in case of inflammation as well as tumours associated with the brain. In urine, elevated levels are

usually associated with glomerulonephritis. Electrophoresis is used to separate the protein fractions.

# **SDMA (Symmetric Dimethylarginine)**

Material S. HP 0.5 ml Method **ELISA** 

Species Dog, cat, horse Duration 1 – 2 days

Note

SDMA is formed during protein degradation, is excreted exclusively via the kidneys and is helpful for the early detection of renal dysfunction, even in the creatinine-blind area. In cats, a significant inverse correlation between the glomerular filtration rate and SDMA has been described. Determining the SDMA concentration is recommended if an early stage of renal dysfunction is suspected, e.g. because of beginning polyuria/polydipsia. An elevated SDMA level can already indicate a 30% reduction in glomerular filtration rate (GFR).

#### **Taurine**

Material EP 1 ml (cooled)

Method LCMS
Species Dog, cat
Duration 1 week

Note

Chronic taurine deficiency causes dilatative cardiomyopathy in cats. In general, most commercial diets contain sufficient amounts of taurine. Taurine deficiency may be caused by chronic malabsorption or by homemade rations.

#### **Triglycerides**

Material S, EP, HP 0.5 ml Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration 1 day

Note

 Triglyceride levels are influenced by food intake and metabolic status, among others. Synthesis takes place in the liver, small intestine and adipose tissue.

Dog: elevation e.g. postprandial, diabetes mellitus, hypothyroidism, hypercortisolism, acute pancreatitis.

• Horse: Hyperlipaemia in ponies, metabolic syndrome and Cushing's disease.

• Cattle: Lipid mobilisation syndrome

#### Troponin I

Material S (cooled, centrifuged) 0.5 ml

Method CLIA

Species Dog, cat, horse

Duration 1 day

Note Acute myocardial cell damage (highly specific myocardial parameter);

can be used to diagnose hypertrophic cardiomyopathy.

**Urea** Material

S, EP, HP 0.5 ml

Method

Photometry

Species Duration

Note

Dog. cat. rabbit, quinea pig. rat. ferret, reptiles, horse, ruminants, pig

1 da

 Urea is the most important degradation product of the protein metabolism in mammals. Serum levels are not only influenced by the renal function but also by extrarenal factors (e.g. diet, increased protein degradation). Thus, creatinine should always be checked in parallel.

 In cattle, the urea level serves mainly as an indicator for energy supply.

#### **Uric Acid**

Material S, EP, HP 0.5 ml Method Photometry Species Dog, birds, reptiles

Duration 1 day

Note

• **Dog**: Due to a metabolic disorder, increased levels of serum uric acid can occur especially in Dalmatians. Uric acid concrements in the urine and a characteristic brownish-yellowish discolouration of the coat (Bronzing syndrome) are of clinical significance. This metabolic disorder is mostly of genetic origin which can be confirmed with the genetic test "Hyperuricosuria (HUU/SLC)" (see Chapter 20.2.1, p. 325).

 Birds: Concentrations above 500 μmol/l indicate nephropathies or exsiccosis

 Reptiles: The levels of uric acid vary, depending on various factors, such as feed intake, protein content of the ration, season and species.

# 4.3 Minerals and Trace Minerals

# Calcium (Ca)

Material S, HP 0.5 ml Method Photometry

Species Dog, cat, rabbit, quinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration 1 day

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Note

- Calcium measurement from EDTA plasma does not provide plausible values.
- More than 99% of the body's calcium is stored in the bones. Further functions include transmission of nerve impulses, muscle contractions and blood clotting.
- Non-parathyroid-related hypercalcaemia often occurs due to tumours (malignant lymphoma, carcinoma) (also see Parathormone-rp, Chapter 8, p. 127).
- Hypocalcaemia is often the cause of parturient paresis in cattle and of an increased tendency for seizures in small animals.
- In case of concurrent hypoalbuminaemia, the calcium level should be corrected.

**Calculation:** Corrected calcium level (mg/dl) = serum calcium level (mg/dl) - (0.4 x serum protein (mg/dl)) + 3.3

#### Calcium, ionised

Material S, HP 0.5 ml

Method ISE

Species Dog, cat, birds, reptiles

Duration 1 day

Note

- Ionised Ca represents the biologically active part of the total
- The sample should be collected without air (vacutainer system). The instructions can be requested from us.

#### Chloride (CI)

S, HP 0.5 ml Material

Method ISF

Species Dog, cat, rabbit, guinea pig, rat, mouse, ferret, birds, reptiles, horse,

ruminants, pig

Duration 1 day

Note

- It is the most important extracellular anion and decisive for maintaining the osmotic balance.
- Increased levels are found in all diseases which also cause hypernatraemia. The most common causes are dehydration and hyperchloremic metabolic acidosis.
- Similarly, decreased levels occur in diseases which cause hyponatraemia, e.g. vomiting, abomasal reflux in case of abomasal displacement, and metabolic alkalosis.

#### Cobalt (Co)

Material Method

Horse, ruminants, New World camelids Species

Duration

1 week

**ICPMS** 

S. H 1 ml

Note

• Central component of vitamin B12 (cobalamin), which is formed in ruminants by ruminal bacteria.

• Cobalt deficiency: reduced growth rate, milk vield and reproductive performance, coarse coat, cachexia, anaemia

# Copper (Cu)

Material Method

S, HP 0.5 ml Photometry

Species Dog, cat, horse, ruminants, pig

Duration 1 dav

Note

- Copper is a component of various enzymes. Decreased levels can lead to depigmentation as well as growth and reproductive
- **Dog:** In the copper storage disease in Bedlington Terriers, copper serum levels are usually normal, elevated levels can only be detected in liver tissue. For the genetic test for copper storage disease in Bedlington Terriers. Doberman and Labrador Retriever see Chapter 20.2.1, p. 311.
- Cattle: Decreased levels also occur in case of recumbency. anaemia and cardiac insufficiency.
- Sheep: In newborn lambs, copper deficiency leads to CNS symptoms. Oversupply, e.g. caused by mineral supplement for cattle, leads to intoxication in sheep.

#### lodine (I)

Material Method S 1 ml (cooled)

**ICPMS** 

**Species** Dog, cat, horse, cattle, others on request Duration Approx. 3 days

In cattle, iodine deficiency has been reported to result in goitre Note

formation, fertility problems, abortions, decline in libido, reduced

semen quality and hairlessness.

#### **Iodine/Creatinine Ratio**

Material Urine 1 ml

Method ICPMS, photometry

Species Dog, cat, horse, others on request

Duration Approx. 3 days

Note Studies in different animal species show that the iodine/creatinine

ratio better reflects the alimentary iodine supply than the determinati-

on of blood iodine levels.

Iron (Fe)

Material S, HP 0.5 ml Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse, cattle,

goat, sheep, pig

Duration 1 day

Note

• It is impossible to determine iron levels from EDTA plasma.

 Iron is found in the body in the form of haemoglobin and myoglobin. Furthermore, it is a component of various enzymes.

 Elevated iron levels in the serum occur due to the destruction of liver parenchyma (acute hepatitis, cirrhosis). In case of rarely occurring haemochromatosis and in connection with increased serum levels, deposits are formed in the liver and muscles.

 Decreased levels are often associated with anaemia but also with infections, malignant tumours and nephrosis.

Iron is a negative acute-phase marker.

Magnesium (Mg)

Material S, HP 0.5 ml Method Photometry

Species Dog. cat. rabbit. guinea pig. rat. ferret. birds. reptiles. horse,

ruminants, pig

Duration 1 day

Note

• Magnesium is essential for the energy metabolism of the cells and

for neuromuscular transmission.

Hypermagnesaemia may occur in case of Addison's disease;
 hypomagnesaemia is the most common cause of grass tetany in cattle and can also be found in case of renal dysfunction.

#### Manganese (Mn)

Material S 0.5 ml Method ICPMS

Species Dog, horse, farm animals, others on request

Duration 2 – 3 days

Note Determination is useful in case of suspected undersupply or

intoxication. Undersupply may be caused by increased levels of iron in drinking water, as iron is antagonistic to manganese. Manganese deficiency in cattle mainly causes dysfunctions in skeletogeny and

reproductive disorders.

#### Inorganic Phosphate (PO4)

Material S, HP 0.5 ml Method Photometry

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration 1 day

Note

- Increased values are found physiologically in young animals.
   Due to high intracellular concentrations, hyperphosphataemia is simulated by haemolysis/in haemolytic samples.
- The most common causes of pathologically elevated serum levels are kidney diseases and hyperthyreosis in cats.
- Endocrinopathies may cause hypophosphataemia.
- Reptiles: The Ca/P ratio should be approximately 2:1.
- Cattle: Hypophosphataemia can lead to chronic rumen acidosis, impaired digestion and recumbency. Hyperphosphataemia causes calcinosis.

#### Potassium (K)

Material S, HP 0.5 ml

Method ISE

Species Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse, cattle,

goat, sheep, pig

Duration 1 day

Note • Potassium is the most important intracellular cation.

Potassium levels are only significant if the serum has promptly

been separated.

 Pseudohyperkalaemia may occur due to the release of potassium from platelets, leukocytes and erythrocytes. Preanalytically, haemolysis also leads to falsely elevated levels.

 The main causes of hyperkalaemia are oliguria and Addison's disease.

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Note

Slight hyponatraemia may be tolerated in small animals as long as the Na/K ratio is > 27:1. If the ratio is < 27:1, Addison's disease is suspected (clarification by ACTH stimulation test).

#### Zinc (Zn)

Material Methode S, HP 0.5 ml Photometry

Species Dog, cat, birds, horse, farm animals

Duration 1 c

Note

- Marked zinc deficiency causes parakeratosis of the skin and mucous membranes. In cats, coat changes are mainly described.
   Serum zinc levels are not necessarily decreased in case of zinc responsive dermatosis.
- Farm animals: Zinc deficiency leads to reduced feed conversion and performance depression, changes in skin and claws, parakeratosis (especially in small ruminants) as well as growth retardation and fertility disorders (incl. underdeveloped reproductive organs).

reflux, diarrhoea, renal dysfunction, Cushing's disease/
glucocorticosteroid therapy.

• Cave: Even in case of absolute notassium deficiency, serum

• The main causes of hypokalaemia are vomiting or abomasal

- Cave: Even in case of absolute potassium deficiency, serum levels may stay within reference range for some time!
- Cattle: Elevated levels of potassium may lead to reproductive disorders and recumbency if there is a relative sodium deficiency at the same time.

# Selenium (Se)

Material

S, EP, HP 0.5 ml

Method AAS

Species Dog, cat, horse, ruminants

Duration 1 - 2 days

Note

- Selenium deficiency may cause nutritional muscular dystrophy in foals. Special emphasis should therefore be placed on the selenium supply of brood mares.
- In cattle, it causes mastitis, puerperal disorder and abortion, in young cattle it causes growth disorder and paralytic myoglobinuria. It leads to calf losses due to newborn myodystrophy, enzootic muscular dystrophy or white muscle disease.

# Sodium (Na)

Material

Species

S, HP 0.5 ml

Method ISE

Dog, cat, rabbit, guinea pig, rat, ferret, birds, reptiles, horse,

ruminants, pig

Duration

1 day

Note

- Sodium is the most important cation of the extracellular space. In dogs and cats, sodium is excreted mainly by the kidneys.
- The main causes of hypernatraemia are loss of water without concurrent loss of electrolytes (diabetes insipidus, diabetes mellitus), retention of sodium (mineralocorticoids) or a high sodium intake with food without the possibility of water uptake.
- The main causes of hyponatraemia are Addison's disease, diarrhoea, vomiting, or diuretics.

# Sodium/Potassium Ratio (Na/K Ratio)

Material S, HP 0.5 ml

Methode ISE
Species Dog, cat
Duration 1 day

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#### Urinalysis 5

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

**Kidney Profiles Horse** see Chapter 2.3.1, p. 55

**Kidney Profiles Pig** see Chapter 2.5.1, p. 66

**Kidney Profiles Ruminants** see Chapter 2.4.1, p. 61

Kidney Profiles Small Animals ➤ see Chapter 2.1.1, p. 34

Kidney-specific individual parameters that are determined from blood (e.g. Indoxyl sulphate, SDMA) > see Chapter 4.2, p. 86

**BRAF Mutation (Urinary Bladder/** 

**Urethral Carcinoma**) see Chapter 18.5, p. 298

#### COLA Test (cystine, ornithine, lysine, arginine)

Material Urine 3 ml (frozen)

Method LCMS Species Dog, cat Duration 5 days

Note

lysine and arginine.

• For the diagnosis of cystinuria in different breeds.

• Elevated in case of nephropathy, glomerulonephritis and renal

Quantitative determination of the amino acids cystine, ornithine,

amyloidosis, among others.

• Additionally, a urine sediment and the determination of the pH

value are recommended.

# Fanconi Screening

Urine 5 ml (frozen) Material

LCMS Method Species Dog Duration 5 days

Note Quantitative determination of the amino acids threonine.

glutamine, proline, glycine, alanine and of the glucose

concentration in the urine.

- For the diagnosis of Fanconi syndrome in dogs.
- Additional examination of urinary sediment is recommended.
- For the genetic test in Basenji see Chapter 20.2.1, p. 319.

#### Fractional Electrolyte Excretion (FE)

1 day

Urine 0.5 ml + S (non-haemolysed) 0.5 ml, samples collected at the Material

same time

Method Photometry Species Horse Duration

Note

- The FE of Na, K, P, Cl are examined.
- If the electrolyte excretion is put into relation with the creatinine excretion (here GFR = excretion), it indicates the FE of the electrolyte.
- The FE is used to diagnose a dysfunction of the renal tubules. In horses with healthy kidneys, the net excretion of an electrolyte in the urine is regulated by the glomerular filtration rate and the tubular reabsorption. If tubular reabsorption fails, the FE of one or more electrolytes usually increases and its FE values will be above the normal range.

#### y-GT/Creatinine Ratio

Material Urine 5 ml Method Photometry Species Horse Duration 1 day

Shows the early stage of a tubular disease and is indicated in case Note

of acute disease.

#### Microalbumin

Material Urine 0.5 m Turbidimetry Method Species Dog, cat Duration 1 day

Note

- It is considered an early way to diagnose renal dysfunction.
- In contrast to the determination of the U-P/C ratio (see below), this test is also useful in patients with no clinical signs.
- Relatively unspecific test which may also yield positive results in case of inflammatory diseases (e.g. borreliosis, leishmaniosis).
- Sample must not contain blood admixture.

### NABE (Fractionated Net Acid-Base Excretion)

Material Urine 15 ml (fresh and cool)

Method Titration
Species Cattle
Duration 2 – 3 days

Note Normal NABE values indicate a physiological acid-base balance.

NABE decreases if feed intake is reduced. Together with the blood parameters ketone bodies (ß-HBA) and free fatty acids (NEFA), NABE represents the minimal spectrum of metabolic control in cattle.

### Protein/Creatinine Ratio (U-P/C)

Material Urine 1 ml
Method Photometry
Species Dog, cat, horse
Duration 1 day

Juralion i day

 Parameter for early diagnosis of renal dysfunction and protein loss via urine.

• Not conclusive in case of bloody urine or active sediment.

 Increased values can also be caused by fever, bacterial and inflammatory processes without there being renal dysfunction.

# Urinalysis incl. Sediment

Material Urine 5 ml

Method Dry chemistry, photometry, microscopic

Species Dog, cat, rabbit, guinea pig, horse, cattle, sheep, goat, pig, others

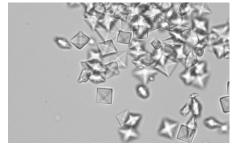
on request

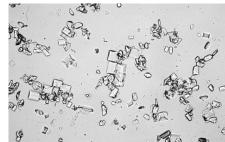
Duration 1 day

Note

Note

- Semi-quantitative measurement of clinical chemical and cellular parameters (protein, haemoglobin/myoglobin, pH value, bilirubin, urobilinogen, glucose, nitrite, ketone bodies as well as erythrocytes, leukocytes, bacteria, yeasts, cylinder, epithelia, crystals) and measurement of the specific gravity.
- Diagnostic clarification of urinary tract diseases and superior diseases (liver or kidney disease or metabolic disorders) which may lead to changes in urination (polyuria, stranguria and oliguria).
- Samples arriving on Saturday will be analysed the same day; delivery of the findings will take place on Monday.
- This test is also offered in combination with cultural urinalysis. In this case, 6 ml of urine are needed for the examination.
- If characteristic crystals (see figure) are found in the sediment, the animal species, urine pH and specific gravity should be included to draw clear conclusions about the chemical composition. Larger concretions can be analysed by FTIR (see Urinary Calculi).





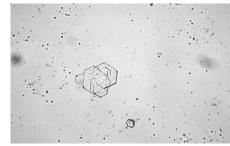
calcium oxalate

calcium carbonate/calcium oxalate

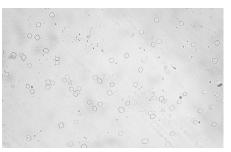


ammonium urate

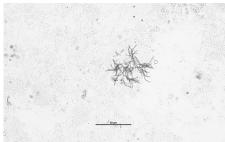
struvite



ammonium biurate



cystine



uric acid

bilirubin

Crystals from urinary sediment (microscopy, 40x obj.)

100% struvite



**Urinary Calculi** 

Material Calculi, concretions, dry min. 5 g
Method Infrared spectroscopy (FTIR)

Species Dog, cat, reptiles, horse, cattle, sheep, goat, others on request

Duration 1 day

Note The analysis of concretions is necessary for a targeted dietary

therapy and for prophylaxis.

Depending on the chemical composition, urinary calculi produce characteristic curves in infrared spectroscopy (see figure for examples). The analysis is also suitable for the description of other concretions such as gallstones. If you have special material or special questions,

please consult the laboratory.

### Urine Bacteriological Culture ➤ see Chapter 14.1, p. 268

### **Urine Protein Electrophoresis**

Material Urine 1 ml

Method Polyacrylamide gel electrophoresis

Species Dog, cat, rabbit, guinea pig, ferret, horse, cattle, goat, sheep, pig

Test frequency 1 x per week

Note

• Differentiation of glomerular and interstitial/tubular nephropathies.

• Only useful in case of increased U-P/C ratio.

Not recommended if urine is contaminated with blood or if prostate cvsts are suspected.

• Detection of Bence-Jones protein in case of suspected myeloma.

FTIR spectrum of cystine

FTIR spectrum of cystine

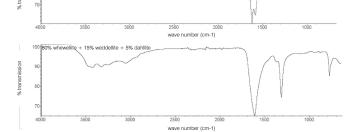
FTIR spectrum of calcium carbonate

FTIR spectrum of calcium of calcium oxalate

FTIR spectrum of calcium oxalate

FTIR spectrum of calcium oxalate and cystine

FTIR spectrum of calcium oxalate and dahllite



# Analysis of urinary calculi by infrared spectroscopy:

FTIR spectra of struvite, cystine, calcium oxalate, calcium carbonate and mixed forms

Recording the transmission of infrared light at specific frequencies. Transmission is directly related to the oscillation energy of the molecular binding.

There are characteristic curves for each type of calculus – including mixed forms.

llite + 50% cvstine

# 6 Allergy

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

# 6.1 Allergy Testing

Allergy Profiles (Dog/Cat)

(Food Allergen Profile, Pruritus Profiles ) > see Chapter 2.1.2, p. 38

Allergy Profiles (Skin, Repiratory Horse) > see Chapter 2.3.2, p. 57

# **Allergy Screening Test**

Material **Dog, cat:** S 2 ml

Horse: S 1.5 ml

Method **Dog, cat:** ELISA, Fcε-receptor technology

Horse: ELISA

Species Dog, cat, horse

Duration 2 days

Note

- Cost-effective screening test to determine for which allergen group the main test should be performed or whether it is already possible to test again after cortisone administration.
- The pollen, mite and mould groups are tested in all species.
- Dog, cat: including flea saliva
- Horse: including insects
- Ideal testing time at the time of exposure (no earlier than 3 4 weeks after the onset of the signs).

All samples sent in are stored by us for 14 days. Hence, in this time frame, if there has been a positive test result, it is possible to request further tests from a sample sent in for a screening test.

#### Perennial Panel (dog, cat)

Material S 0.5 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat Duration 2 days

Note Differentiation or detection of individual mould and mite allergens.

Moulds: Alternaria alternata, Aspergillus fumigatus, Cladosporium

herbarum, Penicillium notatum

<u>Mites:</u> Dermatophagoides farinae, Dermatophagoides pteronyssinus, Acarus siro, Tyrophagus putrescentiae

#### Perennial Panel (horse)

Material S 0.5 ml
Method ELISA
Species Horse
Duration 2 days

Note Differentiation or detection of individual mould and mite allergens.

 $\underline{\underline{\mathsf{Moulds:}}}$  Alternaria alternata, Aspergillus fumigatus, Aspergillus niger,

Cladosporium sp., Epicoccum sp., Helminthosporium sativum, Penicillium sp., Fusarium spp., Ustilago sp., Rhizopus sp. Mites: Dermatophagoides farinae. Dermatophagoides

pteronyssinus, Acarus siro, Tyrophagus putrescentiae, Glycophagus

domesticus, Lepidoglyphus destructor

### Seasonal Panel (dog, cat)

Material S 1 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat Duration 2 days

Note Differentiation or itemisation of seasonal allergens.

Pollen: 6-grass mix (orchard grass, perennial ryegrass, Timothy grass, meadow fescue, Kentucky bluegrass, meadow soft grass); rye, mugwort, ragweed, English plantain, nettle, common sorrel,

birch, hazel, willow

Ideal testing time at the time of exposure (no earlier than 3 – 4 weeks

after the onset of the signs)

The Seasonal Panel for dogs and cats also includes the CHO test and, if necessary, blocking of antibodies directed against cross-

reactive carbohydrate determinants (anti-CCD IgE).

#### Seasonal Panel (horse)

Material S 0.5 ml
Method ELISA
Species Horse
Duration 2 days

Note Differentiation or itemisation of seasonal allergens.

Pollen: 6-grass mix (orchard grass, perennial ryegrass, Timothy grass, meadow fescue, Kentucky bluegrass, meadow soft grass); rye, mugwort, lamb's quarters/goosefoot, English plantain, nettle, sorrel, dandelion, rape, ragweed, hazel, alder, poplar, birch, beech, willow

#### **Further main tests**

### Feathers/Hairs/Epithelia

Material S 0.5 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat, horse

Duration 7 days

Note Detection of single epithelial allergens: cat, dog, rabbit, guinea pig,

parrot feathers, feather mix

#### Flea Saliva (IgE)

Material S 0.5 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat Duration 2 days

Note A combination of flea saliva and recombinant flea saliva allergen is

used as an allergen.

### **Food Allergens Basic**

Material S 0.5 ml
Method ELISA
Species Dog, cat
Duration 2 days

Note

Note

Determination of IgG and IgE antibodies against 19/16 single allergens.
 <u>Dog:</u> beef, pork, lamb, chicken, turkey, duck, soy, wheat, corn, rice, egg, milk, barley, potato, oats, whiting, salmon, rabbit, deer <u>Cat:</u> beef, lamb, pork, chicken, turkey, duck, potato, soy, wheat,

corn, rice, egg, milk, salmon, tuna, whiting

Basis for the specific selection of fitting dietary components for an

elimination diet.

# **Food Allergens Exotic**

Material S 0.5 ml

Method ELISA (microarray)

Species Dog, cat Duration 7 days

> Determination of IgE and IgG antibodies in dogs and cats against 15 "exotic" single allergens (trout, goat, camel, buffalo, quail,

hermetia, sweet potato, sunchoke, buckwheat, bean, carrot, pumpkin, zucchini, pea, yeast).

Basis for the specific selection of fitting dietary components for an

elimination diet.

# Food Allergens Extended

Material S 0.5 ml
Method ELISA (microarray)

Species Dog, cat Duration 7 days

Note

 Determination of IgE and IgG antibodies against 8 single allergens which are fed rather infrequently.

<u>Dog:</u> horse, ostrich, wild boar, reindeer, amaranth, millet,

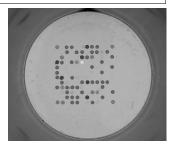
kangaroo, parsnip Cat: horse, ostrich, wild boar,

reindeer, amaranth, millet,

venison, rabbit

 Basis for the specific selection of fitting dietary components for

an elimination diet.



Microarray technology:

A variety of allergens and reference controls are placed in a well of the plate. Each allergen has a specific position in the well and is tested in triplicate.

### Food Panel (horse)

Material S 1 ml Method ELISA Species Horse Duration 7 days

Note

- Determination of IgE and IgG antibodies against 8 single alimentary allergens: soy, molasses, oats, corn, barley, wheat, barn, lucerne.
- Basis for the specific selection of fitting dietary components for an elimination diet.

# Hvmenoptera\*

Material S 0.5 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat Duration 10 days

Note Detection of individual allergens of bee, wasp, hornet, paperwasp.

This service also includes the CHO test and, if necessary, blocking of antibodies directed against cross-reactive carbohydrate determi-

nants (anti-CCD lgE).

# Insect Panel (dog, cat)

Material S 0.5 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat Duration 7 days

Note Detection of individual allergens of deerfly (Chrysops), mosquito

(Culex sp.), horse fly (Tabanus sp.), stable fly (Stomoxys sp.) and

cockroach (Blatella germanica).

#### Insect Panel (horse)

Material S 1 ml Method ELISA Species Horse Duration 3 days

Note Detection of individual allergens of black fly (Simulium sp.),

mosquito (Culex sp.), horsefly (Tabanus sp.), housefly (Musca sp.),

biting midges (Culicoides sp.).

#### Malassezia

Material S 0.5 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat Duration 7 days

Note • Detection of a sensitisation (IgE) to Malassezia.

Can be added to the ASIT.

#### **Mediterranean Panel**

Material S 2 ml

Method ELISA, Fcε-receptor technology

Species Dog, cat Duration 7 days

Note Detection of individual Mediterranean allergens:

 Perennial allergens (Mites: Dermatophagoides farinae, Dermatophagoides pteronyssinus, Acarus siro, Tyrophagus putrescentiae. Moulds: Alternaria alternata, Aspergillus fumigatus, Penicillium notatum)

 Seasonal allergens (Timothy grass, perennial ryegrass, Bermuda grass, yellow dock, English plaintain, mugwort, lamb's quarter/ goosefoot, pellitory, dandelion, nettle, ragweed, olive, cypress, pine tree, plane tree, common privet, birch)  Ideal testing time at the time of exposure no earlier than 3 – 4 weeks after the onset of the signs.

 This service also includes the CHO test and, if necessary, blocking of antibodies directed against cross-reactive carbohydrate determinants (anti-CCD IgE).

# 6.2 Allergen-specific Immunotherapy

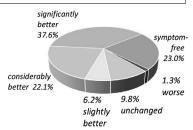
# Allergen-specific Immunotherapy (ASIT)

Material Species Duration

Anmerkung

Not necessary Dog, cat, horse Approx. 2 – 3 weeks

- Hyposensitisation to seasonal or perennial antigens according to test outcome (serum test or intradermal testing).
- Please note: Food and Hymenoptera allergens cannot be added to the therapy!
- Therapy has to be carried out for at least one year, if successful, it is continued for a lifetime (patient-specific solutions).
- The composition of an ASIT can also be done on the basis of any other test result (e.g. intradermal testing).
- A maximum of 8 allergens or allergen mixtures per set; if more than 8 allergens/mixtures are needed, the allergens will be spread over a double set (2 sets), for which the double price of a single set is charged.



**Dog:** 89% success if ASIT is started within 2 years of the onset of the disease.

Sign	Success ASIT
Pruritus	75 %
Respiratory disease	80 %
Respiratory disease within 2 years	86 %

Impressive success rates of ASIT in **horses**, especially in respiratory diseases and if therapy is started after a short duration of disease.

- Please enclose a **veterinary prescription** with your order!
- The starter set will last for approx. 6 months, the refill set for approx. 10 months.



# **Digital Material on Allergies**

# App 4Paws

The Laboklin app 4Paws for vets and animal owners reminds users to administer and to stock up on medication. This ensures adherence to the treatment plan and is particularly helpful in allergy treatment. Additionally, diagnoses and other important data about the animal can be saved in the app in compliance with data protection regulations. The app can be installed for free from the app stores.

# Immunological Tests/Markers for Inflammation

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

### **Acetylcholine Receptor Antibodies**

Material S 1 ml **IFAT** Method Species Dog Duration 1 day

Note

- This test is used to detect myasthenia gravis in dogs. In this disease, antibodies against acetylcholine receptors are formed. It is characterised by weakness of the striated muscles which is enhanced by stress.
- The weakness of the muscles may be generalised or locally limited to few muscle groups, such as those of the oesophagus (megaoesophagus).

### **Antinuclear Antibodies (ANA)**

S. EP. HP 0.5 ml Material

**IFAT** Method

Dog, cat, horse Species

Duration 1 day

This test is used for the serological detection of autoimmune Note

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diseases (e.g. lupus erythematodes). If the test yields a negative result, a biopsy should be taken and examined, as the serological testing can be negative especially in case of local changes. Low positive titres may also occur in many general diseases

#### Coombs Test (direct)

FB 0.5 ml Material Method Agglutination Species Dog, cat, horse Duration 1 day

Note • This test is used for the detection of autoimmune haemolytic

· Positive reactions also occur in almost all infections with blood parasites.

#### C-reactive Protein (CRP)

Material S 0.5 ml
Method Photometry
Species Dog
Duration 1 day

Note Inflammatory mediator (acute-phase protein) that is used to

diagnose non-obvious inflammation and for therapy monitoring.

#### **Gluten Sensitivity**

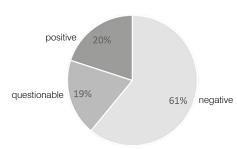
Material S 0.5 ml (cooling preferred)

Method ELISA
Species Dog
Duration 10 days

Note

- Detection of IgA against tissue transglutaminase and IgG against modified gliadin peptides.
- Gluten and its subfraction gliadin are found in wheat, spelt, rye and barlev.
- Gluten intolerance leads to different clinical pictures depending on the breed: gluten-sensitive enteropathy in the Irish Setter and canine epileptoid cramping syndrome (CECS, Spike's disease, paroxysmal gluten-sensitive dyskinesia) in the Border Terrier. Mixed forms and gluten intolerance in other breeds have been described in the literature.

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In one study, Laboklin was able to prove a clear or questionable gluten sensitivity in 39% of the dogs (see figure). The affected breeds were mainly mongrels, French Bulldogs, German Shepherds and Labrador Retrievers.

Gluten sensitivity in the diagnosis of food allergies

Haptoglobin

Material S, HP 0.5 ml Method Photometry

Species All Duration 1 day

Note Haptoglobin is an acute-phase protein. Its levels increase due to

inflammation. Haptoglobin is much more sensitive than fibrinogen.

#### Cellular Immune Status

Material EB, HB 3 ml (not older than 48 h)

Method Flow cytometry Species Dog, cat, horse Duration 1 day

Note

- The cellular immune status includes a complete blood count as well as the determination of B-cells (CD 21+), T-cells (CD3+, CD5+). T-helper cells (CD4+) and cytotoxic T-cells (CD8+).
- Blood used for this examination must not be older than 48 hours!
- In dogs, determination of the immune status is useful for monitoring leishmaniasis. Furthermore, the immune status can be helpful for monitoring pyoderma (German Shepherd), demodicosis and systemic lupus erythematodes as well as T-cell deficiency.
- In cats, the cellular immune status is used to determine the current phase of the disease in FIV-positive animals. The test is also used in FIV-positive cats with stomatitis.
- In **horses**, it is used to clarify frequent and prolonged infections.

# Immunoglobulin A (IgA)

Material S 0.5 ml
Method ELISA
Species Dog
Test frequency 2 x per week

Note

- In animal serum, IgA is found in lower concentrations than the other immunoglobulins. It is considered the most important immunoglobulin in the outer conjunctival secretions and in the urine, and is present in secretory form.
- In dogs, IgA is an important diagnostic marker of steroidresponsive meningitis-arteritis.

lymphocytes - CD21 CD45

### Immunoglobulin G (IgG)

Material S 0.5 ml

Method Capillary electrophoresis

Species Dog, cat, horse, foal, cattle, calf, lamb,

pig (only on request)

Duration 1 day

Note

- IgG is the strongest immunoglobulin fraction in blood serum.
   The greatest significance of IgG lies in the antibody-mediated immune response. Due to its small size, IgG can diffuse from the capillaries and, thus, has an additional relevance in immune reactions in tissue spaces and on the body surface.
- Newborns: At birth, foals, calves and piglets have only marginal IgG content in the blood. They mainly take up IgG via the colostrum. IgG content is therefore an indicator for a sufficient supply of colostrum.
- Foals: The lack of maternal antibodies is one of the most important predisposing factors for infectious diseases in foals.
   lgG determination in the blood of newborn foals allows for a timely diagnosis – before infections occur – as well as the initiation of therapeutic measures.

# Immunoglobulin M (IgM)

Material S 0.5 ml Method Turbidimetry

Species Dog, cat, pig and others (only on request)

Duration 2 days

Note Most notably, the importance of IgM lies in the mediation of the

primary immune response. IgM is also involved in the secondary immune response but its significance is lower. The secondary

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immune response is mainly mediated by IgG.

Insulin Antibodies > see Chapter 8, p. 125

### Leukaemia Immunophenotyping

Material Lymph node aspiration (in NaCl plus some drops of serum),

peripheral blood (EB, HB 2 ml; not older than 48 h) + cytology/

blood smear

Method Flow cytometry
Species Dog, cat, horse
Duration 1 – 2 days

Note

 It is recommended to send in more sample material if possible. Up to 5 ml of sample volume are required if the total leukocyte count is low.

With > 30.000 lymphocytes or positive clonality in the PARR test (see Chapter 18.4, p. 297), leukaemia immunophenotyping may allow to differentiate between lymphoproliferative neoplasia (lymphoma or leukaemia; B- and T-cell) and myeloid leukaemia. In dogs, it can additionally be differentiated between acute and chronic forms. This differentiation provides an indication of prognosis and treatment.

Leukaemia immunophenotyping is also part of the Leukaemia/

Lymphoma Profile (see Chapter 2.1.1, p. 35).

# 

# Scatterplot of lymphocyte immunophenotyping

The lymphocyte population in this example is positive for the panleukocyte marker (CD45) and the B-cell marker (CD21). Here, it is a B-cell lymphoma.

# Leukaemia/Lymphoma Profile ➤ see Chapter 2.1.1, p. 35

### **Rheumatoid Factor (Waaler-Rose Test)**

Material S 0.2 ml

Method Haemagglutination

Species Dog, cat
Duration 1 – 2 days

Note This test can be used to determine rheumatic locomotor disorders.

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It should be performed during an acute episode because serology

might yield negative results in symptom-free episodes.

# LABOKI

# Serum Amyloid A (SAA)

Material S 0.5 ml Method Photometry

**Species** Dog, cat, horse, cattle, others

1 day (cat, horse) Duration

2 – 3 days (dog, cattle)

Note

• Inflammatory mediator (acute-phase protein) that can be used to diagnose non-obvious inflammation and for therapy monitoring.

• For horses, SAA can also be requested in combination with the large screening.

### **Serum Protein Electrophoresis**

S, EP, HP 1 ml, birds and reptiles: S, HP 0.5 ml Material

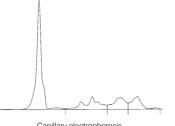
Method Capillary electrophoresis

**Species** Dog, cat, rabbit, ferret, birds, reptiles, horse, cattle, others on request

Duration

Note

- Includes the separation of the protein fractions albumin,  $\alpha$ -,  $\beta$ -, y-globulins and the albumin-globulin ratio. The protein fractions are also represented in form of a graph (see **figures**).
- Acute inflammation leads to an increase in the alpha- and/or beta-globulin fraction. Polyclonal hypergammaglobulinaemia is caused by infectious, immune-mediated or neoplastic diseases. Especially in case of feline infectious peritonitis (FIP), the test is used to support the clinical suspicion.
- · Albumin, alpha- and beta-globulin fractions are lowered in case of severe liver diseases.

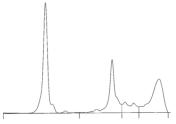




Fraction	%	g/I	Dog:	
Albumin	56.7	37.54	Alb:	47-59%
Alpha	17.2	11.39	α glob:	9-15%
Beta	13.8	9.14	β glob:	14-24%
Gamma	12.3	8.14	γ glob:	8-18%

1.31 Alb/alob = Total protein:



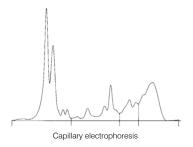


Capillary electrophoresis

Fraction	%	g/I	Cat:	
Albumin	40.2	42.57	Alb:	45-60%
Alpha	23.9	25.31	α glob:	8-15%
Beta	8.3	8.79	β glob:	10-20%
Gamma	27.6	29.23	v aloh:	10-28%

Alb/glob = Total protein: 105.9 g/

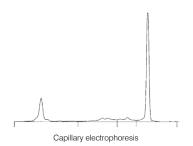
pronounced α2 peak, polyclonal γ peak suspicion: acute infectious process



Fraction	%	g/I	Horse:	
Albumin	45.4	31.05	Alb:	45-60%
Alpha	16.9	11.56	α glob:	10-20%
Beta	11.4	7.80	β glob:	10-25%
Gamma	26.3	17.99	γ glob:	8-22%

Alb/glob = Total protein:

split albumin peak frequently found in ponies in case of lipoproteinaemia



Fraction	%	g/l	Cat:	
Albumin	22.1	28.20	Alb:	45-60%
Alpha	10.6	13.53	α glob:	8-15%
Beta	7.5	9.57	β glob:	10-20%
Gamma	59.8	76.30	γ glob:	10-28%

Total protein:

monoclonal gammopathy in leukaemoid disease

Serum protein electrophoresis - examples for normal results, lipoproteinaemia, suspected acute infection and leukaemoid disease

### Thyroglobulin Antibodies > see Chapter 8, p. 131

# **Thrombocyte Antibodies (Platelet Antibodies)**

Material EB, HB 0.5 ml (not older than 72 h)

Method Flow cytometry Species Dog, cat Duration 1 day

Note

- If possible, it is recommended to send at least 1 ml EB as the required sample amount depends on the total platelet count.
- There are two possible ways for the development of thrombocyte AB:
- Autoantibodies against platelets are formed. Only in this case, a positive test result can be expected.
- In the course of another underlying disease, immune complex formation occurs causing secondary damage to the platelets.
- Evaluation: ≤ 10% negative, > 10% positive
- Detection of platelet AB is also part of the Thrombocytopenia Profile (see Chapter 2.1.4, p. 41).

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# Thrombocytopenia Profile > see Chapter 2.1.4, p. 41

# 8 Endocrinology/Tumour Markers

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

#### **ACTH (Adrenocorticotropic Hormone)**

Material EP 0.5 ml (centrifuge, pipette off and cool promptly after collection)

Method CLI

Species Dog, cat, horse, others on request

Duration 1 day

Note • ACTH remains quite stable in EP.

Indications

Diagnosis of Cushing's disease in horses and therapy monitoring

of dopamine receptor antagonists in horses

Differentiation of primary or secondary Addison's disease in dogs.

#### AFP > see Tumour Marker AFP, p. 132

#### Aldosterone

Material EP 0.5 ml (cooled)

Method LCMS
Species Dog, cat
Duration 5 days

Note Diagnosis of hyperaldosteronism due to unilateral tumours of the

adrenal cortex; common signs are hypertension, acute vision loss,

hypokalaemic polymyopathy.

#### **Androstenedione**

Material S 0.5 ml (cooled)

 $\begin{array}{ll} \text{Method} & \text{ELISA} \\ \text{Species} & \text{Dog, ferret} \\ \text{Test frequency} & 1-2 \text{ x per week} \end{array}$ 

Note Used for detecting an endocrine active hyperplasia/neoplasia of the

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adrenal gland.

#### Anti-Müllerian Hormone (AMH)

Material S, HP 0.5 ml (cooled)

Method CLIA

Species Dog, cat, horse, cattle, others on request

Duration 1 day

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Note

AMH is secreted in the granulosa cells of the maturing follicle and the Sertoli cells in the testes. It is, thus, a highly sensitive marker for the diagnosis of granulosa cell tumours, cryptorchidism, differentia-

tion castrated/intact.

### NT-pro-BNP (B-Type Natriuretic Peptide)

Material EP 0.5 ml (cooled)

Method **ELISA** Species Dog, cat Test frequency 2 x per week

Note

 The serum concentration of BNP depends on changes in blood pressure in the ventricle. Its determination mainly serves the early diagnosis of dilated cardiomyopathy. The myoendocrine cells of the heart start to secrete BNP as soon as the myocardium exhibits vasodilative wall tension. BNP increases the renal excretion of sodium and water, lowers the intracardiac pressure and has vasodilatory effects.

• This test is suitable as a screening test for elder patients or predisposed breeds (e.g. Doberman).

### see Tumour Marker CEA, p. 132

#### Cortisol

Material S, HP 0.5 ml

Method CLIA

Species Dog, cat, rabbit, guinea pig, rat, mouse, ferret, horse, cattle, others

on request

Duration 1 day

Note

• Depending on the clinical issue, the following Function Tests (see Chapter 9, p. 135) can be performed:

ACTH stimulation test

Dexamethasone suppression test (low or high dose)

• Determination of cortisol following administration of Vetoryl; see

Vetoryl Therapy Control in this chapter, p. 133.

• Single value determination has extremely low diagnostic value – in dogs, due to episodic secretion, in cats, because high concentrations may be stress-related.

### see also Saliva Cortisol, p. 128



# **CPSE (Canine Prostate-Specific Arginine Esterase)**

Material S. EP. HP 0.5 ml (centrifuged!, cooled)

Method ELISA Species Dog. male Test frequency 1 – 2 x per week

Note

• For the early detection of benign prostatic hyperplasia and for the detection of bacterial prostatitis and prostate carcinoma.

• The enzyme CPSE is secreted by the prostate cells under the control of sex hormones. In hyperplastic prostate cells, CPSE levels increase significantly.

**Erythropoietin** 

Material S 0.5 ml (cooled or, even better, frozen)

Method **ELISA** 

Species Dog, cat, others on request

Test frequency 1 x per week

Note Used for the diagnosis of renal-related non-regenerative anaemia

and to confirm or exclude polycythaemia.

#### fT3 and fT4 > see below T3 and T4, p. 129

# IGF-1 (Insulin-like Growth Factor 1, STH Equivalent)

Material S 0.5 ml (cooled)

Method CLIA

ELISA (horse, cattle)

Species Dog, cat

Horse, cattle on request

Duration 1 dav

Note

• Secretion is stimulated directly by the somatotropic hormone (= growth hormone), IGF-1 can therefore be interpreted as equivalent to STH.

• Indications are growth disturbances in young animals, changes in coat structure, acromegaly in adult animals, therapy-resistant diabetes mellitus in cats.

• Single value: only slightly decreased in case of growth disturbances; if the result is questionable, a function test should be performed (xylazine stimulation test/STH stimulation test).

• In cattle, IGF-1 is suitable as a laboratory parameter for the early diagnosis of ovarian cysts and laminitis.

S 0.5 ml (cooled)

# LABOKI IN

### Inhibin B\*

Material

S 3 ml (centrifuged, cooled)

Method Species RIA Horse

Duration 4 weeks (will be passed on to a partner laboratory)

Note

• Is used for the detection of granulosa cell tumours.

• Because of the long testing time, we recommend the determination of the AMH level. The diagnostic value regarding this clinical issue is comparable.

#### Insulin

Material

S 1 ml (centrifuged promptly, cooled)

Method CLIA

Species

Dog, cat, ferret, horse, cattle

Duration 1 day

Note

- In case an insulinoma is suspected; concentration is only diagnostically conclusive if glucose is determined at the same time.
- The serum must be immediately centrifuged, pipetted and cooled before shipping.
- 12-hour fasting period prior to sampling no longer recommended for horses, but no concentrated feed or cereal grains – only hay.
- Dog/cat: Determine insulin/glucose ratio or AIGR (amended insulin/ glucose ratio). An insulin/glucose ratio < 52 or an AIGR < 30 are considered normal, see also Function Tests, Chapter 9, p. 135.
- Equine metabolic syndrome (EMS): EMS leads to a disorder of carbohydrate and fat metabolism with insulin dysregulation (ID). Increased insulin secretion (partly) compensates for decreased insulin efficiency. Insulin-dysregulated horses therefore have considerably increased fasting insulin levels. At the same time, fasting glucose is physiological (compensated) or elevated (not compensated). Further tests: glucose tolerance test, combined glucose insulin test and oral glucose test (see Chapter 9, p. 141).
- Simultaneous measurement of the glucose level makes it possible to calculate the proxies
  - insulin/glucose ratio,
  - reciprocal inverse square of insulin (RISQI) "insulin sensitivity"
  - modified insulin-to-glucose ratio (MIRG) "β-cell function (pancreas)".

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#### Insulin Antibodies\*

Material

**ELISA** Method Species Doa

Duration 7 – 10 days

### Normetanephrine/Metanephrine\*

Material EP 5 ml (frozen!)

Method HPLC

Species Dog, others on request

Duration 5 – 8 days

Note

• Normetanephrine and metanephrine are metabolites of adrenaline and noradrenaline. High levels, particularly of normetanephrine. are indicative of the presence of pheochromocytoma.

Reference values for dogs only.

# Normetanephrine/Metanephrine-Creatinine Ratio\*

Material Urine 10 ml cooled, acidified, protected from light – test tube in

aluminium foil

Method HPI C

Species Dog. others on request

Duration 5 – 8 days

Note High ratios of normetanephrine to creatinine and of metanephrine to

creatinine are indicative of the presence of pheochromocytoma.

#### Oestradiol-178

Material Method

S 0.5 ml **LCMS** 

Species

Dog. cat. rabbit. ferret, horse, cattle, others on request

Test frequency

Note

- 2 x per week
- - The test is performed in case of disorders of the sexual cycle (repeated determination), neoplasia of the ovaries, ovary cysts, suspected Sertoli cell tumour.
  - In dogs and ferrets, permanently elevated levels can lead to thrombocytopenia and anaemia due to bone marrow depression.
  - Male dogs: often feminisation syndrome if levels are elevated.
  - Ferrets: part of the Ferret Profile for the diagnosis of hyperadrenocorticism.

# Oestrone Sulphate (= E1S)

Material S 1 ml Method LCMS

Species Horse, donkey, llama, alpaca, others on request

Duration 5 days

Note • Mare: To determine an intact pregnancy

- In pregnant mares, oestrone sulphate is secreted in increasing concentrations from the 50<sup>th</sup> day onwards. The oestrone sulphate level drops to basal level after abortion or resorption within a few days. Reliable diagnosis is possible from day 110 onwards.
- Stallion (from 3 years of age): Diagnosis of cryptorchidism (horse only; please indicate sex on submission form). Because of the higher sensitivity and as it is independent of age, we recommend the determination of Anti-Müllerian Hormone (AMH) instead.

 Llama/alpaca: diagnosis of late pregnancy (from 10<sup>th</sup>/11<sup>th</sup> month onwards).

### PAG (Pregnancy-Associated Glycoproteins)

Material S, HP 1 ml Method ELISA

Species Cattle, sheep, goat

Duration 2-3 days

Note Can be used from the 28<sup>th</sup> day after conception to determine

pregnancy in cows.

#### Parathormone (iPTH, intact Parathyreoid Hormone)

Material S 1 ml (centrifuged promptly after collection, shipped cooled)

Method CLI

CLIA

Species Dog, cat, horse, others on request

Duration 1 day

Note • iPTH is an unstable hormone.

- Determination is used to diagnose hyper-or hypoparathyroidism.
- Concentration should only be assessed when ionised Ca (and possibly phosphate) levels are determined simultaneously.
- Possible causes for high levels of PTH are low levels of calcium, e.g. in case of renal dysfunction, and disorders of vitamin D metabolism.

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#### Parathormone-rP (Parathormone-related Protein)\*

Material EP 1 ml (cooled)

Method RIA
Species Dog, cat
Duration 10 days

Note • PTHrP is a parathormone-like protein.

The hormone is formed physiologically during growth and during

pregnancy.

 Pathologically, it is secreted by different tumours, e.g. by some kinds of lymphoma, lymphosarcoma and anal sac adenocarcinoma.

# PMSG = eCG (Pregnant Mare Serum Gonadotropin resp. Equine Chorionic Gonadotropin)

Material S, HP 0.5 ml Method ELISA

Method ELISA
Species Horse, donkey

Test frequency/ Horse 1 – 2 x per week
Duration Donkey 7 – 10 days\*

Note
 Determination of pregnancy between day 45 and 100.

PMSG can be detected for a while after resorption or abortion,

although there is no live foetus anymore.

# Progesterone

Material S, HP 0.5 ml

Method CLIA

Species Dog, cat, horse, cattle, sheep, goat, alpaca, others on request

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Duration 1 day

Note • Control of luteal function.

- Can be used during early pregnancy to confirm conception in cattle, horse, sheep and goat. However, as there are also increases in progesterone levels in the regular cycle, this difference is only diagnostically useful during the cycle-dependent decrease in progesterone. In horses and cattle, only samples which were taken on the 18<sup>th</sup> and 19<sup>th</sup> day after conception are meaningful regarding pregnancy.
- Female dog: Determination of the ovulation time, determination
  of the optimum time for mating, diagnosis of corpus luteum insufficiency (repeated determination), measurement of corpus luteum
  function.

# 17 OH-Progesterone

Material S 0.5 ml Method ELISA

Species Dog, ferret, others on request

Test frequency

2 x per week

Note

 Clarification of steroid-producing adrenocortical hyperplasia and neoplasia in ferrets and dogs.

• In female animals in the luteal phase, considerably high concentrations may be measured.

• In case of doubt, it is necessary to perform an ACTH stimulation

test.

#### Saliva Cortisol

Material Saliva 0.1 ml Method ELISA

Species Dog, cat, guinea pig, horse, cattle, others on request

Duration 5 days

Note • Only on request.

· Test vessels will be provided.

• Measurement also possible as part of an ACTH stimulation test.

#### Serotonin

Material S 0.5 ml (centrifuge promptly within 1 hour and separate serum,

send cooled or, even better, frozen, protected from light – test tube

in aluminium foil)

Method HPLC

Species Dog. others on request

Test frequency

1 x per week

Note

• At least 6 hours of **fasting** before sample collection.

 The test will not be performed if the samples do not arrive wellcooled at the laboratory (shipping is recommended in a styrofoam box with 2 – 3 frozen cold packs).

 For the clarification of behavioural disorders. Decreased serotonin levels have been found in case of aggression as well as in separation anxiety and other behavioural problems.

The additional determination of serotonin can be helpful in diagnosis and therapy monitoring.

 The determination of serotonin levels is also part of the Behaviour Profile (see Chapter 2.1.1, p. 32).

#### Somatotropin

Material S, HP 0.5 ml

Species Cattle, test is performed only on request

#### Thyroid Profiles Dog or Cat ➤ see Chapter 2.1.1, p. 37

# T3 (Total Triiodothyronine)

Material S 0.5 ml Method CLIA

Species Dog, horse, others on request

Duration 1 day

Note

 Additional parameter for the diagnosis of hyperthyroidism or hypothyroidism as the peripheral transformation of T4 to T3 only takes place when necessary and T3 is only secreted thyroidally to a small extent.

• If presence of T4 AB is suspected.

 Therapy monitoring: Blood sampling 3 hours after oral intake of T3 medication (dog).

# fT3 (Free Triiodothyronine)

Material S 0.5 ml Method CLIA

Species Dog, cat, horse, others on request

Duration 1 day

Note T3 in the serum is 99.7% reversibly bound to transport proteins. fT3

levels correlate with metabolic activity. Measuring free T3 is indicated when changes in the concentration of transport proteins for T3 result

in changes in total T3 concentration.

# T4 (Total Thyroxine)

Material S, HP 0.5 ml resp. 0.4 ml (small mammals)

Method CLIA

Species Dog, cat, rabbit, guinea pig, ferret, birds, reptiles, horse, cattle,

others on request

Duration 1 day

Note

Dog: For the diagnosis of hypothyroidism. As a single value, it
has only limited diagnostic significance. Determination should
therefore always be performed in conjunction with fT4 and TSH.
Alternatively, a TRH stimulation test can be performed.

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- Cat: For the diagnosis of hyperthyroidism, which is the most common hormonal disease in elder cats and which entails extensive sequelae (tachycardia, chronic diarrhoea, cachexia).
   Usually sufficient as a single parameter, in questionable cases, TSH can be determined additionally.
- Therapy monitoring dog/cat: Blood sampling 4 hours after administration of thyroxine (dog) and 3 weeks after therapy start (cat).
- Birds and reptiles: Values are physiologically often very low (below the detection limit of the test system used); in this case, determination of fT4 is recommended.
- Horse: If (very rarely occurring) hypothyroidism is suspected, determination of T4 and T3 with a subsequent TRH stimulation test is recommended.

### fT4 (free Thyroxine 4)

Material S (possibly HP) 0.5 ml or 0.4 ml (small mammals)

Method CLIA

Species Dog, cat, rabbit, guinea pig, horse, others on request

Duration 1 day

Note • S

- Single value determination: fT4 and fT3 are both strongly affected by the current metabolic state.
- Just like T4, fT4 is affected by underlying diseases.
- Fasting for 10 hours prior to sampling is recommended.
- In cases of doubt: TRH stimulation test or, in dogs and cats, measure TSH concentration.

#### fT4 Dialysis\*

Material S 0.5 ml (frozen)
Method Equilibrium dialysis

Species Dog, cat Duration 10 – 14 days

# fT4 + TSH + Thyroglobulin Antibodies (dog) > see Chapter 2.1.1, p. 37

Testosteror	ie – – – – – – – – – – – – – – – – – – –
Material	S, HP 0.5 ml
Method	CLIA, horse: LCMS
Species	Dog, cat, rabbit, guinea pig, rat, mouse, ferret, horse, cattle, others
	on request

Duration/ 1 day

Test frequency 2 x per week (horse)

Note

 Used for checking the endocrine testicular function, for the diagnosis of ovarian tumours in mares and to differentiate between cryptorchid and castrated male animals.

### **Thymidine Kinase**

Material S 0.5 ml (cooled)

CLIA

Method

Species Dog, cat, guinea pig, horse, others

Duration 3 – 4 days

Note

- Thymidine kinase is an enzyme which helps introduce the nucleoside thymidine into the DNA by converting deoxythymidine to deoxythymidine phosphate (dTMP). It is thus essential for DNA synthesis.
- The activity of serum thymidine kinase 1 (sTK1) correlates closely with DNA synthesis and cell proliferation. The main indications for the determination of thymidine kinase in malignant haematopoietic neoplasms are therapy monitoring and early detection of relapse.
- Its concentration is a measure for the activity of cell division.
   Thymidine kinase is therefore used as a tumour marker. Elevated concentrations are primarily measured in malignant diseases of the haematopoietic and lymphatic system.
- Thymidine kinase is mainly excreted renally. Therefore, if levels are increased, it is necessary to exclude impaired renal function in differential diagnosis.
- Patients with hepatic cirrhosis may also show elevated concentrations
- Growing patients physiologically have higher concentrations.
- Thymidine kinase is also part of the profile Tumour Diagnostics (see Chapters 2.1.1, p. 38 and 2.3.1, p. 57).

# Thyroglobulin Antibodies (TgAb)

Material S, EP 0.5 ml Method ELISA Species Dog

Duration Approx. 3 days

For the diagnosis of autoimmune thyroiditis.

 Detection of thyroglobulin Ab is also part of the Thyroid Profile (dog) and of: fT4 + TSH + Thyroglobulin Antibodies (dog), as well as of: T4 + TSH + Thyroglobulin Antibodies (dog), see Chapter 2.1.1, p. 37.

2022/23 Endocrinology/Tumour Markers

# **LABOKLIN**

#### **Thyroid-stimulating Hormone (TSH)**

Material S 0.5 ml
Method CLIA
Species Dog, cat
Duration 1 day

Note

- **Dog:** For the diagnosis of hypothyroidism only useful if T4 or fT4 are determined simultaneously, as TSH values are within the normal range in > 25% of the dogs with hypothyroidism.
- Therapy monitoring during diagnostic treatment. The dosage of thyroid hormones should be reduced if the concentration is < 0.03 ng/ml.</li>
- · Cat: For therapy monitoring.

# **Tumour Marker AFP (Alpha Fetoprotein)**

Material S (possibly also EP, HP) 1 ml

Method CLIA

Species Dog, cat, horse, others on request

Duration 1 da

Note

- To some extent, slightly increased values also occur in case of benign liver diseases in dogs.
- In case of hepatopathies, values are not or only slightly elevated.
- In case of liver tumours, values are slightly to significantly elevated.
- Values are increased physiologically during pregnancy.
- Therapy monitoring: In case of previous positive findings after surgical and/or chemotherapy, concentration should be within normal range. Relapse monitoring (every 6 months). Elevated in mares with placentitis.

# **Tumour Marker CEA (Carcinoembryonic Antigen)**

Material S (possibly also EP, HP) 1 ml

Method CLIA

Species Dog, cat, others on request

Duration 1 day

Note

- Values increase due to tumours of the gastrointestinal tract and the mammary glands, but also due to inflammatory processes.
- Therapy monitoring: In case of previous positive findings after surgical and/or chemotherapy, concentration should be within normal range. Relapse monitoring (every 6 months).

#### Vetoryl® Therapy Control

Material S 0.5 ml
Method CLIA
Species Dog
Duration 1 day

Note

- For monitoring Vetoryl treatment on day 28 in case of hyperadrenocorticism.
- Cortisol determination pre-pill or pre- and post-pill. For pre- and post-pill determination, please send in 2 x serum, likewise for the determination of 2 x pre-pill (collection 1 hour before and directly before pill administration).
- If the time of taking the pills has to be changed because of the blood sampling, it needs to be done at least one day in advance.
- An additional determination of cortisol 3 hours after the administration of the pill can further facilitate the analysis.
- The determination of 2 x pre-pill is especially recommended for animals in which previous therapy monitoring pre- and post-pill did not produce sufficiently clear results and if fluctuations before pill administration are suspected.
- Separate cortisol reference values apply for monitoring Vetoryl (pre-pill: 14 – 50 ng/ml, post-pill: 14 – 23 ng/ml).
- Not suitable for patients in poor general condition and for animals from which blood cannot be sampled stress-free. In these cases, the ACTH stimulation test is still recommended.



#### 9 Function Tests/Calculations

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

Preferably, the patients should have fasted for at least 12 hours prior to every function test. Stress and excitement should be avoided.

In horses, fasting is only necessary in exceptional cases!

#### **ACTH Stimulation Test**

Diagnosis

- Initial diagnosis of Addison's disease
- latrogenic Cushing's disease
- Cushing's disease

**Species** Material Test procedure

Dog, cat, horse, ferret, others on request S 2 x 0.5 ml

- First blood collection = baseline value
- **Dog, ferret:** injection of 5 µg ACTH/kg as Cosacthen® i.v./i.m.
- Cat: injection of 5 μg/kg Cosacthen®/animal i.v./i.m.
- Horse: injection of 100 I.U. ACTH i.v. (only hypoadrenocorticism)
- Second blood collection 1 hour post ACTH injection = stimulation value in dogs and cats
- Second blood collection 2 hours post ACTH administration = stimulation value in horses

Parameter to be determined Cortisol

Interpretation

- Addison's disease/iatrogenic Cushing's disease: cortisol concentration post stimulation < 10 ng/ml (20 ng/ml in 8% of the cases, in central Addison's a moderate stimulation > 20 ng/ml can be expected in dogs).
- In Cushing's disease (hyperadrenocorticism), the cortisol concentration post stimulation exceeds 150 ng/ml or is more than 3 times the baseline value, as long as it is in the middle of the normal range. Chronic stress and other underlying diseases (e.g. diabetes mellitus) can also lead to an abnormal ACTH response. According to the literature, a stimulation value > 220 ng/ml is, to a very high percentage, associated with Cushing's disease.
- It should be noted that approx. 15% of the dogs with pituitary and approx. 40% of the dogs with adrenal hyperadrenocorticism show a normal, i.e. not significantly elevated increase.
- Therapy monitoring in Cushing's disease by means of Vetoryl Therapy Control (see Chapter 8, p. 133).

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• Horse: In healthy animals, the cortisol level increases by approximately 80%; horses with hypoadrenocorticism show very low baseline values which do not or only slightly increase after stimulation.

# LABOKI IN

#### **ACTH Stimulation Test Extended**

Diagnosis

Material

• Endocrine-active adrenal neoplasia, adrenal hyperplasia

· Early Cushing's disease

Dog Species

S 2 x 0.5 ml

Test procedure

• First blood collection = baseline value

• Injection of 5 μg ACTH/kg as Cosacthen® i.v./i.m.

• Second blood collection 1 hour post ACTH injection = stimulation

value

Parameter to be determined

Cortisol and 17-OH-progesterone

Interpretation

• Corresponds to the basic ACTH Stimulation Test regarding the interpretation of cortisol.

• To clarify steroidal adrenal pathologies, cortisol and 17-OHprogesterone can be determined simultaneously. This is also possible in case of questionable results when determining the cortisol level after ACTH stimulation.

· In dogs with physiological steroid hormone synthesis, the concentration of 17-OH-progesterone increases to up to 180 ng/dl in the ACTH stimulation test.

• Dogs with a possible imbalance in the synthesis, which is found, for example, in case of enzyme defects or adrenal tumours. show an increased baseline concentration and significant hyperstimulation.

 Dogs with pituitary-dependent hyperadrenocorticism also show hyperstimulation. If the cortisol level is also significantly increased after stimulation, it indicates classic hyperadrenocorticism.

 Because of the test properties, patients that are already being treated with Vetoryl® cannot be tested for 17-OH-progesterone.

#### **Bile Acid Stimulation Test**

Diagnosis Detection of a portosystemic shunt Species Dog, cat

Material S 2 x 0.5 ml

Test procedure • First serum sample = value after fasting (10 h)

• Second serum sample 2 hours after feeding = post prandial value

Parameter to be determined

Bile acids

Interpretation Stimulation values  $> 50 \mu \text{mol/l}$  are indicative of a portosystemic

Feeding of 100 g meat plus 5 g fat/10 kg bdw

shunt, stimulation values  $> 40 \mu \text{mol/l}$  are considered suspicious.

#### Protein-corrected Calcium Concentration

Diagnosis

• Hypercalcaemia that is not caused by hyperparathyroidism can typically be attributed to tumours.

 Hypocalcaemia often causes parturient paresis in cattle and seizure disorders in small animals.

 If hypoalbuminaemia or hyperproteinaemia exists, the calcium value should be corrected.

Species Dog, cat Material S 0.5 ml

Parameter Calcium, total protein

Calculation Protein-corrected **calcium concentration** (mg/dl) = serum calcium

level (mg/dl) – (0.04 x serum protein (g/l)) + 3.3

#### **Cortisol Creatinine Ratio**

Diagnosis of Cushing's disease including differentiation between Diagnosis

adrenal and pituitary forms

Species Dog

Material Morning urine 1 ml

• Collection of morning urine day 1 = sample 1 Test procedure

• Collection of morning urine day 2 = sample 2

• Administration of dexamethasone on day 2: orally 3 x 0.1 mg/kg bdw throughout the day

• Collection of morning urine day 3 = sample 3

Parameters to be determined

Cortisol, creatinine

Interpretation

• Interpretation of the ratio of day 1 and day 2:

< 40: Normadrenocorticism, Cushing's disease is unlikely.

40 - 60: Questionable result

> 60: Hyperadrenocorticism is possible and should be verified by a low-dose dexamethasone test.

• Interpretation of the ratio of day 3:

(An increased ratio on day 1 and day 2 is a prerequisite)

> 50% of the average value of the first two samples indicate a cortisol-producing adrenocortical tumour. The presence of non-

suppressible pituitary Cushing's disease is possible.

< 50% of the average value of the first two samples indicate a pituitary-dependent Cushing's disease or another disease which causes increased cortisol secretion (diabetes, stress, gastro-

intestinal diseases, diseases with protein loss).

Dexamethasone Suppression Test (high dose)

see below Dexamethasone Suppression Test (low dose)

# **LABOKI** IN

### Dexamethasone Suppression Test (low dose) Cat

Diagnosis **Species** Material

Screening test to confirm the diagnosis of Cushing's disease

Cat

S 2 x 0.5 ml or 3 x 0.5 ml

Test procedure

- First blood collection = baseline value
- Injection of 0.01 mg of dexamethasone/kg bdw i.m. or i.v.\*
- Blood collection 4 hours post injection = 1<sup>st</sup> suppression value
- Blood collection 8 hours post injection = 2<sup>nd</sup> suppression value

Parameter to be determined Interpretation

Cortisol

 Normal: baseline value in reference range or slightly elevated (due to stress), 4 hours post injection reduction by 50% or to < 10 ng/ml and 8 hours post injection to < 10 ng/ml ( $> 1 \mu\text{g/dl}$ ).

- Cushing's disease: baseline value in reference range or elevated and one or both suppression values > 10 ng/ml.
- The additional blood collection 4 hours p.i. gives information on whether Cushing's disease is pituitary or adrenal.
- Pituitary: baseline value in reference range or elevated, 4-hour value reduced by 50% or to < 10 ng/ml and 8-hour value > 10 ng/ml.
- Adrenal tumour: baseline value in reference range or elevated, no adequate reaction to the administration of dexamethasone after 4 and 8 hours.

# Dexamethasone Suppression Test (low dose) Dog

Diagnosis **Species** 

Screening test to confirm the diagnosis of Cushing's disease

S 2 x 0.5 ml or 3 x 0.5 ml Material

Test procedure

- First blood collection = baseline value
- Injection of 0.01 mg of dexamethasone/kg bdw i.m. or i.v.
- Blood collection 4 hours post injection = 1<sup>st</sup> suppression value
- Blood collection 8 hours post injection = 2<sup>nd</sup> suppression value

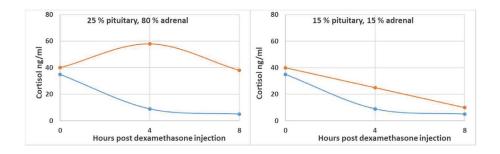
Parameter to be determined

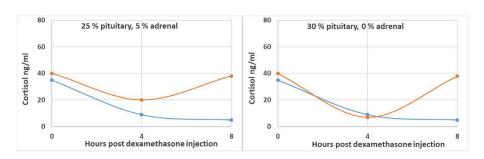
Cortisol

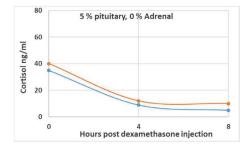
Interpretation

- Normal: baseline value in reference range or slightly elevated (due to stress), 4 hours post injection reduction by 50% or to < 10 ng/ml and 8 hours post injection to < 10 ng/ml.
- Cushing's disease: baseline value in reference range or elevated and one or both suppression values > 10 ng/ml.
- The additional blood collection 4 hours p.i. gives information on whether Cushing's disease is pituitary or adrenal.

- Pituitary: baseline value in reference range or elevated, 4-hour value reduced by 50% or to < 10 ng/ml and 8-hour value > 10 ng/ml.
- Adrenal tumour: baseline value in reference range or elevated, no adequate reaction to the administration of dexamethasone after 4 and 8 hours.







Low-dose dexamethasone suppression test in dogs: Possible results and their frequency in pituitary and adrenal Cushing's disease.

# Dexamethasone Suppression Test (low dose) Horse (Overnight Dexamethasone Suppression Test)

Diagnosis

PPID (formerly called Cushing's disease)

Species

Horse

Material Test procedure S 2 x 0.5 ml or 3 x 0.5 ml

 First blood collection = baseline value (blood sampling at around 4 – 6 pm)

- Injection of 2 mg/50 kg bdw of dexamethasone i.v.
- Blood sampling approx. 15 hours after administration of dexamethasone = 1<sup>st</sup> suppression value – may be omitted
- 2<sup>nd</sup> suppression value after approx. 18 20 hours (at around 10 am 1 pm) obligatory
- Because of the circadian rhythm, the indicated times of the day should be observed.

Parameter to be determined

Cortisol

Interpretation

- PPID: one or both suppression values > 10 ng/ml
- Cave: In late summer/autumn, healthy horses, too, possibly suppress insufficiently.

Note

PPID (pituitary pars intermedia dysfunction) in horses is caused by "pituitary adenoma" (hyperplasia of the pars intermedia). The hyperplastic cells have no cortisol receptors, that is why in PPID, the exogenous administration of dexamethasone does not suppress the endogenous secretion of corticoids as it does in healthy horses.

# **Dexamethasone Suppression Test (high dose)**

Diagnosis

Differentiation between adrenal and pituitary Cushing's disease

Species

Dog, cat

Material

S 2 x 0.5 ml or 3 x 0.5 ml

Test procedure

- First blood collection = baseline value
- Injection of 0.1 mg (dog) or 1.0 mg (cat) of dexamethasone/kg
- bdw i.m. or i.v.
- An additional sample 4 hours post injection gives information concerning a delayed decrease of cortisol.
- Blood sample 8 hours post injection = suppression value

Parameter to be determined

Cortisol

Interpretation

- Pituitary: one or both suppression values < 10 ng/ml (in rare cases suppression values > 10 ng/ml)
- Adrenal: both suppression values > 10 ng/ml

#### **Glucose Tolerance Test**

Diagnosis

Equine metabolic syndrome (EMS)

Species

Horse

Material Each time NaFB 1 ml

Test procedure

- The horse must fast overnight (hay/straw can be given), in the morning, collect fasting blood sample.
- Glucose infusion: 0.5 g/kg bdw of a 50% glucose solution i.v.

(within approx. 5 min)

• Blood samples 2 to 7 after 30, 60, 90, 120, 150 and 180 min

Parameter to

be determined

Glucose

Method Photometry

Interpretation

The glucose level should be back at the fasting glucose level after

3 hours.

For information regarding EMS see Insulin (Chapter 8, p. 124).

#### Combined Glucose-Insulin Test

Diagnosis

Equine metabolic syndrome (EMS)

Species

Horse

Material Earth Test procedure •

Each time NaFB 1 ml

 The horse must fast overnight (hay/straw can be given), in the morning collect fasting blood sample.

- morning, collect fasting blood sample.
- Glucose infusion: 150 mg/kg bdw of a 50% glucose solution i.v.
- Directly followed by 0.1 units/kg bdw of insulin i.v.
- Blood samples after: 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135 and 150 min

Parameter to

be determined Method Glucose Photometry

Interpretation

In healthy horses, the glucose level should return to the baseline value within 45 min. Horses that do not reach these values may be considered insulin resistant/insulin dysregulated. The time glucose needs to return to the baseline value is a measure for insulin resistance.

**Particularity:** Determining the fasting insulin level from the baseline sample makes sense and can provide further insights. To do so, serum would have to be sent in – parallel to the baseline NaFB sample (see above). The same applies to the sample after 45 min. If the insulin level is above 100  $\mu$ U/ml at that time, it indicates insulin resistancy/insulin dysregulation.

**Cave:** There is always a small risk of hypoglycaemia, thus, a glucose infusion solution should always be kept at hand.

#### Oral Glucose Test 1

Diagnosis Equine metabolic syndrome (EMS)

Species Horse

Material S 1 ml (centrifuged, cooled)

Test procedure Fasting overnight, hay/straw can be given. In the morning

(1) 1 g/kg bdw of glucose

or

(2) 0.5 g/kg bdw of glucose per os (can be done by the animal owner)

Parameter to

be determined Insulin Method CLIA

Interpretation Healthy horses stay below these cut offs:

(1) < 85 mU/l insulin (2) < 68 mU/l insulin

EMS horses show higher values.

#### Oral Glucose Test 2

Diagnosis Insulin dysregulation (ID)

Species Horse

Material S 1 ml (centrifuged promptly, cooled)

Test procedure Fasting, only hay/straw

Give 0.15 ml/kg bdw of Karo Light Corn Syrup®, blood collection

after 60 - 90 minutes

Parameter to

be determined Insulin Method CLIA

Interpretation Insulin > 45  $\mu$ U/ml indicates insulin dysregulation.

#### **GnRH Stimulation Test**

Diagnosis • Detection of endocrine active tissue of the gonads (ovary, testis)

Ovarian remnant syndrome (dog)

Cryptorchidism

Species Dog, horse
Material S 2 x 1 ml
Test procedure Female dog:

• First blood collection = baseline value (oestradiol)

• Injection of 0.32 µg of GnRH buserelin (Receptal®)/animal i.v.

• Second sample after 3 hours = stimulation value

Male dog:

• First blood collection = baseline value (testosterone)

Injection of 0.32 μg of GnRH buserelin (Receptal®)/kg animal i.v.

• Second sample after 1 hour = stimulation value

#### Male horse:

• In the morning: first sample = baseline value (testosterone)

• Injection of 0.04 mg of GnRH/horse i.v.

• Second sample after 1 hour = stimulation value

Parameter to be determined

Oestradiol (female dog)/testosterone (male dog, male horse)

Interpretation

• In dogs and cats, the determination of the AMH level (see Chapter 8, p. 121) largely replaces the GnRH Stimulation Test.

• Female dog: depending on the current cycle phase – significant increases are only expected in diestrus and late anoestrus.

• Intact male dog: post stimulation concentrations > 1 ng/ml of testosterone are expected.

• Horse: depending on the clinical issue

### **HCG Stimulation Test Dog/Cat**

Diagnosis
 Detection of endocrine active tissue of the gonads (ovary, testis).

Ovarian remnant syndrome (dog)

Cryptorchidism

Species Dog, cat Material S 2 x 0.5 ml

Test procedure

 First blood collection = baseline value (male dog: testosterone, female dog: oestradiol)

• Injection of 500 IU of HCG (Ovogest®)/animal i.v.

 Second sample after 1 hour = stimulation value (perhaps additional sample after 30 minutes)

Parameter to be determined

Testosterone (male) or oestradiol (female and male)

Interpretation

 In most cases, the determination of the AMH level (see Chapter 8, p. 121) provides comparable information.

 Male dog: post-stimulation testosterone concentrations > 1.0 ng/ml indicate that there is testicular tissue.

 Female dog: Stimulation of oestradiol secretion strongly depends on cycle phase. Significant increases are expected in diestrus and late anoestrus.

#### **HCG Stimulation Test Horse**

• Detection of endocrine active tissue of the gonads (testis)

Cryptorchidism

Species Horse
Material S 2 x 0.5 ml

Test procedure

• First blood collection = baseline value (testosterone)

• Injection of 5000 – 10000 IU of HCG (Ovogest®)/animal i.v.

• Second sample after 1 hour = stimulation value

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Parameter to be determined

Testosterone

Interpretation

• Stallion: Testosterone concentrations between 0.05 and 0.1 ng/ml after stimulation are considered as borderline and need further clarification – e.g. by determining the anti-Müllerian hormone. Higher values indicate the existence of testicular tissue. In most cases, the determination of the AMH level (see Chapter 8, p. 121) provides comparable information.

#### Insulin Glucose Ratio

Diagnosis

Calculated parameter for the detection of an insulinoma

Species

Dog, cat

Material

S 1 ml (promptly centrifuged, cooled)

Test evaluation

Ratio = (serum insulin (μU/ml) x 100)/(serum glucose (mg/dl))

 Modified ratio (AIGR = amended insulin glucose ratio) = (serum insulin (µU/ml) x 100)/(serum glucose (mg/dl) – 30)

Parameters to

be determined Insulin, glucose

Interpretation

Ratios of < 52 or an AIGR < 30 are considered normal.

### STH (GH) Stimulation Test

Diagnosis

Determination of IGF-1 as indirect parameter for the secretion of growth hormones; IGF secretion is stimulated directly by the growth hormone (GH).

 Changes are caused by STH deficiency and STH reactive dermatosis (without decrease of STH).

• Perform function test after exclusion of other endocrinological causes as reference range and pathological range overlap.

Species

Dog, cat

Material

S 0.5 ml (centrifuged, cooled)

Test procedure

- First blood collection = baseline value
   Injection of xylazine (100 μg/kg) i.v.
- Second blood collection after 30 minutes = stimulation value

Parameter to be determined

IGF-1

Interpretation

A significant increase is expected:

• > 2 times if baseline value is low

• > 1.5 times if baseline value is high

Note

The determination of IGF needs to be requested individually for

every blood sample and will be invoiced per sample.

#### TRH Stimulation Test Dog I (3 x fT4)

Diagnosis

- Hypothyroidism
- The test is a compromise between the single determination of T4, fT4 and TSH and the TSH stimulation test

Species Dog

Material S 3 x 0.5 ml

Test procedure

- First blood collection = baseline value
- Injection of Thyroliberin® i.v. (100  $\mu$ g up to 3 kg bdw,

200  $\mu$ g with a bdw > 3 kg)

- Second sample after 90 min = 1<sup>st</sup> stimulation value
- Third sample 3 hours post injection = 2<sup>nd</sup> stimulation value

Parameter to

be determined fT4

...

Interpretation • Euthyroid: at least 1 x > 25 pmol/l

• Questionable: at least 1 x 20 – 25 pmol/l,

all other samples < 25 pmol/l

• Hypothyroid: all samples < 20 pmol/l

#### TRH Stimulation Test Dog II (2 x T4)

Diagnosis

- Hypothyroidism
- The test is a compromise between the single determination of T4, fT4 und TSH and the TSH stimulation test

Species Dog

Material S 2 x 0.5 ml

Test procedure • First blood collection = baseline value

• Injection of Thyroliberin® i.v. (100  $\mu$ g up to 3 kg bdw,

200  $\mu$ g with a bdw > 3 kg)

- Second sample 4 hours post injection = 1st stimulation value
- Determination of T4 in first and second sample

Parameter to be determined

T4

Interpretation

- $\bullet$  Euthyroid: elevation of T4 concentration by at least 0.5  $\mu \text{g}/\text{dl}$  to
- at least 2.5  $\mu$ g/dl
- Questionable: elevation of T4 concentration by less than  $0.5 \mu g/dl$  to  $> 2.5 \mu g/dl$  or by more than  $0.5 \mu g/dl$  but to  $< 2.5 \mu g/dl$

# TRH Stimulation Test Dog, Extended (2 x T4 + 2 x TSH)

Diagnosis Hypothyroidism

Species Dog

Material S 3 x 0.5 ml
Test procedure • First blood

• First blood collection = baseline value

• Injection of Thyroliberin® i.v. (100  $\mu$ g up to 3 kg bdw,

200  $\mu$ g with a bdw > 3 kg)

2022/23 Vitamins

• Second sample 20 min post injection

• Third sample 4 hours post injection = stimulation value

Parameters to be determined

• T4 (samples 1 and 3)

• TSH (samples 1 and 2)

Interpretation

• T4 (see above)

• The stimulation value is of limited significance if there is no TSH

rise after 20 min.

### TRH Stimulation Test Horse I (2 x T4)

Hypothyroidism Diagnosis

**Species** Horse Material

S 2 x 0.5 ml

Test procedure

• First blood collection = baseline value

• Injection of Thyroliberin® 0.5 mg/pony up to 1 mg/horse, slowly i.v.

• Second sample 4 hours post injection = stimulation value

Parameter to

be determined

T4

Interpretation Euthyroid: 2- to 3-fold rise after 4 hours

#### TRH Stimulation Test Horse II (2 x ACTH)

Diagnosis

- PPID (Cushing's disease)
- Test with high sensitivity and specificity; indications: if the results of ACTH determination or of the suppression test do not correlate with clinical findings or are not conclusive.

Species Horse

Material

EP 2 x 0.5 ml (centrifuged, cooled)

• First blood collection = baseline value Test procedure • Slow injection of 1 mg of TRH i.v.

• Second sample 10 min post TRH injection = stimulation value

Parameter to

be determined **ACTH** 

Interpretation Cut off value 10 min after stimulation: < 110 pg/ml;

borderline: 110 - 220 pg/ml; positive: > 200 pg/ml

These values are valid for mid-November to mid-July; so far, this test

is not recommended for mid-July to mid-November.

# Xylazine Stimulation Test ➤ see STH Stimulation Test, p. 144

# 10 Vitamins

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

β-Carotene		
Material Method Species Duration	S, HP 0.5 ml HPLC Cattle, others on request 1 – 2 days	
Note	In cattle, $\beta\mbox{-carotene}$ deficiency can lead to fertility disorders.	
Folic Acid		
Material Method Species	S (possibly also EP, HP) 0.5 ml CLIA Dog, cat, others on request	

Duration	1 day
Note	Can be used to differentiate between malabsorption and bacterial
	overgrowth or abnormal bacterial colonisation of the small intestine.

· Haemolysis is seen as the cause for falsely elevated values of folic acid.

Vitamin A	
Material	S, EP, HP 1 ml (centrifuged, cooled)
Method	HPLC
Species	Dog, cat, horse, cattle, others on request
Duration	Approx. 3 days

#### Vitamin B1

Material	EB, HB 1 ml (cooled)		
Method	HPLC		
Species	Dog, cat, others on request		
Duration	5 days		
Note	<ul> <li>Analysis must be requested within one day after the samples have arrived at the laboratory</li> </ul>		

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- Calf, lambs: cerebrocortical necrosis (CCN) often based on thiamine deficiency

Vitamin B2

Material EB, HB 1 ml (cooled)

Method HPLC

Species Dog, cat, others on request

Duration 5 days

Note Analysis must be requested within one day after the samples have

arrived at the laboratory.

Vitamin B6

Material EB, HB 1 ml (cooled)

Method HPLC

Species Dog, cat, others on request

Duration 5 days

Note Analysis must be requested within one day after the samples have

arrived at the laboratory.

Vitamin B12

Material S (or possibly HP) 0.5 ml

Method CLIA

Species Dog, cat, cattle, others on request

Duration 1 day

Note

- Can be used to differentiate between malabsorption and bacterial overgrowth or abnormal bacterial colonisation of the small intestine.
- To clarify the need for a parenteral substitution of B12 in exocrine pancreatic insufficiency.
- Cattle: Synthesis of vitamin B12 in the rumen can only take place insufficiently if too little cobalt is ingested with the feed. Vitamin B12 deficiency leads to disorders of the energy metabolism with lack of appetite, apathy, growth and performance depression and anaemia as well as possible diarrhoea.

### Vitamin D (25 OH)

Material S (or possibly HP) 0.5 ml (cooled)

Method CLIA

Species List Dog, cat, ferret, birds, reptiles, ruminants, others on request

Duration 1 day

Vitamin E

Material

S, EP, HP 1 ml (centrifuged, cooled)

Method HPLC

Species Dog, cat, horse, cattle, sheep

Duration Approx. 3 days

Note Concentration in horses living in a stable 1 – 2 mg/l, when grazing

2 - 3 mg/l;

in cattle > 3 mg/l.

Vitamin Profiles ➤ see Chapter 2.1.1, p. 38 (dog/cat),

Chapter 2.3.1, p. 57 (horse) or Chapter 2.4.1, p. 63 (ruminants)

# 11 Drug Level

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

Bromide	
Material	S, EP, HP 1 ml
Method	ICP MS
Species	Dog, cat
Duration	4 days
Note	Therapy monitoring under bromide treatment.

Cyclosporine	
Material	EB (only!) 1 ml
Method	CLIA
Species	Dog, cat
Duration	1 day
Note	<b>Therapy monitoring:</b> The determination is suitable for monitoring treatment with cyclosporine.

Digoxin	
Material	S 1 ml
Method	CLIA
Species	Dog, cat, others on request
Duration	1 day
Note	<b>Therapy monitoring</b> at the earliest 7 days after initial application and approx. 6 – 8 hours after the last drug administration.

Levetiracetam* (Keppra)		
Material	S 1 ml	
Method	LCMS	
Species	Dog	
Duration	10 days	
Note	Therapy monitoring, effective levels only determined for dogs	

#### Phenobarbital

Material S (possibly also EP, HP) 1 ml

Method CLIA
Species Dog, cat
Duration 1 day

Note **Therapy monitoring:** Determination is suitable for monitoring

phenobarbital and primidone therapy. In dogs, primidone is immediately metabolised to phenobarbital. Determination should take place at the earliest one week after starting long-term therapy. Sampling can be done regardless of when the drug is administered.

Especially for horses, there is a series of drug level tests available as part of the doping analysis. We will be pleased to answer any questions that you may have.

- Screening for doping-relevant substances
- Antiphlogistics Screening
- Glucocorticoid Screening
- NSAID Screening
- Sedativa/Tranquilizer
- Stimulants
- Tricyclic antidepressants

# 12 Intoxication

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

Heavy Metal Toxicity Screening > see Chapters 2.1.1, p. 34 (dog, cat),

2.3.1, p. 55 (horse) and 2.4.1, p. 61 (ruminants)

Lead

Material EB, HB at least 1 ml

Method AAS

Species Dog, cat, small mammals, birds, reptiles

Horse, cattle on request

Duration 1 day

Note Because it is stored in the bones, lead can only be detected in higher

concentrations in the blood in case of acute poisoning. Over 95% of lead in the blood is bound in erythrocytes, so as test material, whole blood is absolutely necessary. An elevated iron level in the serum is

an additional indication of possible lead poisoning.

Poison Screening\*

Material Vomit/stomach contents, blood (EB), urine, tissue

Method GCMS

Species Dog, cat, horse, farm animals, others on request

Duration 10 days

Note Global screening test, detection of, for example, coumarin derivatives.

Medical history is a must: please add information about medication

prior to sample collection.

Thallium (Rodenticides)

Material S, H 1 ml, hairs

Method ICPMS

Species Dog, cat, horse, cattle

Duration 1 week

Note Thallium is a cumulative cytotoxin that can cause systemic

intoxication. Cases of acute intoxication can be detected using

serum, urine or hairs.

# 13 Infectious Diseases: Pathogenic Agents and Antibody Detection

For abbreviations and additional information concerning the test descriptions see p. 11 and following.

The obligation to notify the authorities upon suspicion or upon diagnosis of a disease applies to Germany.

# 13.1 Viruses

The following pages will provide you with details about the diagnosis of viral infections. You will get information on the required material, the method used, the species examined and on the duration of the test. Antibody and antigen detection are included as well as molecular biological methods (PCR, realtime PCR, droplet digital PRC) and pathology (histology, cytology) for pathogen detection.

If the listed diagnostic procedures cannot be used in viral infections in **reptiles** or have not yielded any findings that match the clinical picture, we also offer virus cultivation by means of **cell culture**. For this, we need tissue or a swab, each put into **cell culture medium** (which we can provide you with) or, if need be, in some sterile physiological saline solution. For cell culture, samples should be sent refrigerated; the test duration is expected to be at least 4 weeks. There will be no further reference to this examination option in this document.

#### 13.1.1 Adenoviruses

Adenoviruses are non-enveloped double-stranded DNA viruses, which are characterised by a high tenacity. They belong to the linear double-stranded DNA viruses. Adenoviruses are strictly host-specific and only in exceptional cases infection of related or unrelated animal species occurs. Adenoviruses mostly cause mild respiratory symptoms and are involved in many multifactorial disorders.

# Dog

# Hepatitis contagiosa canis (HCC)

HCC is caused by **canine adenovirus 1 (CAV-1).** The virus is shed in urine and faeces and transmission occurs directly or indirectly. After oronasal infection, the virus first multiplies in the tonsils and subsequently in the endothelium of the blood vessels, in hepatocytes as well as in cornea and uvea. Deposition of immune complexes can result in glomerulonephritis and uveitis with a corneal oedema ("blue eye"). HCC can be acute or chronic. Especially in unvaccinated puppies, HCC can take a peracute or



acute course and can be fatal. Not only dogs, but also all other species of the family Canidae are susceptible to an infection with CAV-1.

As consistent vaccination against HCC has been carried out in Germany for some time now, the virus CAV-1 has largely disappeared from dog populations. However, CAV-1 still occurs in Eastern European countries.

#### Infectious laryngotracheitis

Infectious laryngotracheitis is caused by **canine adenovirus 2 (CAV-2).** The virus has a strong affinity to the epithelia of the respiratory tract and is a component of the "kennel cough complex".

# Reptiles

Adenoviruses, which have mostly been documented in lizards and snakes, play an important role in reptiles. Literature particularly describes adenoviruses in bearded dragons (Pogona). The clinical picture is often non-specific. In **Pogona**, mainly young animals are affected. Clinical signs which are often seen are anorexia, apathy, diarrhoea and opisthotonus. Boas, colubrids and vipers belong to the **snake** families that are often affected. Gastrointestinal symptoms are very typical. The liver, too, is very frequently affected. Transmission probably occurs through the faeces, but also a vertical transmission is being discussed.

#### Adenovirus - Pathogen Detection

Material Dog: CAV-1: EB 0.2 ml, tissue (e.g. liver), urine, (faeces)

CAV-2: swab without medium (e.g. eye, nose, pharynx),

bronchoalveolar lavage

Birds: swab without medium (cloaca or pharynx), tracheal lavage,

tissue (e.g. intestine or liver)

**Reptiles:** swab without medium (cloaca), tissue (intestine, liver)

Method Realtime PCR (dog), PCR (birds, reptiles)

Species Dog, birds, reptiles
Duration 1 – 3 days (dog)

2 – 4 days (birds, reptiles)

# **Adenovirus – Antibody Detection**

Material S, EP, HP 0.5 ml

Method IFAT

Species Dog, others on request

Duration 1 day

Note Differentiation between infection and vaccination can only be done

by testing serum pairs (taken minimum 2 – 3 weeks apart from each other). Because of extensive vaccination, the disease has become

extremely rare.

# 13.1.2 African Horse Sickness Virus (AHSV)

African horse sickness (AHS) is an endemic viral disease in Equidae particularly in Central Africa; sporadic outbreaks have been observed in the Middle and the Near East as well as in Southern Europe (export-relevant test). Generally, the disease is transmitted by Culicoides spp. or by Culex, Anopheles, Aedes and ticks. All secretions, intestines and the blood of infected animals are infectious. A distinction is made between a subclinical, febrile form, a subacute cardiac form, an acute pulmonary form and a mixed form; CNS manifestation is rare. All organ manifestations are accompanied by oedema and haemorrhage. The mortality rate is 70 – 95% in horses, approximately 50% in mules and about 10% in donkeys.

In Germany, the disease is **notifiable upon suspicion**.

#### AHSV - Antibody Detection\*

Material S 2 ml
Method cELISA
Species Horse
Duration 5 - 7 days

#### 13.1.3 Arenaviruses

**Inclusion body disease of boid snakes (IBD)** is caused by arenaviruses and particularly affects **boas and pythons.** Clinical signs comprise tremor, opisthotonus and loss of the reflex to turn. In young animals, it is often an acute infection with a mortality rate of nearly 100%. In adult animals, the course of the disease is usually chronic and protracted. Early signs are a slight tremor of the head, apathy and less flickering of the tongue.

Progression of the disease is often faster in pythons than in boas. Many times, regurgitation is the first clinical sign in boas. In pythons, the typical course of the disease is stomatitis accompanied by progressive pneumonia, which, showing signs of CNS disease, leads to death. Over the past years, an increase of the disease has been observed in boas, whereas it does not occur as often in pythons anymore. To date, little is known about the transmission in reptiles. Transmission through close contact as well as through mites is being discussed. In some cases at least, a vertical transmission from infected parents to young animals seems to occur.

Diagnosis is either made through the detection of the characteristic intracytoplasmic inclusions in the tissues of affected animals or through the detection of reptarenaviruses using PCR. In both cases, detection is easier in boas; in pythons, inclusions as well as the virus are often only found in the brain. Particularly in boas, inclusions can be detected histologically, most notably in the pancreas, liver, kidneys, oesophageal tonsils and the brain. The same organs are suitable for PCR virus testing. In live animals, inclusions and viral RNA can be detected through blood smears or whole blood as well as through biopsies of the liver, kidney or the oesophageal tonsils. Especially in boas, oesophageal swabs are very useful for PCR virus detection.

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#### Arenaviruses/IBD - Pathogen Detection

Material EB. tissue (e.g. liver, pancreas, kidney, brain).

swab without medium (oesophagus)

Method PCR

Species Snake (boa, python)

Duration 1 – 3 days

Note The PCR detects different reptarenaviruses. These viruses of the

family Arenaviridae are associated with the inclusion body disease

(IBD) in boid snakes (boas, pythons).

A negative test result does not completely rule out IBD, as there may be other virus variants, which have not vet been described, leading

to this disease.

### Aujeszky's Disease Virus > see Herpesviruses, p. 174

### 13.1.4 Avipoxvirus

Poxvirus (Orthopoxvirus) see > Orthopoxvirus, p. 184

Avipoxviruses are normally only known as pathogens which cause **avian pox** in birds. They occur in many different bird species. Susceptibility of domestic and wild birds to avian pox infections is only partly understood. Avipoxviruses are primarily transmitted through insects and aerosols. Breeders also become infected through contaminated animals or food and possibly through blood-sucking parasites as well. Introduction into the population mainly happens when buying additional animals or following exhibitions. In wild birds, infection also occurs directly when picking each other's beaks.

There are different characteristic forms. The cutaneous form is the most common one and is characterised by papular efflorescences of the non-feathered skin areas (eyes, beak, comb, lower leas). Mild forms often develop benian skin tumours (head, leas) as a result of the long convalescence period (weeks/months).

The mucous form is characterised by similar lesions on the mucosa of the beak cavity. tongue, pharynx or larynx (fowlpox). The septicaemic form typically displays general symptoms such as ruffled feathers, somnolence, cyanosis and anorexia without exterior pox lesions. Avian pox infections are usually not fatal (exception: canarypox => often fatal). In Germany, there is an **obligation to notify the authorities** when avipoxviruses are detected.

# Poxvirus (Avipoxvirus) - Pathogen Detection

Tissue (skin lesions, pigeon: small intestine; canary: oesophagus) Material

Method Species Birds Duration 1 – 3 days

Note The diagnosis can also be made by the histological detection of

inclusion bodies.

# 13.1.5 Bluetongue Virus (BTV)

Bluetongue virus (BTV), an Orbivirus, is transmitted by biting midges and causes bluetongue disease in cattle, sheep and goats. Bluetongue disease first occurred in Germany in 2006. So far, serotypes 6 and 8 have been recognised in Germany. Bluetongue disease is characterised by fever, circulatory disorders, head oedema, and ulcerations of the mucosa of the head as well as the teats and the feet. The disease can also be accompanied by severe pneumonia. In sheep, the mortality rate can be 50%. Llamas and alpacas may become infected, too. In Germany, the disease is **notifiable upon suspicion**.

#### Bluetongue Virus (BTV) - Pathogen Detection

FB Material

Method Realtime PCR Ruminants, pig Species Duration 1 – 3 days

Detection of BTV 1 - 24 Note

#### 13.1.6 Borna Disease Virus

#### **Mammals**

Numerous species of mammals are susceptible to this virus. It is of clinical relevance particularly in horses, in cats (where the disease is also called "staggering disease") and in sheep.

The virus has a strong neurotropism and triggers non-purulent meningoencephalitis, associated with anorexia, apathy, somnolence and multiple neuronal dysfunctions. Animals suffering from Borna disease develop motor and behavioural disorders. In horses and sheep, in addition to the symptoms listed above, a lowered head posture. separation from the herd, empty chewing and salivation have been described and, at a later stage, recumbency and flailing movements. Cats frequently suffer from hind-leg ataxia and lumbosacral pain.

There is often little or no immune response, which makes it difficult to diagnose by testing for antibodies. The incubation period is unknown. The progression of a clinically manifested infection is lethal (duration of the disease usually 1 – 3 weeks). Clinically inapparent infections are also possible. It has been a controversial issue

whether this virus also infects humans and is there linked to neuropsychiatric disorders. A seasonal increase of the disease from March to September has been described in horses and sheep; in cats, increases can be found from December to May.

The modes of transmission have not yet entirely been clarified; infection probably occurs through the nerve endings of the nasal and pharyngeal mucosa. Infections from horse to horse (sheep to sheep, cat to cat) are experimentally possible, but very unlikely. Shrews constitute the virus reservoir.

Since 2020. Borna disease virus infections have been notifiable upon diagnosis in Germany.

#### Birds

**Proventricular dilatation disease (PDD)** is a globally distributed, serious disease particularly affecting psittacines (large parrots) like macaws, amazons or grey parrots. In 2008, identification of the previously unknown avian Borna virus (ABV) in birds infected with PDD was achieved for the first time. An etiological relationship could then be proven in infection experiments.

PDD either affects the gastrointestinal tract, the central nervous system or both areas. This means, on the one hand, there may be digestive disorders such as diarrhoea, vomiting or regurgitation as well as anorexia and the excretion of undigested seeds in faeces. On the other hand, PDD can manifest itself through neurological dysfunctions like ataxia and coordination disorders, tremor or paresis. Both symptom complexes are associated with depression, general weakness and excessive loss of weight. In addition to peracute and acute deaths, especially in older birds, chronic progressions of the disease can also be observed. Moreover, clinically inapparent birds can be infected with the virus. Breeding flocks and new additions should thus be tested for an infection with ABV.

Avian Borna viruses are RNA viruses which show high genetic divergence. A negative result does therefore not entirely rule out PDD, as there may be other virus variants, which have not yet been described, leading to this disease.

The safest way to detect an ABV infection requires a combination of antibody and pathogen detection. In some birds, only viral RNA can be detected, in others only anti-ABV antibodies are detectable, while others again react positively on both tests. Both test results should always be interpreted together with the clinical signs.

### Borna Virus - Pathogen Detection

Material Mammals: CSF 0.2 ml, tissue (brain), EB 0.2 ml (viraemia),

intraocular fluid (horse), retina (horse)

Birds: swab without medium (crop and cloaca), tissue (brain,

gastrointestinal tract)

Method Realtime PCR

Species Cat, birds (especially large parrots), horse, sheep, alpaca

Duration 1-3 days

# Borna Virus - Antibody Detection\*

Material S 0.5 ml

Method IFAT

Birds: FLISA

Species Cat, birds, horse, sheep

Duration 1 week

# 13.1.7 Bovine Respiratory Syncytial Virus (BRSV)

Bovine respiratory syncytial virus (BRSV) is an enveloped RNA virus of the family Paramyxoviridae and causes respiratory tract diseases in cattle and sheep. It is mainly calves that develop this disease. Infection primarily occurs in the winter months and is characterised by sudden fever, slight hyperpnoea, apathy, rhinitis and cough. Mild bronchiolitis, multifocal lesions and interstitial pneumonia with syncytia formation will develop. The duration of the disease is 3 – 10 days. In severe cases, it can result in death, otherwise the fatality rate is low. Persistent infections have been described; they may be the reason for maintaining the infection within a herd.

In cattle, BRSV is involved in enzootic bronchopneumonia; it predisposes calves and lambs to the adherence of Mannheimia haemolytica.

There are different vaccines available. However, reinfections may occur after some months.

#### **BRSV** – Pathogen Detection

Material Swab without medium (nose, pharynx), lavage sample, tissue

(e.g. trachea or lung)

Method Realtime PCR

Species Cattle
Duration 1 – 3 days

Note Particularly in the first phase of infection, BRSV can be detected by

means of PCR.

This pathogen detection can be requested individually and it is also part of the PCR test Bovine Respiratory Profile 1 (see Chapter 2.4.3.

p. 64).

#### BRSV - Antibody Detection

MaterialS 1 mlMethodELISASpeciesCattleDuration3 - 5 days

Note This antibody detection is part of the serological Bovine Respiratory

Profile (see Chapter 2.4.2, p. 63).

# 13.1.8 Bovine Viral Diarrhoea Virus (BVDV)

Bovine viral diarrhoea virus, a pestivirus, is the causative agent of bovine viral diarrhoea/mucosal disease (BVD/MD) in cattle, two diseases that are prevalent worldwide. Sheep, goats, wild ruminants and pigs are also susceptible to the virus. BVD virus has 2 genotypes (BVDV1 and BVDV2) and the biotypes cytopathogenic (cp) and non-cytopathogenic (ncp).

Infection in cattle results in different symptoms depending on the time of infection.



**Transient infections** (temporary infections of already born animals) are often asymptomatic, but may lead to diarrhoea, fever, cough and erosions of the mucous membrane particularly in calves, and to reduced milk yield, fertility disorders (repeat breeding, abortions) and malformations (e.g. oculo-cerebellar syndrome) in cows. Transiently infected animals temporarily shed the virus to a certain extent (nasal discharge, saliva, faeces, semen).

**Persistently infected calves (PI animals)** develop during the infection of the mother between the 40<sup>th</sup> and the 120<sup>th</sup> day of gestation, because the immune system of the calf does not recognise the virus as "foreign". PI calves are usually born without abnormalities and shed large quantities of the virus in all secretions and excretions throughout their lives. PI animals are typically seronegative, but can also form antibodies after being infected with a heterologous BVD strain.

If a PI animal is additionally confronted with a cp virus strain through mutation of the prenatally acquired strain or a new, postnatal infection, it will develop a severe and always fatal **MD**.

In Germany, there is an obligation to notify the authorities upon suspicion.

#### **BVDV - Pathogen Detection**

Material EB 0.2 ml, milk, faeces, tissue (e.g. ear tissue tag samples, spleen,

brain, abortion material)

Method Realtime PCR

Species Cattle
Duration 1 – 3 days

# **BVDV – Antibody Detection\***

Material S, milk 0.5 ml

Method ELISA
Species Cattle
Duration 3 – 5 days

#### 13.1.9 Caliciviruses

Caliciviruses see also 

European Brown Hare Syndrome Virus (EBHSV), p. 171

Rabbit Haemorrhagic Disease Virus (RHDV), p. 193

There are numerous strains of the **feline calicivirus (FCV)** with only slight serological differences but large genetic divergence, resulting in wide variations in virulence. Symptoms of FCV may vary from inappetence and fever to joint pains and muscular pain. In rare cases, interstitial pneumonia occurs. The typical proliferate and exudative ulcers in the oral cavity are often aggravated by secondary bacterial infections, i.a. with pasteurella.

#### Calicivirus Cat (FCV) – Pathogen Detection

Material Swab without medium (conjuctiva, oral cavity or pharynx), EB 0.2 ml

(only during the viraemia phase)

Method Realtime PCR

Species Cat

Duration 1 - 3 days

Note Due to genetic divergence, not all strains can be detected by means

of PCR. Detection in the blood is only possible during the viraemia

phase.

#### Calicivirus Cat (FCV) - Antibody Detection

Material S, EP, HP 0.5 ml

Method IFAT Species Cat Duration 1 day

Note Differentiation between infection and vaccination is usually only

possible by analysing serum pairs (taken 3 – 4 weeks apart from

each other). Therefore, detection by PCR is preferable.

# 13.1.10 Caprine Arthritris Encephalitis Virus (CAEV)

The pathogen which causes caprine arthritis encephalitis (CAE) is a retrovirus and belongs to the genus Lentivirus, just like the maedi visna virus. It is a viral disease in goats which causes encephalitis, arthritis and/or mastitis, depending on the age of the animals affected. Development of clinical signs is slow. Neurological changes and interstitial pneumonia will result in ataxia, lameness, paralysis and respiratory distress. Only about one third of the seropositive animals will contract the disease.

The main transmission route is an infection of newborn animals via the colostrum. A horizontal and intrauterine transmission is possible, but of secondary importance.

# **CAE Virus – Antibody Detection**

Material S 0.5 ml
Method ELISA
Species Goat, sheep
Duration 3 days

Note Particularly in affected herds, detection serves to eliminate positive

carrier animals. Positive animals are considered to be infected and potentially shedding (especially when lactating). Negative animals should be checked regularly (at least annually) since recent infection

or low antibody titres can mimic a pathogen-free condition.

# 13.1.11 Carp Edema Virus (CEV)

Carp Edema Virus was first described in Japan in 1974 and in Germany in 2014 and belongs to the family Poxviridae. Three different CEV lines have been identified that can lead to clinical signs in koi carp and carp. **Koi sleepy disease** normally occurs at a range of 15 – 25 °C, but especially in carp, temperatures may also be much lower. The incubation period depends on the water temperature. Signs are lethargy and lying motionless on the ground. Additionally, there may be erosive or haemorrhagic skin lesions with oedema of the underlying tissue as well as an excessive production of mucous on skin and gills. Secondary infections are possible.

In differential diagnosis, koi herpesvirus (KHV), spring viraemia of carp (SVC), a high organic load of the water and infestation with ectoparasites need to be considered.

#### Carp Edema Virus (CEV) - Pathogen Detection

Material Tissue (gill biopsy)
Method Realtime PCR

Species Fish
Duration 1 – 3 days

# 13.1.12 Chronic Bee Paralysis Virus (CBPV)

Chronic bee paralysis virus is an RNA virus which, so far, cannot be assigned to any family. This virus affects adult bees. Infected animals are unable to fly, they crawl on the ground, often have a bloated abdomen and diarrhoea; the affected bees die 5-10 days after the onset of the disease. **Loss of hair** and an asymptomatic progression are possible. Transmission occurs through bee faeces. Whether the Varroa mite is involved in spreading or in worsening the course of the disease is debatable. There are often self-healing processes in the colonies. If the progress is particularly severe, an artificial swarm can be created with brood hatched in an incubator.

#### **Chronic Bee Paralysis Virus - Pathogen Detection**

Material Bee heads Method Realtime PCR

Species Bees
Duration 1 – 3 days

#### 13.1.13 Circoviruses

# Dog

#### **Canine Circovirus**

Canine circovirus was first detected in canine blood samples in the USA in 2012/2013 and was described in a dog with necrotising vasculitis and granulomatous lymphadenitis. In a subsequent study, it was mainly found in faecal samples from dogs with diarrhoea. In 2014, it was detected in Italy and in 2015 in Germany as well. Circoviruses can also be found in healthy dogs; further studies will be necessary to clarify questions on the pathogenesis and epidemiology.

Dog circovirus should be considered in the differential diagnosis in case of diarrhoea/vomiting, fatigue, hepatic diseases, haemorrhage and vasculitis. Co-infections with other, mainly enteropathogenic agents are frequently observed. Similarly, an infection with canine circovirus can further complicate other infectious diseases.

#### **Psittacidae**

### Psittacine Beak and Feather Disease (PBFD)

PBFD is characterised by an impaired growth of the beak, the feathers and claws. The disease is globally distributed; more than 40 species of macaws, agapornis, grey parrots, amazons and parakeets are affected.

Nestlings mostly die peracutely, while the course of the disease is acute in fledglings. Animals show clinical signs of lethargy, loss of appetite as well as vomiting and/or diarrhoea. Death is possible within 1-2 weeks. Changes in the developing feathers are pathognomonic – but usually only visible in chronic forms. Symmetric feather loss occurs or the feathers get stuck in the shaft and will then break off. Lesions on the beak and, rarely, on the claws will only occur later on.

Transmission of the virus mainly takes place horizontally. The virus is spread with the faeces, the shedding of developing feathers and with the crop content of feeding parent birds. Thus, nestlings can be infected very early on. Vertical transmission is also possible, but of secondary importance. Here, hatching birds are infected through egg shells that are contaminated with circoviruses.

# Pigeon

# Pigeon Circovirus (PiCV)

Circovirus infections mainly occur in pigeons aged 6 weeks to 12 months (young pigeon disease syndrome). The clinical picture is non-specific; signs include lethargy, anorexia, diarrhoea, wasting and PBFD-like changes in the feathers. The disease is accompanied by immune suppression, and organ alterations occur, particularly in the central immune system and the spleen. In addition to the clinically manifest form especially in young pigeons, there is also a high number of subclinically or persistently infected animals.

# Pig

#### Porcine Circovirus 2 (PCV-2)

Porcine circovirus type 2 (PCV-2) is associated with the so-called **post weaning multisystemic wasting syndrome (PMWS)**. PMWS is usually observed in weaners and less frequently in suckling piglets. Affected animals show a progredient loss of weight as well as respiratory disorders with coughing, which are often complicated by secondary bacterial infections. PCV-2 can be detected in the tissue of infected piglets by means of PCR. In conjunction with PMWS, co-infections of PCV-2 with porcine parvovirus or PRRSV are being discussed.

Circovirus – Pathogei	n Detection
PCV-2 (pig)	

Material Dog: faeces, EB 0.2 ml (viraemia), tissue (above all liver, lym-

phoid tissue, intestine, kidney)

**Psittacidae:** 2 – 3 feather shafts (recently extracted),

blood (1 – 2 drops on a filter paper), (faeces)

**Pigeon:** 2 – 3 feather shafts (recently extracted), swab without

medium (cloaca), blood (1 – 2 drops on a filter paper, viraemia!), faeces, tissue (bursa Fabricii, spleen, liver)

Pig: EB 0.2 ml, swab without medium (nose or pharynx),

lavage sample (BAL), tissue (e.g. lung, trachea, abortion

material or foetal organs)

Method Realtime PCR (dog, Psittacidae, pig)/PCR (pigeon)

Species Dog, Psittacidae, pigeon, pig

Duration 1 - 3 days

Note The PCR test Circovirus (PBFD) for Psittacidae does not detect

circovirus infections of other groups of birds.

#### 13.1.14 Coronaviruses

SARS-CoV-2 see > Chapter 13.1.45, p. 197

Coronaviruses are enveloped RNA viruses which have club-shaped appendages on their surface that look like a crown under the electron microscope and have thus given the virus family its name. As they are genetically highly variable, transmission of coronaviruses to and among different species is possible. They belong to a large group of RNA viruses that can cause respiratory and/or enteral diseases in various animal species and in humans.

#### Dog

An infection with **canine (enteric) coronaviruses (CCoV, CCV, more recently also CECoV)** is usually asymptomatic or may lead to mild, non-haemorrhagic diarrhoea. In puppies, however, severe courses of the disease with haemorrhagic gastroenteritis are possible. Loss of intestinal villi, flattening of the epithelial cells of the small intestine and detachment of the goblet cells occur. The most noticeable signs are vomiting and watery to bloody diarrhoea, accompanied by severe dehydration. The virus is excreted via the faeces; the duration of excretion is usually less than two weeks.

CCV is also infectious to other canids as well as to cats and pigs, but the pathogenicity of these species is not yet known.

**Canine respiratory coronavirus (CRCoV)** was first detected in a dog in 2003. It appears to have originated from bovine coronavirus, since both viruses share very close similarities. In general, respiratory coronavirus can frequently be detected in the majority of dogs suffering from kennel cough (also known as canine infectious respiratory diseases (CIRD) complex).

In many dogs with mild or moderate signs, such as cough or nasal discharge, but also in asymptomatic dogs, the virus can mainly be found in the trachea.

#### Cat

Feline coronaviruses (FCoV) can be divided into two pathotypes: the weak to non-virulent feline enteric coronavirus (FECV), which infects the intestinal epithelial cells and is considered a mere "diarrhoea pathogen", and the usually fatal **feline infectious peritonitis virus** (FIPV), which mutates in cats and can replicate massively in macrophages.

A cat from a multi-cat household is more likely to excrete the virus than a cat from a single-cat household. The higher the infection pressure, the more likely it is that enteric coronaviruses mutate and cause FIP.

If FIP develops, two different clinical manifestations can be observed: the wet exudative form and the dry granulomatous form. In the wet form, severe polyserositis develops with the formation of a highly viscous, yellowish, fibrin-containing ascites fluid. In the dry form of FIP, pyogranulomatous swellings form on the serosa and in the organs. This mainly affects the liver, lungs and kidneys. Inflammatory nodules form on the spleen and the lymph nodes. Cats often develop anaemia with icterus, emaciation and high fever. There may also be CNS symptoms and, due to the deposition of precipitates, uveitis. A positive titre indicates that the cat had been in contact with coronaviruses. This is the case with most adult animals. In a clinically healthy animal, high titres are usually not important. They do not suggest that this cat will contract FIP. FECV excretors can be identified by means of PCR. In animals suffering from FIP, there are often only low to negative antibody titres. In this case, the antibodies have been bound in immune complexes; thus, antibodies are no longer detectable. For further evaluation, a serum protein electrophoresis and the determination of the albumin-globulin ratio can be included. Diagnostic information is provided by an increase in the gamma globulin fraction and an albumin globulin ratio below 0.6. The albumin globulin ratio is 92% specific.

#### **Ferret**

**Ferret enteric coronavirus** can cause epizootic catarrhal enteritis (ECE) in ferrets with mucoid, greenish, malodorous diarrhoea, especially in adult animals. It is shed in saliva and faeces.

**Ferret systemic coronavirus** can cause a disease similar to FIP, which mainly affects ferrets under 18 months of age. Signs may be non-specific (including diarrhoea, weight loss, lethargy, hyporexia/anorexia, vomiting). In some cases, neurological signs, such as paresis, ataxia, tremor or seizures, can be observed.

Information on the detection of SARS-CoV-2 can be found in Chapter 13.1.45, p. 197.

#### Horse

Equine coronavirus **(ECoV)**, a beta coronavirus, was first detected in the USA in 1999 in the faeces of a foal suffering from diarrhoea. Recent studies in the USA, Japan and Europe confirm it is associated with fever, colic and diarrhoea particularly in adult horses. Infections caused by ECoV mostly occur in the cold season (November to May); involvement of the respiratory system has not yet been proven.

Clinical signs include anorexia, lethargy, fever and changes in faecal consistency. Diarrhoea and mild colic symptoms may also occur. Neurological abnormalities (ataxia, depression, recumbency) have rarely been described, they were, however, secondarily caused by hyperammonaemia. The blood count shows leukopenia (neutropenia/lymphopenia) and hypoalbuminaemia.

Infections with ECoV seem to be self-limiting but can be secondarily complicated (e.g. dehydration or intestinal displacement). Transmission is mainly via the faecal-oral route.

#### Cattle and Wild Ruminants

Bovine coronaviruses **(BCoV)** cause enteric and respiratory diseases in cattle and wild ruminants. These include calf diarrhoea, winter dysentery in adult cattle and respiratory diseases in cattle of different ages.

# Pig

In pigs, coronaviruses cause highly contagious, sometimes epidemic **transmissible gastroenteritis (TGE)**. TGE virus presents an economic problem in all countries with intensive pig production. Loss of income occurs in the affected farms as a result of piglet losses, growth retention and reduced weight gain.

An infection with porcine coronavirus leads to a local infection of the intestinal tract, mostly in the jejunum and ileum. As the disease progresses, the villous epithelium is rapidly lost. Clinically, this is manifested in a watery malodorous diarrhoea. In Germany, TGE is a **notifiable** animal disease.

### Coronavirus - Pathogen Detection

Material Cat: Qualitative detection: FECV faeces/

FIP: puncture fluid 0.2 ml, CSF 0.2 ml, intraocular fluid,

tissue (e.g. kidney or omentum) <u>Quantitative detection:</u> faeces **Cattle:** faeces, swab without medium (nose), tissue (e.g. lung)

**Dog:** CCoV: faeces

CRCoV: swab without medium (pharynx, nose, trachea), BAL

**Ferret:** faeces, EB (viraemia phase), CSF (neurological abnormalities), tissue (intestine, lymph nodes)

Horse: faeces

**Pig:** faeces, tissue (e.g. intestine)

Method Realtime PCR; PCR (ferret), droplet digital PCR (quantitative PCR)

Species Dog, cat, ferret, horse, cattle, pig

Duration 1 - 3 days

Note

 In small animals, a pooled faecal sample is recommended as this increases sensitivity.

- Cat: In cat populations, quantitative PCR allows for the formation
  of groups of animals (animals shedding many or few pathogens
  or pathogen-free animals). This way, infection pressure is reduced
  and rehabilitation may be possible. A cat is considered free if PCR
  was negative in 3 tests at weekly intervals and each time pooled
  faecal samples from 3 days were examined. Quantification can
  also be requested following a qualitative PCR.
- Ferret: differentiation between enteric and systemic coronaviruses is performed automatically.
- Pathogen detection by means of an antigen test is part of the Virological Faecal Profile (EIA) or the Calf Faecal Profiles.
- For pathogen detection of SARS-CoV-2 see Chapter 13.1.45, p. 197

#### Coronavirus – Antibody Detection Feline Coronavirus (FCoV) – Antibody Detection

Material S, HP, Ascites 0.5 ml Method ELISA (cat); IFAT (dog, pig)

Species Dog, cat, pig

Duration 1 day

Note

A positive titre merely confirms contact with coronaviruses.

On specific request, detection in cats can also be done by IFAT.
 For antibody detection of SARS-CoV-2 see Chapter 13.1.45,
 p. 197

# 13.1.15 Cytomegalovirus

Cytomegaloviruses belong to the herpesviruses and have been detected in different rodents. They are considered strictly host-specific. In guinea pigs, salivary and lacrimal glands become inflamed and respiratory symptoms may occur as well. In rare cases, there might also be signs of paralysis.

#### Cytomegalovirus - Antibody Detection\*

Material S 0.5 ml Method IFAT

Species Guinea pig, rat, mouse

Duration 3 – 5 days

# 13.1.16 Deformed Wing Virus (DWV)

Deformed wing virus (DWV) is an RNA virus from the genus Iflavirus, which causes wing deformities in adult honey bees. All developmental stages can be affected. If already the larva is infected, it will develop crippled wings, a bloated abdomen and discolouration during metamorphosis, and the animal dies shortly after emerging. The virus causes a latent infection that persists. Thus, bees without any symptoms can still be carriers. The Varroa destructor mite is among the vectors of this virus and its move to other colonies poses a major problem in the spreading of the disease. There is no causal therapy.

#### Deformed Wing Virus (DWV) - Pathogen Detection

Material Bees

Method Realtime PCR

Species Bees
Duration 1 – 3 days

# 13.1.17 Distemper Virus

Canine distemper virus (CDV) belongs to the genus Morbillivirus (measles-distemper-rinderpest group). All animals of the families Canidae (such as dog, fox, wolf), Procyonidae (like raccoon and panda), Mustelidae (such as ferret, badger, marten) and Felidae (tiger, lion) can get infected. Distemper is enzootic worldwide. Transmission takes place orally or airborne via secretions and excretions of infected dogs or clinically healthy carriers. Intrauterine infections are also possible. Distemper is a febrile general disease with an acute to subacute course. A respiratory, an intestinal, a central nervous and a cutaneous form of the disease can be distinguished.

Virus excretion begins after approx. 7 days (up to 60 to 90 days p.i.), during which a typical cyclic infection with leukocyte-associated (possibly also non-cell-bound) viraemia occurs. Depending on the ability of the immune system to produce neutralising antibodies, distemper can take a mild or fatal course.

#### **Distemper Virus - Pathogen Detection**

Material Qualitative PCR: swab without medium (eye, nose, pharynx or ton-

sil), EB 0.2 ml (viraemia), CSF, urine, faeces Quantitative PCR (dog): swab without medium

Method Realtime PCR; quantitative PCR: droplet digital PCR

Species Dog, ferret, big cats, racoon, other species

Duration 1-3 days

Note Dog: Quantitative PCR can also be requested following qualitative

PCR. The pathogen load can indicate whether the viruses are field or vaccine viruses. A high viral load indicates a field infection, even if the dog has previously been vaccinated with live vaccine against distemper. A low pathogen load denotes a vaccine strain, if a distemper vaccination was given in the last few weeks. Otherwise, it may be a beginning or abating field infection. For medium viral load, we offer Distemper Virus-Vaccine PCR – unless vaccination was

carried out with Vanguard (Zoetis).

#### **Distemper Virus – Antibody Detection**

Material S, EP, HP 0.5 ml, CSF

Method IFAT

Species Dog, ferret, big cats

Duration 1 day

Note Detection can be done in CSF, serum or plasma. Vaccine and infec-

tion titres in serum can only be differentiated by testing serum pairs, while in CSF, only infection titres are present and therefore individual samples are diagnostically conclusive (no blood in the sample!).

# 13.1.18 Equine Arteritis Virus

Equine Viral Arteritis (EVA) is a worldwide distributed, contagious viral infection of Equidae caused by the equine arteritis virus (EAV). Confirmed outbreaks seem to have increased in recent years. The majority of naturally acquired infections is subclinical; however, seroconversion still occurs. When clinical signs appear, they vary in type and severity: fever, depression, anorexia and peripheral oedema, conjunctivitis ("pink eye"), urticaria and abortion. In young animals, pneumonia and pneumoenteritis may also be seen. The virus is mainly transmitted through ejaculate. Persistently infected carrier stallions carry the virus in their accessory sex glands and intermittently shed it in the genital secretions. Geldings, prepubescent stallions and mares cannot be carriers. Especially in animals with systemic disease, excretion can also occur through other body secretions, such as aerolised secretions of the respiratory tract, urine, abortion material, etc.

In Germany, when EVA is detected in Equidae (horses, donkeys, etc.), there is an **obligation to notify the authorities.** 

#### **Equine Arteritis Virus - Pathogen Detection**

Material Swab without medium (conjunctiva, pharynx), EB 0.2 ml (viraemia),

sperm, urine, abortion material

Method Realtime PCR

Species Horse, donkey, other Equidae

Duration 1-3 days

#### **Equine Arteritis Virus – Antibody Detection**

Material S 0.5 ml
Method VNT
Species Horse
Duration 5 days

Note This detection is mainly required for export.

It may be necessary to test paired samples after 3 – 4 weeks.

# 13.1.19 Equine Infectious Anaemia Virus (EIAV)

Equine infectious anaemia (EIA) is a worldwide distributed disease in Equidae, caused by a retrovirus, with acute lethal to chronic recurrent forms. Characteristic signs are recurrent fever, anaemia, thrombocytopenia, oedema and considerable weight loss. Transmission takes place via infected blood, blood-sucking insects, iatrogenic through infected injection equipment, but also intrauterine.

Once infected horses remain infectious and seropositive throughout their lives. All horses older than 6 months that are seropositive are thus considered carriers; younger horses can be seropositive through maternal antibodies. Normally, the incubation period is 1 – 3 weeks, but may also last up to 3 months.

In Germany, there is an **obligation to inform the authorities upon suspicion,** as EIA is an **epizootic disease!** 

# **Equine Infectious Anaemia Virus – Antibody Detection**

Material Agar gel diffusion test (Coggins test): S 0.5 ml

cELISA: S 2 ml

Method Agar gel diffusion test (Coggins test), cELISA

Species Horse, other Equidae

Duration Agar gel diffusion test (Coggins test): 3 days

cELISA: 1 day

Note First antibodies can be detected 2 – 3 weeks post infection. If the

results of the serological examination are negative but animals are suspected of being infected, the test should be repeated, possibly

several times, at intervals of 3 to 4 weeks.

13.1.20 European Brown Hare Syndrome Virus (EBHSV)

European brown hare syndrome (EBHS), also called viral hepatitis of hares, is a disease of the hare species Lepus europaeus and Lepus timidus.

The disease was first described in Scandinavia in the 1980s and has since occurred in numerous European countries; several cases have been reported in Germany as well. The causative agent of EBHS is a calicivirus (genus Lagovirus), which only causes the disease in hares. As far as is known, rabbits (and other animal species, too) are not affected. The virus is shed in all secretions and excretions and is very environmentally stable. Transmission presumably occurs directly, particularly faecalorally or indirectly through contaminated water and feed. The disease is peracute to acute and is characterised by a very high morbidity and mortality rate (up to 100%). If at all possible, signs are rarely observed in free-living wildlife. They include: weakness, apathy, disorientation, loss of shyness and movement disorders (e.g. paralysis of the hind legs). There is no known therapy.

#### **EBHS Virus - Pathogen Detection**

Material Faeces, tissue (e.g. liver), (urine)

Method Realtime PCR
Species Hare (not rabbit!)
Duration 1 – 3 days

# 13.1.21 Feline Immunodeficiency Virus (FIV)

Feline immunodeficiency virus (FIV) belongs to the family Retroviridae. It is closely related to the human immunodeficiency virus (HIV) but is not infectious for humans. Since FIV is mainly transmitted through bite injuries, the prevalence of infected animals is highest in the group of uncastrated male cats over five years. FIV infection is spread worldwide. The prevalence in Germany is at approximately 3 – 5.5%. The virus persists for life. It has a clear tropism for T lymphocytes and macrophages. Similar to the clinical symptoms of HIV-infected patients, the course of FIV infection is often divided into four stages, with the final stage resembling human AIDS. However, transitions are smoother and the phase with no clinical signs is often longer than in humans. Detection should be performed in chronic recurrent and treatment-resistant infections, particularly in the oral cavity and the respiratory tract.

#### FIV Provirus – Pathogen Detection

Material EB 0.2 ml

Method Realtime PCR, qualitative or quantitative\*

Species Cat

Duration Qualitative PCR: 1 – 3 days

Quantitative PCR: 7 – 14 days

Note Quantitative PCR: estimation of provirus load (therapy monitoring).



#### **FIV – Antibody Detection**

Material S, EP, HP 0.5 ml

Method ELISA Species Cat Duration 1 day

Note A positive result indicates FIV infection, but positive titres are also

possible in kittens with maternal antibodies. In questionable cases (exclusion of false positive results), the test should be repeated after

2 - 4 weeks.

Further validation can be provided by an FIV blot at a partner laboratory. Here, a separate antibody detection of two antigens typical for

FIV is performed.

A negative result does not completely rule out an infection. False negative results can, for example, occur at the beginning or at the

final stage of the disease.

# 13.1.22 Feline Leukaemia Virus (FeLV)

Like feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV) belongs to the retroviruses.

Prevalence of FeLV in Germany is less than 1%, with regional differences. Not only cats, but also other felids are susceptible to FeLV. In particular, kittens and cats from multi-cat households are affected, as FeLV is transmitted directly from cat to cat. The main source of transmission is saliva, but other secretions and excretions can also be infectious. In most cases, cats develop an oropharyngeal infection. The virus penetrates the mucous membranes and multiplies there as well as in the tonsils and the retropharyngeal lymph nodes. While some cats with abortive infection are able to fight the virus in a way that no viraemia occurs, other cats develop viraemia which can be detected by an antigen test. If it can be overcome by the cat, it is called transient viraemia. It is therefore always advisable to retest a cat that is positive in the antigen test at a later time. However, some cats do not succeed in fighting off the virus sufficiently, so that the bone marrow becomes infected as well. As a result, provirus PCR is positive, regardless of whether the virus continues to circulate in the blood (progressive infection) or not (regressive infection).

Cats with progressive infection usually have the worst prognosis, younger animals are more often affected by progressive infection than older ones.

#### FeLV Provirus - Pathogen Detection

Material EB 0.2 ml, bone marrow

Method Realtime PCR

Species Cat
Duration 1 – 3 days

Note Detection of provirus can confirm a positive antigen result. Latent

infections can also be detected if no antigen is present in the blood.

#### FeLV - Antigen Detection

Material S, EP, HP 0.5 ml

Method ELISA Species Cat Duration 1 day

Note In order to distinguish transient from persistent infections, a positive

detection should always be controlled. This can be done after 4 – 6

weeks at the earliest, but better after 16 weeks.

As this is an antigen detection, a "cross reaction" in vaccinated cats

can be excluded.

#### FIP > see Coronaviruses, p. 164

#### 13.1.23 Hantavirus

In rats and mice, hantaviruses lead to a persistent infection and it is assumed they are permanently excreted in the urine, as well as through faeces and saliva, without the animals showing any symptoms. Hantaviruses are specific to individual rodent species and only very rarely spread to other species. Rodents are infected in the burrow, in territorial fights or while rearing the young.

It is a zooanthroponosis which leads to severe medical conditions in humans. In Europe, the clinical picture is mainly dominated by fever and nephropathies (dialysis required) or haemorrhages.

#### Hantavirus - Antibody Detection\*

Material S 0.5 ml
Method IFAT
Species Rat, mouse
Duration 3 – 5 days



# 13.1.24 Herpesviruses

Herpesviruses cause epidemic as well as latent or persistent diseases in almost all animal species. The name is derived from the Greek word "herpein" (to creep). Common to all herpesviruses is lifelong latency in the host organism.

#### Herpesviruses dog

The so-called "puppy death" in dogs is caused by canine herpesvirus 1 (**CHV**-1). Puppies under 3 weeks of age die of haemorrhagic systemic disease. There is massive lytic virus replication at a subnormal body temperature of 36 – 37 °C and death occurs within 48 hours. The morbidity rate is 100%, the mortality rate is almost 95%! Older puppies usually show mild respiratory symptoms, that is why an aetiological involvement in kennel cough complex is attributed to CHV. Adult animals usually go through clinically inapparent infections. CHV-1 leads to a latent infection; after a primary cell-lytic infection, the viruses retreat into the trigeminal and lumbosacral ganglion cells. In stressful situations (e.g. birth or incipient lactation), viruses may be reactivated and then shed in saliva as well as nasal and ocular secretion. Female dogs can transmit the virus in utero to the foetuses; abortions and stillbirths are rare. In adult immunocompromised animals, a peracute course of the disease with fatal outcome is possible. A diagnosis of breeding animals is recommended. An infection with suid herpesvirus 1 causes **Aujeszky's disease (pseudorabies)**. For more information see Herpesviruses pig, p. 177.

# Herpesviruses cat

The main signs of feline herpesvirus 1 **(FHV-1)** are respiratory symptoms such as rhinitis and sinusitis with ocular and nasal discharge. Conjunctivitis, corneal ulcers, dyspnoea and anorexia may occur. Co-infections, for example with feline caliciviruses and bacteria, are possible. After the primary infection, a lifelong latent infection develops which may be reactivated under stress at any time and thus lead to recurrent symptoms. In kittens, apart from very high fever and general weakness, there may also be deaths (fading kitten syndrome).

# Herpesviruses birds

There are many different herpesviruses that are found in birds, including commercial poultry, ornamental, wild and zoo birds. New viruses are also regularly found in these animal groups. Several herpesviruses have been described in parrots, too. The best-known and perhaps clinically most relevant one is psittacid herpesvirus 1 (PsHV-1). PsHV-1 is responsible for Pacheco's disease in parrots and is therefore also called Pacheco's virus. The clinical course depends on the genotype or serotype and the affected psittacine species. For budgerigars and cockatiels, mild to subclinical courses with virus shedding are reported. In large parrots, such as macaws, amazon parrots, cockatoos or grey parrots, an infection often leads to death. If symptoms occur, they are usually non-specific and consist of anorexia, apathy and poorly developed feathers. Changes in faeces and an increase in uric acid excretion may occur, too. Occasionally,

CNS symptoms are also observed. The disease particularly breaks out in stressful situations, e.g. capture and quarantine of imported birds, change of owner, hospitalisation, beginning of breeding or the onset of sexual maturity. Therefore, a suitable preliminary examination of birds that are to be integrated into the flock is recommended in order to avoid posing a threat to the other birds.

An examination for herpesviruses may also be appropriate for other animals with systemic diseases, diseases of the respiratory system, the liver or with skin lesions or lesions of the mucous membrane at the cloaca or around the beak.

In amazon parrots and cockatoos, psittacid herpesviruses can also be detected in papillomas in the throat and the cloaca.

#### Herpesviruses reptiles

Herpesvirus infections are most common in a variety of chelonians, including tortoises, terrapins and sea turtles. In the veterinary practice, herpesviruses of **tortoises** of the genus Testudo play an important role. As this is a highly contagious virus infection, animals should be routinely examined for infection before being introduced into a population.

Clinical signs include nasal and ocular discharge, regurgitation, anorexia and lethargy. Necrotic plaques in the oral cavity and on the tongue are also typical.

So far, 4 different types of herpesvirus, **testudinid herpesviruses (TeHV)** 1-4, are known in tortoises. In Europe, especially TeHV-1 and TeHV-3 are found. TeHV-3 has a broad host range among tortoises and infections are usually associated with very high morbidity and mortality rates. TeHV-1 can mostly be detected in Russian tortoises (Testudo horsfieldii). These are often diseases of individual animals, since TeHV-1 has a considerably lower tendency than TeHV-3 to spread in the population. Individual cases of TeHV-2 (especially in desert tortoises) and TeHV-4 (in African tortoises) have been detected in Europe in recent years.

In **turtles**, herpesvirus infections are mainly associated with hepatic inflammation. In live animals, dry pharyngeal and cloacal swabs, and in dead animals, liver samples can be examined by PCR.

In **sea turtles**, herpesviruses cause fibropapillomatosis and other diseases. Virus detection by PCR is possible from altered tissue.

In lizards, herpesviruses are mainly seen in connection with oral lesions.

# Herpesviruses horse EHV-1 and EHV-4

In horses, donkeys, mules and zebras, infections with EHV-1 as well as with EHV-4 are caused by droplet infection or direct contact. The severity of the clinical symptoms depends on the age and immune status of the infected animal. Particularly infections with EHV-1 are able to spread beyond the respiratory mucosa and cause severe manifestations of the disease: abortions, perinatal foal death, neurological diseases. In case of foals infected with EHV-4, morbidity rates of up to 100% are possible, especially during the weaning period. More than 80% of the isolates come from animals with rhinopneumonitis.



Once horses are infected with herpesviruses, they remain carriers of the virus throughout their lives, and the virus can be reactivated endogenously under unfavourable conditions (stress etc.). Lymph organs, the leukocyte fraction and trigeminal ganglion cells are the main latency organs. If the vaccinated horses are also taken into account, seroprevalence in the horse population is high.

In recent years, EHV-1-associated neurological diseases, for which a "neurotropic" strain of EHV-1 is held responsible, have been reported with increasing frequency and severity of the clinical disease. This much-feared clinical picture is referred to as **EHM (equine herpesvirus myeloencephalopathy)**.

Two different variants of EHV-1 have been described in horses (DNA $_{pol}$  D $_{752}$  vs. DNA $_{pol}$  N $_{752}$ ). Each is associated with a different level of neuropathogenicity. The D752 variant is associated with most outbreaks of neurological disease and is considered neuropathogenic. However, only a fraction of the horses infected with this virus will develop neurological signs.

The N752 variant is most commonly isolated in conjunction with abortions, but also in a smaller number of neurological diseases. The differentiation is particularly interesting from an epidemiological point of view.

#### EHV-2 and EHV-5

The involvement of EHV-2 and/or EHV-5 in keratoconjunctivitis has long been suspected and these viruses are indeed regularly detected in conjunctival swabs. In recent years, it has increasingly been shown that EHV-2 and 5 are precursors of other viral and bacterial infections of the respiratory tract. Especially in young animals, EHV-2 and/or EHV-5 were detected in treatment-resistant, partly catarrhal-purulent, partly necrotising or abscessing bronchopneumonia. EHV-5 was recently presented as aetiological agent of "equine multinodular pulmonary fibrosis" (EMPF).

#### EHV-3

Coital exanthema caused by equine herpesvirus type 3 (EHV-3), which only sporadically occurs in Germany, is a mildly progressing breeding infection in horses. Clinically, blisters, pustules and erosions appear on the mucous membrane of the vestibulum, penis or prepuce as well as on adjacent skin areas. Healing takes place spontaneously after approximately 2 – 3 weeks, but can be complicated by secondary infections. Transmission mainly occurs through mating, but is also possible through close contact as well as rectal and vaginal examinations. Infected animals remain carriers of the virus for life.

# Herpesviruses cattle

Bovine herpesvirus 1 **(BHV-1)** is the causative agent of infectious bovine rhinotracheitis (IBR), which – depending on where the disease occurs in the individual organ systems – is also known as infectious pustular vulvovaginitis (IPV) and infectious balanoposthitis (IBP) In Germany, it is an **epizootic disease** that is **notifiable upon suspicion!** 

### Herpesviruses pig

Suid herpesvirus 1 causes **Aujeszky's disease (pseudorabies)** and is also called Aujeszky's disease virus (ADV) or pseudorabies virus (PrV). Pigs are the natural host; they develop different clinical signs depending on age and can survive the infection, while the infection is fatal in other animals. In domestic pigs, Germany has been free of Aujeszky's disease since 2003, but the virus occurs in the wild boar population and can mainly infect hunting dogs – also via meat waste of healthy, but latently infected wild boars. In dogs, an infection causes central nervous disorders, mostly itching and death after 1 – 3 days.

In Germany, Aujeszky's disease is **notifiable upon suspicion** in domestic cattle and domestic pigs.

#### Herpesviruses koi

Koi herpesvirus **(KHV)** is a highly infectious virus that has caused epidemic disease in carps (koi and common carps) in recent years, depending on the water temperature. Morbidity and mortality rates can be as high as 100% within 1-2 weeks after the pathogen has been introduced. The incubation period ranges from a few weeks to several months. It depends on various external and internal factors such as stress and condition of the fish. Fish of all age groups are affected at water temperatures between  $18-29\,^{\circ}\text{C}$ . Clinically, the main signs are gill necrosis, increased mucus production, haemorrhages of the skin, liver, spleen and kidneys. Surviving fish probably remain latent carriers of the virus for years and represent a potential hazard in the trade with live fish in pond management and hobby animal keeping. Immunisation by means of live attenuated vaccine is currently rejected from a scientific point of view.

In Germany, it is an epizootic disease that is notifiable upon suspicion!

Herpesvirus - Pathogen Detection	
BHV-1 (cattle)*	

Material

**Dog:** abortion material, tissue of dead puppies (lung, liver,

kidney), swab without medium (nose, pharynx, eye, genital

tract), EB (viraemia)

**Cat:** swab without medium (eye, nose, phayrynx, genital tract),

EB (viraemia!), abortion material, tissue (e.g. kidney, liver)

**Birds:** 2-3 plucked pinfeathers, blood (EB or 1-2 drops on

a filter paper), swab without medium (triple swab: eye + pharynx + cloaca), faeces, tissue (e.g. liver, kidney,

spleen)

Tortoise: swab without medium (tongue + pharynx), tissue (liver,

intestine, possibly brain)

**Turtle:** swab without medium (pharynx + cloaca), tissue (liver)

Sea turtle: alterated tissue

**Lizard:** swab without medium (lesions, pharynx), tissue (liver)

**Horse:** <u>EHV-1:</u> swab without medium (nose or pharynx),

bronchoalveolar lavage, abortion material incl. placenta, EB 0.2 ml (upon request, the detection from buffy coat is also possible, in this case, at least 5 ml EB is required), CSF. According to recent studies, it is recommended to also examine EB in parallel with the swabs/organ material. EHV-2: swab without medium (eye); foals with respiratory symptoms: swab without medium (nose or pharynx), lavage (BAL)

<u>EHV-3:</u> swab without medium (lesions on vestibule, penis, prepuce or surrounding skin), tissue (lesions)

EHV-4: swab without medium (nose or pharynx), lavage (BAL), EB 0.2 ml (buffy coat, examination of EB + organs: see EHV-1)

EHV-5: swab without medium (eye); foals with respiratory symptoms: swab (nose or pharynx), lavage (BAL), EB 0.2 ml

**Cattle:** BHV-1: swab without medium (eye, nose or genital tract),

tracheal lavage, abortion material, tissue (e.g. brain or

tonsil)

Koi: tissue (e.g. gills, brain, liver, spleen, skin or intestine),

swab without medium (gills or skin)

Method Realtime PCR/PCR (birds, reptiles)

Dog. cat. birds. turtle. tortoise. lizard. horse, cattle. koi

Duration 1 - 3 days

Species

Note

2 – 4 days (birds, reptiles)

7 – 14 days (cattle)

Herpesviruses usually produce only short-term viraemia, thus

detection in EB is limited to the early acute phase.

**Tortoise:** In case of a positive result, differentiation of the virus

strain may be of clinical relevance because of different tendencies to spread in the population and for the prognosis of an infection. Differentiation is possible on

request.

**Horse:** Blood test only secondary and only in febrile phase. From a positive buffy coat result, the conclusion can be drawn

that the horse has come in contact with herpesviruses – whether this is acute or happened longer ago can often not be interpreted. In case of young horses, a blood test

can be useful.

If the PCR test result for EHV-1 is positive, differentiation of the EHV-1 virus variant is carried out automatically and free of charge.

The detection of EHV-1 and/or EHV-4 or EHV-2 and EHV-5 is also part of several profiles (see Chapters 2.3.3, p. 57 and 2.3.4, p. 58).

Herpesvirus – Antibody Detection Equine Herpesvirus 1/4 – Antibody Detection

Material S, HP 0.5 ml

Tortoise: S, HP 0.4 ml

Method Dog, cat: IFAT

Horse: ELISA Tortoise: VNT

Species Dog, cat, tortoise, horse

Duration 1 day

Tortoise: 7 – 14 days Horse: 2 – 3 days

Note Differentiation between infection and vaccination is possible by

means of testing serum pairs.

**Tortoise:** The test detects antibodies against TeHV-1 and TeHV-3. **Horse:** Testing of paired serum at an interval of 10 – 14 days. A clear increase in titre would prove an acute EHV infection.

However, in acute cases we recommend direct pathogen detection by PCR (from a nasal swab without medium) to verify excretion. Vaccination titres cannot be distinguished from infection titres.

#### Aujeszky's Disease Virus (Pseudorabies) - Antibody Detection

Material S 1 ml Method VNT

Species Dog, wild boar

Duration 3 days

BHV-1 – Antibody Detection\* gB – Antibody Detection\* gE – Antibody Detection\*

Material (1) BHV antibodies: S or milk 1 ml

(2) gB/gE antibodies: S 1 ml

Method ELISA Species Cattle Duration 5 days

Note In cattle, vaccination and infection titres can be differentiated by

determining the gE glycoprotein.

Glycoprotein B (gB) has both vaccine strains and field strains in the virion. There is a deletion of the gE gene in the vaccine virus and therefore no glycoprotein E (gE) in the virion, while the field virus has gE in the virion. Antibodies against gE can therefore only be detected in field virus infections, but not after vaccination alone.

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# Pacheco's Virus – Antibody Detection\*

Material S 0.2 ml
Method VNT
Species Birds
Duration 7 – 10 days

Infectious Anaemia > see Equine Infectious Anaemia Virus, p. 170

Infectious Viral Arteritis > see Equine Arteritis Virus, p. 169

#### 13.1.25 Influenza Virus

Influenza A viruses belong to the family Orthomyxoviridae and are mostly found in humans, pig, poultry and horses, but also in many others such as birds or dogs.

#### Horse

Equine influenza is caused by the subtypes influenza equi 1 (H7N7) and A equi 2 (H3N8), although H7N7 has not been very present anymore over the past 30 years. In susceptible Equidae, an infection causes fever and a rough, dry cough. In unvaccinated populations, the virus spreads quickly. Secondary bacterial infections with mucopurulent nasal discharge are frequent and mask the clinical picture, especially in partially immune populations.

#### Pig

Pigs may not only become infected with porcine, but also with human and avian influenza viruses and thus contribute to the creation of reassortant influenza viruses. The influenza pandemics in humans in 1918/19 and in 2009 were caused by porcine influenza viruses. In pigs, primary infections are usually linked to livestock transport. The infection spreads explosively in the population.

#### Influenza A Virus - Pathogen Detection

Material Swab without medium (respiratory tract), lavage (BAL), TBS

Method Realtime PCR

Species Dog, ferret, horse, pig, others (not birds)

Duration 1 - 3 days

# Influenza A Virus – Antibody Detection\*

Material S 0.5 ml Method HAH Species Horse Duration 5 days Note

- Testing is done for A equi 1 (Prague 56) and A equi 2 (American, Florida Clade 1) as well as A equi 2 (European, Newmarket 2/93).
   An increase in titer of at least fourfold is usually associated with acute disease.
- Differentiation between vaccine and infection titre is not possible.

#### 13.1.26 Iridovirus

Iridovirus see also > Ranaviruses, p. 195

**Invertebrate iridoviruses (IIV)** particularly occur in insects. In addition, they are regularly found in lizards, where they might cause skin lesions. The detection of iridovirus may be of interest if the mortality rate in the population of feeder animals or lizards is increased.

#### Iridovirus - Pathogen Detection

Material Lizard: tissue (e.g. skin or liver), swab without medium (skin)

Feeder animals (whole insects)

Method Realtime PCR

Species Reptiles (lizards) and their feeder insects (e.g. crickets)

Duration 1 - 3 days

Note As these viruses are frequently found in feeder insects, virus detec-

tion in pharyngeal or cloacal samples or in the intestine needs to be

interpreted very carefully.

# 13.1.27 Lymphocytic Choriomeningitis Virus (LCMV)

The main reservoir of LCMV, which belongs to the arenaviruses, is the house mouse. Cells infected with LCMV express antigens and are recognised by cytotoxic T lymphocytes. This lymphocyte activity also makes the blood-brain barrier permeable resulting in meninges and neurons being damaged.

Infection of adult **mice** leads to choriomeningitis. In contrast, an intrauterine or neonatal infection generally causes an asymptomatic chronic carrier state in mice, with such animals forming immune complexes in the course of their lifetime that lead to glomerulonephritis. In **guinea pigs** and **hamsters**, LCMV infections often progress subclinically, however, conjunctivitis, blepharitis, respiratory symptoms, tremor, seizures and paralysis have been described. LCMV is transmitted diaplacentally and with all secretions and excretions.

In humans, LCMV rarely leads to choriomeningitis; the infection is usually asymptomatic or shows mild, flu-like symptoms. An infection in the second part of pregnancy can cause severe foetal damage.

### Lymphocytic Choriomeningitis Virus (LCMV) - Antibody Detection\*

Material S, HP 0.5 ml

Method IFAT

Species Guinea pig, mouse, hamster

Duration 3 – 5 days

Note Zooanthroponosis! – Be careful when collecting the sample!

### 13.1.28 Maedi/Visna Virus

Maedi and visna are two different diseases in sheep that are caused by the same virus of the retrovirus family and belong to the so-called "slow virus diseases".

Maedi (meaning dyspnoea) disease is characterised by shortness of breath and cough, which are caused by chronic progressive interstitial pneumonia.

Visna (meaning decay) is a slightly contagious but progressive disease of the central nervous system. The animals show paralysis because of a demyelination of the CNS as well as increasing decay.

In Germany, the disease is **notifiable upon diagnosis** in sheep and goats.

#### Maedi/Visna Virus - Antibody Detection

Material S 0.5 ml Method ELISA

Species Cattle, sheep
Duration 3 – 5 days

# 13.1.29 Myxoma Virus

Myxoma virus is the causative agent of **myxomatosis** in rabbits. It is a large, enveloped DNA virus and belongs to the genus Leporipoxvirus (family: Poxviridae). Despite its envelope, poxviruses are relatively stable in the outside world. However, inactivation can easily be achieved with ordinary disinfectants.

The virus is very host-specific: The European rabbit and domestic rabbit breeds descending from it are most susceptible, but American rabbit species and European hare species can be infected as well. The virus is mainly transmitted through insects (gnats, fleas – mechanical transmission), thus, the disease primarily occurs between the end of July and October. Transmission by direct contact is usually only important in cases of high population density.

After a primary virus replication in the mucous membranes of the head, the regional lymph nodes become infected. Subsequently, there is a cell-associated viraemia (lymphocytes) and the virus spreads to nearly all organs.

After an incubation time of 4-10 days, an infection with myxoma virus causes an acute systemic disease with severe conjunctivitis and hypodermal oedema (especially in the

facial and the anogenital region). Nodular tumours in the skin and subcutaneous tissue may also occur. Respiratory problems and dysphagia result in inappetence and anorexia. The mortality rate is between 25 and 90%. Chances of full recovery are generally very low. Seriously affected animals should be euthanised.

Due to the high mortality rate caused by the disease, the virus was introduced into rabbit populations in Europe, Chile and Australia around 1950 for population control. It has since been endemic in these countries. However, co-evolution of the virus and the rabbits has led to weakened virus strains and virus-resistant rabbits. Thus, the severity of clinical symptoms strongly depends on the virulence of the present virus strain and the susceptibility of the host.

Vaccines are available for prophylaxis.

#### Myxoma Virus – Pathogen Detection

Material Swab without medium (conjunctiva, nose or pharynx), tissue

(e.g. conjunctiva, lung or kidney)

Method Realtime PCR

Species Rabbit
Duration 1 – 3 days

#### Myxoma Virus - Antibody Detection\*

Material S 0.5 ml
Method IFAT
Species Rabbit
Duration 3 – 5 days

Newcastle Disease Virus > see Paramyxoviruses, p. 187

#### 13.1.30 Nidoviruses

Viruses of the order Nidovirales are large, enveloped, single-stranded RNA viruses. Among others, this order includes the families Arteriviridae and Coronaviridae. The nidoviruses detected in **snakes** are most closely related to the family Coronaviridae, subfamily Torovirinae. They are found in pythons and boas, and have most commonly been detected in ball pythons and green tree pythons so far. They are associated with pneumonia and stomatitis and seem to be important pathogens in different python species.

Nidoviruses have also been detected in **shingleback lizards** and other **Tiliqua spp.** However, these are clearly different from the nidoviruses described in snakes and are called **shingleback nidovirus 1** (genus Tiruvirus). Infection is associated with respiratory disease.



Nidoviruses - Pathogen Detection

Material Swab without medium (pharynx or trachea), tracheal lavage, tissue

(e.g. lung or trachea)

Method PCR

Species Snake (python, boa)

Duration 1-3 days

Nidovirus (Shingleback Nidovirus) - Pathogen Detection

Material Swab without medium (pharynx), tracheal lavage

Method Realtime PCR

Species Tiliqua spp. (blue-tongued skinks)

Duration 1 - 3 days

# 13.1.31 Orthopoxviruses

Poxvirus (Avipoxvirus) see > Avipoxvirus, p. 156

The genus Orthopoxvirus belongs to the family Poxviridae. Due to their structure and their viral enzymes, these viruses have a special position within the viruses. Poxviruses are able to mature into infectious viruses in the cytoplasm of the host cell without the cell nucleus being involved. Poxviruses have a relatively large genome with a double-stranded linear DNA.

Orthopoxviruses have a broad host spectrum and can therefore be called cowpox, catpox, elephantpox or ratpox. Cattle, carnivores, rodents and humans are particularly susceptible.

In Germany, the authorities must be notified.

#### Cat

An infection with cowpoxvirus can cause catpox in both cats and humans. Cats are usually infected by their prey animals such as mice and rats. The virus penetrates the skin through bite or scratch injuries, which are usually located on the head, neck or forelimbs. To some extent, necrotising, extremely itchy smallpox appears at these sites. In most cases, self-healing occurs after a few weeks, but in immunocompromised people and cats (e.g. FIV infection), a systemic infection with severe to fatal pneumonia can develop.

The vaccination against human pox administered until the 1970s does not provide any protection against an infection, but seroconversion with the vaccinia virus used for vaccination can probably lead to an attenuated clinical picture. These vaccinations were discontinued in the mid-1970s and a more frequent occurrence of this infection becomes more likely.

A PCR analysis of skin crust material can provide a quick and reliable diagnosis. Self-protection during sampling and treatment of an infected cat should not be neglected. In addition, veterinary personnel and, if necessary, the owners should be educated. In most cases, if a person becomes infected with smallpox, it can be diagnostically evaluated whether or not the pet is a carrier.

#### Rat

The occurrence of Orthopoxvirus bovis infections in pet rats and the resulting transmission to humans has only recently been described. The rats show necrotising lesions on limbs and in the head and tail area.

#### Poxvirus (Orthopoxvirus) - Pathogen Detection

Material Scurf Method PCR

Species Cat, rabbit, rat, mouse, cattle and other susceptible species

Duration 1-3 days

Note Zoonosis – be careful when collecting the sample!

Pacheco's Virus > see Herpesviruses, p. 174

# 13.1.32 Papillomaviruses

# Dog/Cat

Canine papillomatosis is a rare viral disease in dogs characterised by numerous benign warts (papillomata) in the head area. The causative agent is canine papillomavirus. Although papillomaviruses occur in many animal species and in humans, they are strictly host-specific, so they do not pose a risk to humans or other animals. Papillomata are mainly observed in the oral cavity and are less frequent on the conjunctiva, the cornea and the eyelids. Warts are benign and usually heal without treatment within one to five months. If feed intake is severely affected by them, surgical excision might be indicated. A current study has attested a good effectiveness to the administration of azithromycin.

#### Papillomavirus - Pathogen Detection

Material Tissue (skin)
Method PCR
Species Dog, cat
Duration 1 – 3 days

#### Horse

The **equine sarcoid** is one of the most common skin tumours in horses (about 2-12% of all horses are affected). The causative agent is bovine papillomavirus – especially type 1, more rarely type 2. The tumour cells are modified fibroblasts; the skin and subcutaneous tissue are affected. Equine sarcoids are considered semi-malignant



tumours, i.e. they do not metastasise, but have a strong tendency to recur if surgical removal is incomplete. It is presumed that transmission mainly occurs through direct contact as well as flies and horseflies, but also indirectly through wound sites, saddles, blankets and cleaning utensils. The entire skin surface as well as certain blood cells are infected; moreover, the infection remains throughout life. The initial diagnosis is made at the age of 3-12 years.

#### BPV (Bovine Papillomavirus 1 and 2, Equine Sarcoid) - Pathogen Detection

Material Scurfs, hairs (with root), tissue (tumour)

Method Realtime PCR

Species Horse
Duration 1 – 3 days

Note Positive PCR results confirm the suspected clinical diagnosis. Gold

standard for the diagnosis of equine sarcoid is the examination by

histopathology.

#### 13.1.33 Parainfluenza Viruses

# Dog

Canine parainfluenza virus 2 (CPiV-2) plays a crucial role in acute infections of the upper respiratory tract in dogs, which are referred to as kennel cough. In kennels or similar facilities, antibodies can be detected in up to 70% of all animals.

Infections solely with CPiV-2 usually only lead to a mild or clinically inapparent course of the disease. Only if secondary infections with other viruses (mainly canine adenovirus 2/canine herpesvirus 1), bacteria (Bordetella bronchiseptica, streptococci, staphylococci, etc.) or mycoplasma occur and if husbandry and/or hygiene conditions are poor, the known severe courses of the disease develop.

# Parainfluenza Virus (CPiV) - Pathogen Detection

Material Swab without medium (nose, pharynx), lavage (BAL)

Method Realtime PCR

Species Dog Duration 1 – 3 days

# Parainfluenza Virus - Antibody Detection\*

Material S, EP, HP 0.5 ml

Method IFAT Species Dog Duration 1 day

Note As a rule, vaccine and infection titres can only be differentiated by

testing serum pairs.

#### Cattle

**Bovine parainfluenza virus 3 (PI-3, BPIV-3)** plays an important role in acute respiratory tract diseases in cattle, especially in the development of the multifactorial disorder **enzootic bronchopneumonia**. Monoinfections cause only mild symptoms or are clinically inapparent. Only secondary infections with other viruses (e.g. bovine adenovirus), bacteria (pasteurella, mycoplasma) and factors that reduce resistance (cold weather, stress, poor stable hygiene) lead to the development of severe symptoms in the form of endemic bronchopneumonia. The parainfluenza virus 3 is shed with the nasal secretion and the transmission is airborne. The clinical picture is characterised by fever, breathing difficulties and salivation. About 5% of the animals die within 3 – 4 days. Various vaccines are available, but reinfections can occur after a few months.

#### BPIV-3 – Pathogen Detection

Material Swab without medium (nose or pharynx), lavage sample, tissue (e.g.

trachea or lung)

Method Realtime PCR

Species Cattle
Duration 1 – 3 days

Note This detection can be ordered individually and is also part of the

PCR test Bovine Respiratory Profile 1 (see Chapter 2.4.3, p. 64).

### **BPIV-3 – Antibody Detection**

Material S 1 ml Method ELISA

Species Cattle, sheep
Duration 3 – 5 days

Note This detection is part of the serological Bovine Respiratory Profile

(see Chapter 2.4.2, p. 63).

# 13.1.34 Paramyxoviruses

Paramvxoviruses see also

➤ BRSV. p. 159

> Distemper Virus, p. 168

> Parainfluenza Viruses, p. 186

Sunshine Virus, p. 198

Paramyxoviruses are enveloped, single-stranded RNA viruses. They mainly cause respiratory disorders in humans and many animal species, but are also the causative agents of severe systemic diseases.

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#### Birds

#### Avian Paramyxovirus 1 (aPMV-1, Newcastle Disease Virus)

Newcastle disease virus is an avian paramyxovirus which can infect many different avian species. In fowl, Newcastle disease is also called atypical fowl pest. There are different pathogenic strains which produce clinical signs of varying severity, from subclinical to peracute diseases. Most notably, affected animals can develop respiratory and CNS symptoms; loss of performance, diarrhoea and sudden deaths are also possible. Newcastle disease virus is zoonotic and can cause conjunctivitis, fever, headache and aching limbs in humans.

aPMV-1 is considered the cause of Newcastle disease once it exceeds a certain pathogenicity index. In Germany, Newcastle disease is **notifiable upon suspicion**. In Germany, poultry must be vaccinated.

#### Paramyxovirus (aPMV-1) - Pathogen Detection\*

Material Swab without medium (cloaca, trachea), tissue (trachea, lung, brain,

liver, spleen)

Method Realtime PCR

Species Birds

Duration Approx. 1 week

#### Paramyxovirus (aPMV-1) - Antibody Detection\*

Material S. EB 0.2 ml

Method HAH Species Birds

Duration Approx. 7 – 10 days

#### Reptiles

# Paramyxoviruses/Ferlaviruses

Ferlavirus infections most notably occur in snakes. These infections are rarely found in lizards and chelonians. Vipers, elapids, colubrids, boas and pythons are particularly affected. Signs of the disease include nasal discharge, breathing with open mouth and breath sounds. In addition to respiratory changes, CNS signs are often observed. They include poor muscle tone, compulsive movements, head tremor and opisthotonus. Transmission can occur horizontally from one animal to another, by aerosols or through faeces.

In live animals, the virus can best be detected by obtaining a tracheal wash sample or through a combined pharyngeal and cloacal swab. The most suitable organ samples are lung, followed by brain, pancreas as well as liver, intestine and kidney.

# Paramyxoviruses/Ferlaviruses - Pathogen Detection

Material Swab without medium (pharynx, cloaca), tracheal lavage, tissue

(e.g. brain, lung, liver, kidney, pancreas, intestine)

Method PCR

Species Reptiles (especially snakes, but also lizards and chelonians)

Duration 1 - 3 days

#### Paramyxovirus/Ferlavirus - Antibody Detection\*

Material S, HP 0.2 ml

Method HAH Species Reptiles

Duration Approx. 1 week

#### 13.1.35 Parvoviruses

Parvovirus is a very small non-enveloped DNA virus with extreme environmental stability. It can persist in the environment for up to a year and is also very temperature-resistant. Animals become infected oronasally with parvoviruses. First, virus replication occurs in the mucous membranes, then followed by viraemia. The lymphatic system and organs become infected.

# Dog

In dogs, **parvovirus infection** usually progresses as a cyclic systemic disease with a manifestation in the intestinal epithelium and the resulting clinical picture of anorexia, fever, vomiting and persistent bloody diarrhoea. The disease is most severe in puppies. Different clinical forms of parvovirus infection can develop. The peracute form results in death within a few hours, usually without any serious signs. The acute form, however, is characterised by severe symptoms. High fever, severe bloody diarrhoea and vomiting occur. Due to the high affinity of the virus to tissues with high mitotic activity, severe leukopenia occurs simultaneously. If the leucocyte count falls below 2000 cells/µl, prognosis must be made carefully. Subclinically infected animals represent the pathogen reservoir as they shed the virus via the faeces.

#### Cat

Feline parvovirus infection – **panleukopenia** – is a highly contagious systemic disease of felids. The mortality rate among unvaccinated animals is over 80%. Clinically, the disease is characterised by fever, diarrhoea, vomiting and dehydration. The blood count shows extreme leukopenia. A special case is the intrauterine infection. The mother cat is infected without showing any symptoms, but it leads to the abortion or death of the kittens. If kittens are born alive, there is often a cerebellar hypoplasia, which leads to ataxia and tremor, usually without any impairment of consciousness.

#### Ferret

**Aleutian mink disease** is caused by a parvovirus. This single-stranded DNA virus is non-enveloped and therefore, like canine and feline parvoviruses, extremely resistant. Minks, but also ferrets, skunks, otters, raccoons, foxes, etc. can be affected by this disease.

The virus triggers an immune complex-mediated disease which is mainly characterised by hypergammaglobulinaemia.

The signs vary: Young animals tend to develop pneumonia, adult animals develop glomerulonephritis, arteritis, and/or meningoencephalitis. Bloody diarrhoea, hind leg paresis and fertility disorders have further been described. The outcome is often lethal. As there is currently no vaccine available, many ferrets are vaccinated with dog vaccines; it is unlikely that this will provide protection against an infection with the Aleutian mink disease virus.

Transmission can be both direct and indirect.

#### Horse

**Equine serum hepatitis**, formerly referred to as **Theiler's disease**, is caused by infection with **equine parvovirus-hepatitis virus (EqPV-H)**. EqPV-H is a hepatotropic single-stranded DNA virus that can cause hepatitis in infected horses. Asymptomatic infection is common. Approximately 2% of infected horses develop clinical hepatic disease, ranging from mild disease to acute fulminant liver failure. Clinical signs may include one or more of the following: lethargy, anorexia, jaundice, neurological signs associated with hyperammonaemic encephalopathy, death usually within 72 hours. EqPV-H should be suspected in horses with signs of illness and/or liver disease. Horses between 3 – 6 years of age have a seroprevalence of about 14%, for the age group of 11 – 15 years even a value of about 43% has been reported. EqPV-H-positive horses have often received a blood product 4 – 8 weeks before.

# Pig

Porcine parvovirus (PPV) can be detected in almost all pig populations worldwide. In Germany, a prevalence of 60 – 80% can be assumed.

In an infection with PPV, fertility disorders and embryonic infections with subsequent fetal death (**SMEDI:** stillbirth, mummification, embryonic death, infertility) are the main clinical symptoms. The sows usually show no clinical signs.

# Parvovirus - Pathogen Detection

Material **Dog:** <u>qualitative PCR:</u> faeces, EB 0.2 ml, tissue (e.g. intestine or heart)

quantitative PCR: faeces

Cat: faeces, EB 0.2 ml

Ferret: faeces, EB (viraemia), swab without medium (rectum), tissue

(e.g. spleen, lymph node or bone marrow)

**Horse:** EB, tissue (liver)

Pig: swab without medium (genital tract), EB, tissue (e.g. abortion

material, sperm)

Method Realtime PCR

Droplet digital PCR (quantitative PCR dog)

PCR (ferret)

Species Dog, cat, ferret, horse, pig

Duration

Note

1 – 3 days

- PCR can be positive up to four weeks after vaccination with live vaccine.
- In dogs, differentiation between vaccine strain (CPV-2) and field strains (CPV-2a, CPV-2b, CPV-2c) is possible on request. Please note that when using parvovirus vaccines containing CPV-2b as vaccine antigen (e.g. Zoetis Versican Plus, Virbagen Puppy 2b) we cannot differentiate between vaccine strain and field strain.
- If vaccination was carried out using a vaccine with field strains or
  if the vaccine or vaccination status is unknown and vaccination
  was carried out shortly (less than 4 weeks) before the onset of
  the symptoms, quantitative PCR is recommended and can be
  requested following qualitative PCR. A very high pathogen load
  indicates an acute infection. If the infection has subsided, it is not
  possible to differentiate between the field strain and the vaccine
  strain even by qPCR; in both cases, only a low pathogen load is
  detectable.
- Direct detection of parvoviruses in the blood is possible approx.
   1 5 days after infection.
- In ferrets, rectal swabs lead to a much better sensitivity than faeces.

#### Parvovirus - Antigen Detection

MaterialFaecesMethodEIASpeciesDog, catDuration1 day

Note A faecal sample of the size of a pea is required.

False positive reactions may occur up to 5 – 12 days post vaccination

with live vaccine!

#### Parvovirus - Antibody Detection

Material **Dog, cat:** S, EP, HP 0.5 ml

Pig: S 1 ml

Method Dog, cat: IFAT

Pig: ELISA

Species Dog, cat, pig\*

Duration 1 day

Pig: 5 days

Note Seroconversion occurs 4 – 7 days after infection; vaccine and

infection titres can only be differentiated by testing serum pairs.

#### PBFD > see Circoviruses, p. 163

#### 13.1.36 Picornaviruses

Picornaviruses see also > Sacbrood Virus, p. 197

Picornaviruses are non-enveloped RNA viruses and in **tortoises** also known as **virus** "X". They are detected regularly in tortoises, and found associated with other infectious pathogens, particularly with herpesviruses and mycoplasma.

Clinically, picornavirus in young animals is associated with a softening of the carapace. In adult tortoises, infections are manifested as rhinitis, stomatitis, ascites and sudden death.

Picornaviruses can best be detected by PCR in pharyngeal swabs. Suitable organ samples include intestine, tongue, kidney, liver and other organs.

#### Picornavirus (Virus "X") - Pathogen Detection

Material Swab without medium (pharynx), tissue (e.g. intestine, tongue,

kidney, liver)

Method PCR
Species Tortoise
Duration 1 – 3 days

#### Picornavirus (Virus "X") - Antibody Detection

Material S. HP 0.2 ml

Method VNT
Species Tortoise
Duration 1 – 2 weeks

# 13.1.37 Polyomaviruses

Polyomaviruses are non-enveloped DNA viruses with a diameter of 45 nm and an icosahedral capsid (similar to papillomaviruses). Polyomaviruses are latently present in mammalian cells and mostly cause persistent infections there. They usually form typical intranuclear inclusions in infected cells and after infection of heterologous cells lead to their transformation. They are therefore regarded as oncogenic. Polyomaviruses have a circular double-stranded DNA.

The highly contagious **budgerigar fledgling disease virus (BFDV)** is considered the first **avian polyomavirus (APV).** BFDV causes an infection that may be fatal for psittacine nestlings; in adult birds, septicaemia and hepatitis are observed. In chronic cases, feather malformation and inability to fly occur; affected animals usually hop or run around. Particularly budgerigars are affected. The disease is also called **French moult.** 

#### Polyomavirus - Pathogen Detection

Material 2 – 3 freshly picked pinfeathers, blood (EB or 1 – 2 drops on a filter

paper), faeces, swab without medium (cloaca)

Method Realtime PCR

Species Birds
Duration 1 – 3 days

# 13.1.38 Porcine Respiratory and Reproductive Syndrome Virus (PRRSV)

Today, PRRS – also called swine infertility and respiratory syndrome (SIRS), porcine epidemic abortion and respiratory syndrome (PEARS), mystery swine disease (MSD), or blue ear disease – is among the world's most important diseases in pig production. In Germany, the disease first occurred in the winter of 1990/91.

The virus can spread very quickly through droplet and airborne infection. The disease is characterised by late abortions around the 110<sup>th</sup> day of gestation. Dead or weak piglets can be born. In addition, there may be respiratory tract infections.

#### PRRSV - Pathogen Detection

Material Swab without medium (nose or pharynx), EB, tissue (e.g. abortion

material, lung, trachea or lymph node), lavage (BAL), sperm

Method Realtime PCR

Species Pig

Duration 1 - 3 days

Note Detection by PCR allows for a reliable diagnosis and the

differentiation between EU and US strains (NA/HP-NA).

#### PRRSV - Antibody Detection\*

Material S 1 ml Method ELISA Species Pig Duration 5 days

# 13.1.39 Rabbit Haemorrhagic Disease Virus (RHDV)

Rabbit haemorrhagic disease (RHD), also known as **rabbit calicivirus disease** or **viral haemorrhagic disease**, is a highly contagious disease of European rabbits (Oryctolagus cuniculus). It occurs in both wild and domestic rabbits and causes peracute, acute or subacute diseases.



RHD is caused by caliciviruses, small, non-enveloped, single-stranded RNA viruses. Rabbit haemorrhagic disease virus (RHDV) is closely related to the European brown hare syndrome virus, which causes a similar disease in hares (Lepus spp.). There are several genetically and serologically different variants of RHDV. Until 2010, six different genotypes were known which cross-react serologically. These are called "classic" RHDV or RHDV-1. A new serotype, called RHDV-2 or RHDV-b, was first detected in France in 2010 and has since spread throughout Europe and other parts of the world. The disease caused by RHDV-2 is similar to that of classic RHDV strains but is associated with a slightly lower (but extremely variable) mortality rate. RHDV-2 can also infect some hare species and, unlike RHDV-1, also infects very young rabbits.

From January 2018 to September 2019, only 0.5% of Laboklin samples were RHDV/RHDV-1 positive, but 53.3% were RHDV-2 positive.

RHDV/RHDV-1 and RHDV-2 are mainly transmitted orally. Contaminated herbage can play a role here. Insects also act as mechanical vectors.

RHDV infections often progress peracutely; affected animals die suddenly or within a few days. Clinically, general signs are seen such as anorexia and lethargy, but also neurological symptoms such as opisthotonus, excitement, ataxia or paralysis. Conjunctivitis and respiratory symptoms such as dyspnoea and nasal discharge (possibly bloody) are also frequently observed. In some cases, an increased tendency to bleed can be observed. The chronic form of RHD only occurs in a small number of animals which then develop jaundice.

Hepatomegaly and splenomegaly are the most common pathologies. Histologically, acute necrotising hepatitis can be detected in affected animals. Bleeding and blood stasis in various organs are frequently observed.

In addition to the clinical examination and histopathology, RHD is mainly diagnosed by virus detection using realtime PCR. Due to the genetic differences between the RHDV strains, both RHDV/RHDV-1 and RHDV-2-specific methods must be used.

Treatment is not possible. A prophylactic vaccination is recommended. Several vaccines are available. It must be noted that vaccination should take place against both RHDV/RHDV-1 and RHDV-2. Currently, RHDV-2 cases are mostly observed in Germany, but classic RHDV strains still occur.

### RHDV 1 + 2 - Pathogen Detection

Material Swab without medium (conjunctiva), urine, faeces, EB, bone

marrow, tissue (e.g. liver)

Method Realtime PCR

Species Rabbit
Duration 1 – 3 days

#### 13.1.40 Rabies Virus

Rabies virus **(RABV)** belongs to the genus Lyssavirus of the family Rhabdoviridae and is globally distributed. In Germany, rabies is an **epizootic disease** that is **notifiable upon suspicion**. After intensive control measures, Germany has been considered free of classical rabies (RABV) since 2008.

When travelling, some countries demand proof of the antibody titre.

### Rabies Virus - Antibody Detection\*

Material S 1 ml
Method FAVN
Species Dog, cat
Duration 1 – 2 weeks

Control of vaccine titres, also for export.

Please request a special form if the test results are required for

export.

#### 13.1.41 Ranaviruses

Ranaviruses are enveloped double-stranded DNA viruses and belong to the family Iridoviridae. They are found worldwide and have a very wide host range infecting different animal species and even classes. Transmission is by direct contact, environmental contamination or cannibalism (or eating infected animals).

In **amphibians**, ranaviruses are increasingly detected and can cause systemic disease and mass mortality in these animals. A distinction is made between the haemorrhagic and the cutaneous form. Clinically, erythema, especially on abdomen and upper legs, ulceration on the toes, and increased tendency to bleed are seen. Some animals die without having appeared ill, while others can be inapparent carriers.

In **reptiles,** ranaviruses occur especially in chelonians, where they are associated with stomatitis, rhinitis, pneumonia and liver disease. In lizards, ranaviruses seem to have a role in skin lesions, stomatitis, granulomatous changes and mass mortality. The clinical symptoms of snakes are reported as stomatitis, granulomatous changes and liver inflammation.

Ranavirus is also found in **fish.** In fish, the infection can extend from clinically inapparent to systemic disease with mass mortality.

# Ranaviruses – Pathogen Detection

Material Chelonians: swab without medium (pharynx, cloaca),

tissue (liver, tongue, kidney, intestine, possibly skin),

possibly EB in box turtles

**Lizard:** swab without medium (pharynx, cloaca),

tissue (above all skin, liver)



**Snake:** swab without medium (pharynx), tissue (above all liver) **Amphibians:** biopsy (toe clips, tail clips), EB or drops of blood on filter paper, tissue (above all liver, kidney), perhaps swab

without medium (skin)

Fish: biopsy (gills), blood, tissue (above all liver, kidney),

perhaps swab without medium (skin)

Method **PCR** 

Species Reptiles, amphibians, fish

Duration 1 – 3 days

#### 13.1.42 Reoviruses

#### **Birds**

Reoviruses are non-enveloped, double-stranded RNA viruses that are regularly found in various bird species. They can cause inapparent infections but are also associated with various clinical changes, Liver and intestine are often affected. Respiratory infection also occurs. Especially in Old World parrots, lethal infections can be seen.

### Reptiles

Reoviruses are often found in snakes and lizards, but occasionally also in chelonians. In snakes and lizards, they are associated with respiratory symptoms, particularly pneumonia. However, they could also be involved in skin lesions (papillomatous changes) and enteritis. In turtles, reoviruses are associated with respiratory symptoms and stomatitis.

#### Reoviruses - Pathogen Detection

swab without medium (cloaca), faeces, Material Birds:

tissue (intestine, liver, heart, kidney, lung)

Reptiles: swab without medium (pharynx, cloaca), lung lavage,

tissue (lung, intestine; in chelonians also tongue)

Method PCR

Species Birds, reptiles Duration 2 – 4 days

#### 13.1.43 Rotaviruses

Group A rotaviruses are one of the most important pathogens of nosocomial gastroenteritis in veterinary and human medicine. In Germany, human rotavirus infections are among the most common gastrointestinal diarrhoeal diseases and are a notifiable infection. Rotaviruses are non-enveloped and therefore very environmentally stable viruses. Rotaviruses are transmitted both via the faecal-oral route and airborne. The destruction of enterocytes and electrolyte imbalances lead to diarrhoea and dehydration. Detection is done from faeces; the virus antigen is detected by ELISA.

#### **Rotavirus - Antigen Detection**

Material Faeces **ELISA** Method

Dog. cat. horse, cattle Species

Duration 1 – 2 days

Note A faecal sample of at least the size of a pea is required.

#### 13.1.44 Sacbrood Virus

Sacbrood virus is an RNA virus of the picornavirus family. This virus only affects bee brood: infected adult animals do not show any symptoms. Transmission occurs through the bees that take up the virus when removing dead larvae and afterwards excrete it again through the hypopharyngeal glands when feeding. The virus can survive winter dormancy in the salivary glands.

Infected larvae die shortly before pupation and become small, fluid-filled "sacs" which later dry out and become scab. The brood pattern shows sunken caps, Sacbrood is considered a so-called secondary infection, since the disease usually only takes on a severe course if the colony has been weakened by a primary infection, such as varroosis. For the therapy of the swarm, affected combs can be removed and melted. Transmission occurs through the bees themselves or through the beekeeper.

#### Sacbrood Virus - Pathogen Detection

Material Bee larvae PCR Method Bees Species Duration 1 – 3 davs

### 13.1.45 SARS-CoV-2

Respiratory coronavirus, first detected in 2019 and then temporarily referred to as "2019-nCoV", is now better known as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) or "COVID-19 virus". According to current knowledge, all mammals can be infected with this virus, and cats, lagomorphs, hamsters and ferrets are particularly susceptible to infection with SARS-CoV-2 and have a higher probability of developina clinical sians.

Signs of SARS-CoV-2 infection can range from nasal discharge to sneezing, extensive inflammation of the respiratory tract up to diarrhoea. However, non-specific signs such as lethargy or weight loss have also been described. In Germany, there is an obligation to notify the authorities!

#### SARS-CoV-2 - Pathogen Detection

Material Swab without medium (deep nasal/pharyngeal swab), BAL

faeces (only great apes)

Method PCR

Species All species, particularly cat, ferret, hamster

Duration 1-3 days

#### SARS-CoV-2 - Antibodies

Material S 0.5 ml

Species Dog, cat and other species (except rabbit)

Duration 3 days

Note Detection of IgG

#### 13.1.46 Sendai Virus

Sendai virus, also known as **murine parainfluenza virus 1,** causes infections in rabbits, guinea pigs, hamsters, rats and mice as well as in humans. An introduction into a population leads to severe respiratory symptoms (focally ulcerative/necrotising rhinitis/tracheitis, pneumonia and pleuritis) and mortality rates of up to 100%, especially in mice. If the infection persists in a population, the course of infection is milder or subclinical. Once the infection is overcome, antibodies are detectable throughout life.

#### Sendai Virus - Antibody Detection\*

Material S 0.5 ml Method IFAT

Species Rabbit, guinea pig, rat, mouse, hamster

Duration 3 – 5 days

#### 13.1.47 Sunshinevirus

Sunshine virus is a novel paramyxovirus (PMV), which was first detected in pythons in 2012 in Australia. Sunshine virus is only distantly related to Ferlavirus (formerly known as reptile PMV or snake PMV). It has been found in animals with respiratory and/or neurological symptoms, but can occasionally be detected even in clinically healthy animals. Initial studies show that this virus may also be present in European pythons.

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# Sunshinevirus - Pathogen Detection

Material Swab without medium (pharynx, cloaca), tissue (lung, brain)

Method PCR
Species Python
Duration 1 – 3 days

# 13.1.48 Tick-borne Encephalitis Virus (TBEV)

**Tick-borne encephalitis (TBE)** is caused by an arbovirus. Arboviruses are an inhomogeneous group of viruses whose common feature is the transmission by blood-sucking arthropods. Like the West Nile virus, the TBE virus (TBEV) belongs to the genus Flavivirus and is transmitted by ticks.

In **dogs**, the disease was first described in 1972. Seroepidemiological studies conducted since then have shown that dogs have relatively frequent contact with TBEV (up to 30% in certain areas) without contracting the disease. If the disease is contracted, the symptoms in dogs are a multifocal occurrence involving the cerebrum, brain stem and sometimes also the spinal cord. The disease usually begins acutely to peracutely with a highly elevated body temperature (up to over 41 °C) and a further rapidly progressive course. Changes in behaviour, from being apathetic to overexcited or aggressive, gait disorders up to tetraparesis/tetraplegia and seizures can occur. Various brain nerve deficits are observed, e.g. facioplegia, strabismus, nystagmus, miosis, missing menace reflex. Hyperalgesia in the head and neck area as well as a generally increased painfulness are characteristic. A large part of the disorders ends lethally or by euthanasia within one week. Recently, there have been more and more literature reports on dogs with a chronic course of the disease that have survived. Sometimes slight neurological signs remained, sometimes the dogs were fully recovered.

Diagnosis should be confirmed serologically by antibody detection using ELISA. However, it must be taken into account that the antibodies could be the result of a previous subclinical infection. Antibodies may also appear in the CSF within the first week after infection and can be detected by ELISA.

In the peracute form, PCR can be used to try to detect the virus in the CSF. Due to the very rapid virus elimination from the brain, however, this is only possible in the early phase of infection. **Virus detection** by PCR from a collected **tick** is possible and especially useful if a person is affected by a tick.

TBE is also increasingly detected in neurologically affected **horses**. The clinical picture is similar to the disease caused by the West Nile virus.

#### Tick-borne Encephalitis Virus - Pathogen Detection

Material CSF 0.2 ml, S 0.2 ml, tick

Method Realtime PCR

Species Dog, horse and others

Duration 1 - 3 days

Note Detection from serum (before seroconversion) or cerebrospinal fluid

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is only possible in the early phase of the infection.

# Tick-borne Encephalitis Virus – Antibody Detection

Material S, HP, CSF 0.5 ml

Method ELISA

Species Dog, cat, horse

Duration 3 days

Note

Three different tests are offered: detection of IgM in serum or

detection of IgG in serum or detection of IgG in CSF.

Performing the analysis is advisable if animals have been to

endemic areas and show neurological signs.

#### 13.1.49 West Nile Virus

West Nile virus **(WNV)** is an RNA virus of the family Flaviviridae, which is endemic in various, mainly tropical regions of the world. Yet, through migratory birds, WNV also regularly occurs in non-tropical areas further north and has been detected in Germany since 2018.

Transmission primarily takes places through mosquitoes (mostly species of Culex) between wild birds. However, the mosquitoes can also transmit WNV to people, horses and other mammals. Due to the low viral load, horses and humans are dead-end hosts and do not represent a source of the virus for mosquitoes.

The incubation period for WNV encephalitis in horses is 3-15 days. Most infections are subclinical, only a small percentage of horses develops neurological signs, such as stumbling, hind-leg paralysis, ataxia, tremor or weakness up to recumbency of the animals.

In birds, infections vary from asymptomatic to lethal, depending on the species.

Passerine birds, birds of prey and owl species are most susceptible to the disease.

They may develop severe epidemics with central nervous signs (e.g. vertigo, tremor, inability to fly) and death rates may increase.

In Germany, the disease is **notifiable upon suspicion** in birds and horses.

# West Nile Virus - Pathogen Detection

Material Birds: swab without medium (oropharynx, cloaca), tissue (e.g. brain,

heart, kidney)

Horse: CSF, EB, tissue (e.g. brain, spleen, tonsils)

Method Realtime PCR Species Birds, horse Duration 1 – 3 days

Note The very short viraemia phase (1 – 3 days) ends shortly after or

before the onset of clinical signs.

#### **West Nile Virus - Antibody Detection**

Material Birds: S 0.5 ml; horse: S 1 ml

Method ELISA
Species Birds, horse
Duration Birds: 5 days

Horse: 2 – 3 days

Note • Birds: Detection of IgG

• Horse: Detection of IgM and IgG

# 13.2 Bacteria

Please note: If the detection of a bacterial pathogen is exclusively done by **PCR**, it is **not possible to create an antibiogram**.

# 13.2.1 Actinobacillus pleuropneumoniae (APP)

Actinobacillus pleuropneumoniae is a gram-negative bacillus. It produces exotoxins that can destroy erythrocytes and lung macrophages. The clinical picture of APP infection is characterised by severe respiratory symptoms and significant impairment of the general condition (body temperature may rise up to 42 °C). In intensive pig farming, pleuropneumonia caused by A. pleuropneumoniae is one of the most important infectious diseases. In peracute courses of the disease, it can lead to the death of the animals within 24 hours.

#### **APP – Pathogen Detection**

Material (1) Swab with medium, tissue (lung, tonsils)

(2) Swab without medium (nose), nasal flush, tissue

Method (1) Bacteriological culture

(2) PCR\*

Species Pig

Duration (1) 1 - 3 days

(2) 7 - 14 days

### APP - Antibody Detection\*

Material S, HP 0.5 ml
Method ELISA
Species Pig
Duration 5 days

Actinomyces > see Chapter 14.4, p. 271

# 13.2.2 Anaplasma

Based on genetic analyses, the former species Ehrlichia phagocytophila, Ehrlichia equi and the causative agent of human granulocytic ehrlichiosis (HGE) were unified in the new species Anaplasma phagocytophilum. In addition, infection with Anaplasma platys, the causative agent of infectious canine cyclic thrombocytopenia, plays an increasingly important role in Europe, too.

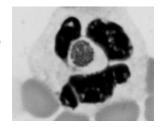
### Anaplasma phagocytophilum

Anaplasma phagocytophilum is a gram-negative, obligate intracellular bacterium, which particularly infects neutrophil granulocytes and forms, when multiplying within the granulocytes, typical inclusion bodies, so-called morulae. In Europe, the main vector is lxodes ricinus. Deer, mice and other rodents are reservoir hosts.

The clinical signs are similar to those of ehrlichiosis, but thrombocytopenia can be observed more often, mainly because of the formation of anti-thrombocyte antibodies. Anaplasma infections can be asymptomatic, can cause non-specific symptoms (fever, inappetence, apathy) or severe symptoms (CNS disorders). In dogs, orthopaedic problems (myositis, joint swellings, lameness) are often associated with Anaplasma infections.

In **horses**, fever, apathy, limb oedema and reluctance to move are initially dominant. Horses older than 4 years show more obvious signs than younger animals. Once the infection is overcome, a resilient immunity is acquired for about 2 years.

In **ruminants**, Anaplasma phagocytophilum can cause **tick-bite fever**. Most infections progress subclinically, but fever and productivity loss or abortions are also possible. Severe cases occur when non-immune animals are introduced into endemically contaminated areas.



Neutrophil granulocyte with Anaplasma phagocytophilum (morula) in the middle of the segmented nuclei (Diff-Quik, 1000x magnification)

# Anaplasma phagocytophilum - Pathogen Detection

Material EB 0.2 ml, bone marrow, synovia, CSF 0.2 ml, tick

Method Realtime PCR

Species Dog, cat, horse, cattle, others

Duration 1-3 days

Note PCR is positive in blood smears 6 to 8 days before and 3 days after

the formation of morulae.

Similar to an infection with Ehrlichia canis, persistent infections in the bone marrow, spleen and liver are considered for Anaplasma phagocytophilum. Thus, a negative PCR result does not completely

rule out an infection.

#### Anaplasma phagocytophilum - Antibody Detection

Material S, EP, HP 0.5 ml

Method IFAT; dog: ELISA (IFAT only on specific request)

Species Dog, cat, horse, cattle

Duration 1 day

#### Anaplasma platys

Anaplasma platys (formerly Ehrlichia platys) is an obligate intracellular, gram-negative bacterium in dogs which multiplies in platelets and leads to cyclic thrombocytopenia and bacteraemia with intervals of approximately 14 days. The disease is called **infectious canine cyclic thrombocytopenia.** Descriptions of this species of Anaplasma come from overseas, but the pathogen is also detectable in the Southern Mediterranean (North Africa, southern Portugal, Andalusia, Sicily, southern Italy, southern Greece). It is transmitted through ticks (Rhipicephalus sanguineus). After the initial infection, there is a decrease in the platelet count within 7 days p.i.; the lowest values are reached between days 14 and 24 p.i.

Basophil inclusions (morulae) in the platelets can particularly be detected 7 – 10 days p.i. The phase of bacteraemia extends approximately over a period of 4 – 14 days p.i., followed by a phase in which the pathogen cannot be detected in the peripheral blood. Subsequently, these phases alternate cyclically depending on the platelet count. In the bacteraemic phase, the pathogen can be detected in blood samples by means of PCR.

#### Anaplasma platys - Pathogen Detection

Material EB 0.2 ml, tick Method Realtime PCR

Species Dog Duration 1 – 3 days

### 13.2.3 Bartonella henselae

Bartonella are gram-negative, facultative intracellular bacteria which are transmitted by fleas and ticks. Bartonella henselae is mostly known as the causative agent of "cat scratch disease" in humans. Infections in cats are predominantly subclinical. Fever, muscular pain, local lymphadenopathy and, rarely, also neurological symptoms can occur, which usually disappear again after a few days. Recently, the involvement of Bartonella henselae in gingivitis and stomatitis in cats has been discussed more frequently. Pathogen detection and antibody detection often do not match and a definitive diagnosis is linked to the detection of the pathogen. A negative PCR result does not exclude an infection with B. henselae and should be repeated in case of clinical suspicion.

**Dogs**, too, can occasionally be affected by Bartonella infection. The disease can cause endocarditis, recurrent granulomatous lymphadenitis, systemic granulomatous processes and meningitis.

Bartonella henselae - Pathogen Detection

Material EB 0.2 ml, CSF 0.2 ml, swab without medium (oral cavity), flea

Method Realtime PCR Species Dog, cat Duration 1 – 3 days

#### Bartonella henselae - Antibody Detection

Material S, EP, HP 0.5 ml

Method IFAT
Species Dog, cat
Duration 1 day

Note Antibodies can usually be detected from the second week p.i.

onwards. Seroprevalence is particularly high in cats with flea infestation and is not indicative of a clinical condition. The direct

detection of the pathogen by PCR is preferable.

# 13.2.4 Bordetella bronchiseptica

Bordetella are small gram-negative bacilli which can move by means of flagella. B. bronchiseptica usually only survives for a rather short period of time outside the respiratory tract. Transmission takes place by direct contact or via aerosols.

Because of its toxins, B. bronchiseptica particularly damages the cilia-bearing cells of the respiratory mucosa and it can persist in the respiratory tract for up to three months. The pathogen is not host-specific and can be transmitted from one animal species (e.g. dog) to another (e.g. cat) and also to humans (zoonosis!).

In dogs, Bordetella are known as a component of kennel cough and they are also responsible for respiratory tract diseases in cats, although coughing is not a characteristic symptom in cats. Typical signs are fever, sneezing, nasal discharge, swelling of the submandibular lymph nodes and intensified breath sounds. Usually, only mild symptoms occur which disappear again after about 10 days. However, life-threatening bronchopneumonia can develop in young kittens.

# Bordetella bronchiseptica – Pathogen Detection

Material (1) Swab must be with medium (Amies) (nose, pharynx)

or bronchial secretion

(2) Swab without medium (nose, pharynx), bronchial secretion, BAL

Method (1) Bacteriological culture (MALDI-TOF)

(2) Realtime PCR

Species Dog, cat, rabbit, cattle, sheep, goat, pig, others

Duration (1) 2 - 3 days

(2) 1 - 3 days

Note When requesting a culture test, please clearly indicate on the

submission form that Bordetella bronchiseptica should be tested, as

special culture media are required.

#### 13.2.5 Borrelia

Borrelia are bacteria which belong to the spirochaete family. Spirochaetes are characterised by contractile axial filaments which are located under a multi-layered outer membrane and that give the spirochaetes their typical spiral shape as well as their motility. Borrelia species which are discussed in connection with Lyme borreliosis in dogs are included in the group Borrelia burgdorferi sensu lato, which currently comprises more than 20 different Borrelia species.

Borrelia are transmitted by vectors (ticks or lice) and except for B. recurrens and B. dutonii they all have a reservoir among wild animals.

The main mode of transmission is a bite of the tick lxodes ricinus (European castor bean tick). The bacteria are located in the intestine of the tick, are activated by the blood meal and migrate to the salivary glands. It then takes up to 24 hours until transmission via the saliva takes place. If the tick is properly removed within this period, the risk of infection can be greatly reduced.

In contrast to humans, the clinical signs of Lyme borreliosis (**Lyme disease**) in dogs are rather non-specific and can easily be overlooked. In dogs, there is rarely an erythema migrans. Fatigue, loss of performance, possibly fever and, after a symptom-free phase of several weeks, reluctance to move, alternating lameness, emaciation, vomiting and oedema occur. Occasionally, neurological deficits are also observed. A serious complication is the development of glomerulonephritis with subsequent kidney failure due to the deposition of immune complexes.

The main vector, Ixodes ricinus, occurs throughout Germany but can be found more frequently in certain areas. In such areas, it is therefore recommended to regularly check for any infestation of the dog with ticks and to have a Lyme disease test carried out if the symptoms mentioned above occur.

Infections and diseases in **cats** and cattle are reported more and more often. Furthermore, Lyme disease is classified as an emerging bacterial zoonosis. Grazing animals are often used for blood meals by borrelia-infected ticks. Clinical diseases appear as well as seropositive animals without any clinical signs, with the evaluation often being difficult.

In **horses**, a variety of signs are associated with borrelia: reduced performance, lameness, changes in the skin, eyes or heart up to neurological deficits and abortions. However, there is still controversy as to whether the infection in horses leads to any clinical signs at all.

Lyme disease in **cattle** is associated with lameness, weight loss and abortion. Pathogen isolation from clinical material is sometimes successful (Borrelia burgdorferi sensu stricto, Borrelia afzelii). Seroconversions have been shown as well as the response to tetracycline therapy.

### **Borrelia - Pathogen Detection**

Material Tick, biopsy (skin), synovia, CSF 0.2 ml

Method Realtime PCR

Species Dog, cat, horse, cattle, sheep, goat, others

Duration 1 - 3 days

Note The diagnostic value of a PCR is limited by the selection of the

appropriate material and the concentration of pathogens. During a chronic infection, pathogen spread can be suspected in many sites, but the concentration of pathogen DNA can be very low and therefore the PCR produces a negative result. While a positive PCR is proof of infection, a negative PCR does not exclude an infection.

### Borrelia – Antibody Detection

Material S, EP, HP 0.5 ml
Method IFAT; dog: ELISA
Species Dog, cat, horse, cattle

Duration 1-2 days

Detection of IgG and, in dogs, cats and horses, IgM as well.

 Positive IgG antibody titres are found in dogs after about 3 – 6 weeks, positive IgM antibodies 3 – 4 days after pathogen contact.

 The distinction between IgM and IgG is used to distinguish an acute infection from a pathogen contact that occurred a long time ago.

After consultation, detection in dogs can also be done using IFAT.

#### Borrelia - Blot

Material S, HP 0.5 ml

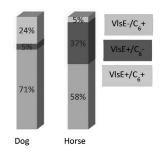
Method Line Immunoassay/western blot

(IgG antibody detection)

Species Dog, horse Duration 1 day

Note The Borrelia blot is used to clarify

numerous questionable antibody titres and to distinguish between vaccine and infection antibodies. The blot also detects antibodies against the VIsE protein and synthetic C6 peptide. VIsE (variable major protein-like sequence, expressed) and its subunit C6 are highly immunogenic surface antigens that express Borrelia during active reproduction. There is no recombination of the VsIE molecule in vitro or in the Borrelia residing in the tick, the detection



VIsE and C<sub>6</sub> antibodies in dogs and horses

(C<sub>6</sub>- and/or VIsE-positive animals)

→ No antibody detects all infections.

of antibodies against the VsIE molecule therefore indicates an infection that has previously occurred. The blot can be carried out at the earliest from the 3<sup>rd</sup> week after

infection onwards.

# 13.2.6 Brachyspira

Brachyspira are gram-negative anaerobic bacteria which, however, have a certain tolerance to oxygen. Reproduction takes place in the goblet cells of the large intestine where Brachyspira can persist after surviving infection (intermittent shedding!). **Pig dysentery,** which is caused by B. hyodysenteriae, is a highly contagious multifactorial diarrhoeal disorder that leads to high economic losses in pig production worldwide. The main sources of infection are infected pigs without clinical signs and rodents as reservoir hosts. B. pilosicoli causes a milder disease, **spirochete diarrhoea** in pigs, which usually occurs directly after weaning.

### Brachyspira hyodysenteriae/pilosicoli – Pathogen Detection

Material Faeces, tissue (large intestine)

Method Realtime PCR

Species Pig Duration 1 – 3 days

Note Differentiation between B. hyodysenteriae and B. pilosicoli is done

by PCR.

#### 13.2.7 Brucella

The causative agent of brucellosis are gram-negative, aerobic bacilli of the genus Brucella. In Germany, brucellosis in cattle, pigs, sheep and goats is **notifiable upon suspicion**. The disease occurs in both animals and humans. Several Brucella species are known, including B. canis (canine brucellosis), B. abortus (bovine brucellosis), B. melitensis (ovine and caprine brucellosis), B. ovis (brucellosis in rams, infectious epididymitis, also notifiable upon suspicion) and B. suis (porcine brucellosis). Host-specificity of Brucella species is only limited.

Brucella canis is transmitted genitally or via the oral route by latently infected animals. After 2 to 4 weeks, bacteraemia develops. In pregnant female dogs, there may be abortions in the last trimester of gestation or weak puppies are born. Male dogs suffer from inflammation of the testicles and epididymis and can become infertile. A rare sign of Brucella canis infection is discospondylitis, so if there is pain in the spine and lameness, especially in dogs from Southeastern Europe, this infection may be an important differential diagnosis. The main signs of brucellosis in ruminants are abortions, birth of weak animals, inflammation of the testicles and epididymis, and infertility. In humans, the infection leads to fever, fatigue, night sweats, headaches and feelings of cold. The occurrence of cases in humans is always related to the disease being present in domestic or wild animals. Apart from direct animal contact, routes of infection also include the consumption of insufficiently heated food (e.g. raw milk or raw milk cheese) obtained from infected animals.

# Brucella canis – Pathogen Detection

Material Swab without medium (cervix, prepuce), EB 0.2 ml, sperm, urine,

(faeces, milk), tissue (abortion material)

Method Realtime PCR

Species Dog
Duration 1 – 3 days

# **Brucella canis - Antibody Detection**

Material S 1 ml Method (1) IFAT

(2) Agglutination Test

Species Dog Duration 1 day

Note Agglutination tests are usually required for entry into and departure

from non-European countries.

#### Brucella abortus and Brucella mellitensis – Antibody Detection

Material S, HP 0.5 ml

Method ELISA

Species Cattle, sheep, New World camelids

Duration 3 days

#### **Brucella suis - Antibody Detection**

Material S, HP 0.5 ml
Method ELISA
Species Pig
Duration 3 days

#### 13.2.8 Burkholderia mallei

**Glanders** is a disease of Equidae caused by Burkholderia mallei, which also has zoonotic potential: Apart from humans, wildcats (zoos!), camels, bears, wolves and dogs are susceptible as well. Cattle, sheep and pig are resistant. The course of the disease is either acute (especially in donkeys and mules) with high fever and respiratory symptoms and death after a few days. Or it is rather chronic, particularly in horses, with nodules and ulcerations on the skin, the mucous membrane and the inner organs. Chronically and subclinically infected animals represent a dangerous source of infection. All secretions of the respiratory tract and the skin are infectious; the incubation period ranges from a few days to many months.

In Europe, glanders is considered eradicated, but it does occur in different Asian, African and South American countries (export-relevant test).

In Germany, there is an obligation to inform the authorities!

# Burkholderia mallei (Glanders) – Antibody Detection\*

Material S 1 ml
Method CFT
Species Equidae
Duration 5 days

# 13.2.9 Campylobacter

Several Campylobacter species could be detected in mammals, birds and also in humans. Some species are part of the normal gastrointestinal microbiota or their pathogenicity is still unclear.

In **cattle**, C. fetus subsp. veneralis causes epidemic abortions and fertility disorders (bovine genital campylobacteriosis, also called vibriosis in cattle; in Germany,

**notifiable upon suspicion**). C. jejuni can lead to diarrhoea or mastitis. In **sheep**, C. fetus subsp. fetus is known as pathogen of the enzootic campylobacter abortion; occasional abortions caused by C. jejuni have also been described. The importance of campylobacter infections in **birds** lies in the contamination of carcasses and the risk of food poisoning associated with it. Most frequently, birds are infected with C. jejuni. Diarrhoea or hepatitis are rare.

In **dogs and cats**, C. jejuni can often be isolated from healthy animals, but can cause diarrhoea especially in young animals. This diarrhoea is often self-limiting. Feeding a barf diet presents a risk of infection.

In **humans**, campylobacter (especially C. jejuni) is one of the most common causes auf bacterial diarrhoea and is usually food-associated (particularly insufficiently heated poultry meat, but also unpasteurised milk and raw minced meat). Rare complications which can occur are Guillain-Barré syndrome (polyradiculitis) and reactive arthritis. Campylobacter of the species C. jejuni, C. coli, C. lani and C. upsalensis are collectively called thermophilic campylobacter. In Germany, campylobacteriosis (thermophilic campylobacter) is **notifiable upon diagnosis** in dogs, cats, ruminants and poultry.

Campylobacter -	Pathogen	Detection
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Material (1) Faeces, swab with medium (intestine, cloaca)

(2) Faeces, swab without medium (intestine, cloaca)

Method (1) Bacteriological culture (MALDI-TOF)

(2) Realtime PCR (only detection of Campylobacter jejuni)

Species No limitations known

Duration (1) 2 - 3 days

(2) 1 - 3 days

Note

- Culture: please send in a faecal sample with a diameter of at least the size of a cherry, otherwise use swab with medium; for PCR: swab without medium.
- A combined detection by culture of Campylobacter and Yersinia is also available.
- Resistances are common; treatment should therefore only be carried out after an antibiogram has been performed. Preparation of an antibiogram is only possible after a culture test.

# 13.2.10 Candidatus Neoehrlichia mikurensis

Officially named in 2004, Candidatus Neoehrlichia mikurensis is an obligate intracellular, gram-negative bacterium. The pathogen is characterised by endotheliotropism but has not been cultivated in vitro so far and thus could not be completely described yet. Cand. N. mikurensis was first found in common rats on the Japanese island of Mikura. It is assumed that small mammals, such as mice and rats, serve as reservoir; transmission most likely occurs through ticks. In recent years, Cand. N. mikurensis has been detected in about 2 to 25% of Ixodes ricinus ticks in Germany.

Since 2007, this pathogen has been associated with diseases in humans. Especially the elderly and immunocompromised people have been affected by the so-called neoehrlichiosis, including two patients from Germany. The symptoms are non-specific, most often high fever and headaches as well as muscular pain and joint pains were seen. The occurrence of vascular complications, like deep vein thromboses, pulmonary embolism and arterial aneurysms, was most noticeable. Laboratory findings particularly indicate an increased level of C-reactive protein, leukocytosis with neutrophilia and anaemia.

In dogs, so far only one single case has been reported in which this bacterium could be isolated. It was an eight-year old female Irish Setter after ovariohysterectomy and mastectomy. Postoperatively, she was lethargic and developed profuse subcutaneous bleeding (Diniz et al. 2011).

#### Candidatus Neoehrlichia mikurensis - Pathogen Detection

Material EB 0.2 ml, tick, tissue (e.g. spleen, kidney, liver)

Method Realtime PCR
Species Dog, tick
Duration 1 – 3 days

# 13.2.11 Chlamydia

Chlamydia are obligate intracellular, gram-negative pathogens. Extracellularly, chlamydia do not have their own metabolism and depend on the enzyme activity in the host cell. Chlamydia relevant in veterinary medicine belong to the family Chlamydiaceae. Some years ago, this family was split into the two genera Chlamydia and Chlamydophila. However, based on recent genetic analyses, this classification is no longer considered justified. Because of this, the single term Chlamydia is used here.

# Dog

In literature, only little data is available on chlamydia infections in dogs. Yet in general, its occurrence must be expected in Europe. Respiratory signs up to bronchopneumonia seem to be dominating here. At the onset of the disease, only progressive loss of condition may appear. High fever can develop. When the disease progresses, central nervous disorders are possible. Other manifestations of chlamydiosis in dogs are conjunctivitis and keratitis. Its involvement in keratitis superficialis chronica in German Shepherd dogs is being discussed.

#### Cat

Originally identified as the causative agent of "feline pneumonitis", C. felis is rather associated with conjunctivitis in cats nowadays. The cardinal symptom is serous conjunctivitis which begins unilaterally and then spreads to the other eye after some days. Especially when there is a secondary bacterial infection, the discharge can become mucopurulent. Chemosis and blepharospasm may also be present. In severe cases, follicular hyperplasia develops or even keratoconjunctivitis with corneal



ulcerations. Conjunctivitis can last for 8 weeks or more. Other acute symptoms include slight rhinitis and fever. Animals between 5 weeks and 9 months of age are most affected, though conjunctivitis neonatalis has also been described. In that case, kittens already suffer a severe conjunctivitis when opening their eyes, often due to a chlamydia infection acquired intrapartum. Transmission occurs directly through conjunctival secretions. Persistent infections are possible, and in some animals also respiratory symptoms may last for several weeks. When the immune system is weakened, the infection can be reactivated.

#### **Birds**

In our birds, infections with chlamydia are of particular significance. Infection rates of 10 to 40% may be prevalent in aviculture. As many birds have a carrier status, the disease can "suddenly" become clinically apparent under stress. Symptoms in birds are manifold and extremely non-specific. Ruffled feathers, apathy and lack of appetite must be mentioned. Basically, every "sick bird" could have a chlamydia infection. Respiratory symptoms with or without conjunctivitis are often seen, but central nervous disorders are also possible. The extent of clinical signs largely depends on the animals' condition; the type of symptoms also varies from one bird species to another. Sudden deaths without prior illness might happen. It is therefore not possible to make a diagnosis based on clinical signs. To make a reliable diagnosis, the identification of the pathogen is always necessary. C. psittaci is a zoonotic agent. Infections in humans are normally airborne, resulting in a flu-like disease. In Germany, it is a **notifiable** disease.

# Reptiles and amphibians

Various species of chlamydia are regularly detected in reptiles and amphibians. In reptiles, they have been associated with granulomatous changes in different tissues as well as with pneumonia, enteritis, hepatitis and myocarditis. In amphibians, they were found in cases of systemic disease.

#### Farm animals

In Germany, chlamydiosis in cattle, sheep and goat, as well as in poultry is a **notifiable** disease.

#### Chlamydia - Pathogen Detection

Material Dog, cat and others: swab without medium (eye, pharynx, cervix,

prepuce), abortion material

**Birds:** triple swab without medium (eye + pharynx + cloaca),

tissue (liver, spleen, lung), if need be faeces

Reptiles: swab without medium (pharynx), lung lavage, tissue

(lesions, lung, liver, spleen, intestine, heart)

Farm animals: swab without medium (eye, nose, cervix), tracheal

lavage, milk, faeces, tissue (lung, liver), abortion material

Amphibians: swab without medium (pharynx), tissue (lesions, lung,

liver, spleen, intestine, heart)

Method Realtime PCR

Species All

Duration 1 - 3 days

Note Pathogen detection identifies all chlamydia of the family

Chlamydiaceae. In case of a positive result in birds, a PCR specific to

C. psittaci is automatically performed.

#### Chlamydia - Antibody Detection

Material S, EP, HP 0.2 ml

Method IFAT

Species Dog, cat, birds, horse, cattle

Duration 1 day

Note Serology can determine whether an infection has occurred.

However, evidence of active shedding can only be provided by

pathogen detection.

#### 13.2.12 Clostridia

Clostridia are obligate anaerobic, gram-positive, spore-forming bacilli. Pathogenic Clostridia cause infectious and intoxication diseases; the latter through enterotoxins and neurotoxins.

# Clostridium botulinum Neurotoxin – Antibody Detection\*

Material S 1 ml Method ELISA

Species Horse, cattle, other farm animals on request

Duration 10 days

Note Botulism is regarded as pure intoxication, in which only the

toxin is absorbed, reabsorbed via the intestine and spread haematogenously. If, in exceptional cases, botulism progresses as a toxin infection, the toxins are formed in the intestine (visceral botulism) or in wounds (wound botulism). The absorption of

botulinum toxin leads to paralysis of the motor nerves.

#### Clostridioides difficile Toxin A and B

Material Faeces Method ELISA

Species No limitations known

Duration 1 – 2 days

Note Determination is particularly indicated in case of colitis.

Clostridium perfringens Enterotoxin

Material Faeces Method ELISA

Species No limitations known

Duration 1 - 2 days

Note Determination is particularly indicated in case of colitis.

In **carnivores**, Clostridium perfringens enterotoxin can cause diarrhoea and vomiting of varying severity; enterotoxaemia is rare. Toxin formation is induced by antibiotic administration, stress, coinfections or especially by an unbalanced diet rich in proteins and

connective tissue.

It plays an increasing role in **farm animals**, especially causing serious disease in calves, lambs (lamb dysentery) or suckling piglets (necrotising enteritis). Older animals are affected by clostridiosis (cattle), pulpy kidney disease (sheep), struck (sheep) or sporadic

catarrhal and haemorrhagic enteritis (pig).

#### Clostridium tetani - Antibody Detection

Material S 2 ml Method ICA

Species Dog\*, horse
Duration Dog: 7 days

Horse: 1 – 2 days

Note For semi-quantitative determination whether a horse has been suf-

ficiently vaccinated.

Detection of further Clostridia on request.

# 13.2.13 Corynebacterium pseudotuberculosis

Corynebacterium pseudotuberculosis is a gram-positive bacterium that belongs to the group of actinomycetes. It is the causative agent of pseudotuberculosis in sheep and goats, which is associated with abscessed lymph nodes and which is widely distributed. Pseudotuberculosis can also be transmitted to other animal species and to humans

#### Corynebacterium pseudotuberculosis – Pathogen Detection

Material Swab with medium

Method Bacteriological culture (MALDI-TOF)

Species Sheep, goat, others

Duration 2 – 3 days

Note For this test, please order the service "Bacteriology".

#### Corynebacterium pseudotuberculosis – Antibody Detection\*

Material S 1 ml Method ELISA

Species Sheep, goat, New World camelids

Duration 1 week

#### 13.2.14 Coxiella burnetii

Coxiella burnetii is an obligate intracellular, gram-negative bacterium and the pathogen that causes **Q fever.** It is highly infectious, even a small amount of pathogens is sufficient to establish an infection.

Coxiella burnetii is spread worldwide and has a large host range, e.g. ruminants, dogs, cats, rodents and birds as well as humans (zoonosis!). An infection in humans is often subclinical but clinically non-specific severe influenza-like symptoms can occur. Furthermore, chronic forms with endocarditis, hepatitis or CNS involvement are described. Especially persons who are in contact with ruminants (e.g. vets, farmers, butchers) are affected.

In ruminants, Coxiella burnetii replicates in the female genital tract and in the utter. It is intermittently or persistently secreted by uterus secretion, amnion fluid and abortion material, but also by urine, faeces or milk. During replication, small spore-like permanent forms are produced, which can survive in the environment for a very long time. Transmission occurs mostly by inhalation of pathogenic dust, but also by direct contact with infected animals. Ticks have also been found to be vectors of Coxiella burnetii, whereby the tick faeces are infectious.

If clinical signs are seen in animals, they include post-natal complications, metritis, foetal death, late abortions, stillbirths with subsequent infertility or birth of weak calves. In Germany, it is a **notifiable disease** in cattle, sheep, goats and other ruminants!

### Coxiella burnetii - Pathogen Detection

Material Swab without medium (reproductive tract), abortion material, milk,

faeces, urine

Method Realtime PCR

Species Mainly ruminants, but also other species

Duration 1 - 3 days

Note As this is a zoonotic disease, the education of the animal owner and

the practice staff on the zoonotic risk is advisable.

## Coxiella burnetii - Antibody Detection

Material S, HP 0.5 ml

Method ELISA

Species Dog, cat, horse, cattle, sheep

Test frequency 1 x per week

Dermatophilus congolensis > see Chapter 14.4, p. 272

E. coli, eae/enteropathogenic > see Chapter 16.1.2, p. 287

#### 13.2.15 Ehrlichia

Infections with ehrlichia occur worldwide. Ehrlichia are gram-negative, obligate intracellular bacteria belonging to the order Rickettsiales and are transmitted by ticks. Depending on the region, the tick species differ and thus also the species of ehrlichia. Whereas in Mediterranean countries and tropical as well as warmer areas. Rhipicephalus sanguineus, the main carrier of E. canis, is prevalent, Ixodes ricinus is found in Central and Northern Europe, However, if R. sanguineus is introduced into Germany, it can survive in heated rooms. Infection with Ehrlichia canis still rather presents a "typical" travel-related disease or can mainly be found in imported animals. An infection with E. canis leads to an infection of the monocytes and thus to canine monocytic ehrlichiosis (CME). The incubation period is about 8 – 20 days, which then changes into an acute phase of 2 – 4 weeks. Clinical signs are mostly nonspecific: fever, anorexia, dyspnoea, anaemia, lymphadenopathy. In rare cases, CNS disorders may occur. In the first 10 – 20 days, thrombocytopenia can be seen, although there is rarely spontaneous bleeding. Subsequently, if untreated, a subclinical stage develops, which lasts for months or years, or chronic infections arise, which are often accompanied by hypergammaglobulinaemia. E. canis can also infect cats!

# **Ehrlichia canis - Pathogen Detection**

Material EB 0.2 ml, bone marrow, tick

Method Realtime PCR
Species Dog, cat
Duration 1 – 3 days

# **Ehrlichia canis - Antibody Detection**

Material S, EP, HP 0.5 ml
Method ELISA (dog), IFAT (cat)

Species Dog, cat Duration 1 day

Note In dogs, detection by IFAT is possible on request.

# ESBL > see Chapter 14.4, p. 272

#### 13.2.16 Francisella tularensis

Francisella (F.) tularensis is a gram-negative, pleomorphic, non-motile, aerobically growing bacillus that is very resistant especially in lower environmental temperatures. This pathogen is the causative agent of the so-called **tularaemia (rabbit fever)**, which is a zoonosis.

Four subspecies have been classified. Two subspecies are of particular clinical relevance: F. tularensis ssp. tularensis and F. tularensis ssp. holarctica, with F. tularensis ssp. tularensis naturally occurring in North America only and being responsible for a more aggressive course of the disease. In contrast, F. tularensis ssp. holarctica can be found in the entire northern hemisphere.

It is predominantly hares, rabbits and rodents (mice) that are affected, but also numerous other animal species, including birds, are susceptible to this pathogen. In dogs, cats and sheep, sporadic cases of illness are known. Cats suffer from the disease more often than dogs, but overall, the disease is rarely contracted.

Signs of an acute disease are apathy, fever, tachypnoea and swelling of the lymph nodes; most animals die of septicaemia within 2 weeks. Furthermore, in a chronic course, emaciation and skin ulcerations occur; in dogs and cats in addition to splenomegaly, hepatomegaly, ulcers on the tongue and icterus.

Here, too, a lethal outcome is possible after 2 – 6 weeks.

The modes of transmission include blood-sucking insects like fleas, midges, lice, ticks, etc., consumption of infected carcass/meat or contaminated water. The infectious dose is very low, only a few bacteria are sufficient (exception: infections through the gastrointestinal tract).

Humans become infected when frequently exposed to hares, rabbits or wild animals. In Germany, there is an **obligation to notify the authorities** when F. tularensis ssp. is detected in hares and rabbits!

# Francisella tularensis - Pathogen Detection

Material Tissue (mainly spleen, liver, lung, kidney), lymph node aspirates,

swab without medium (pharynx, tonsils)

Method Realtime PCR

Species (Dog, cat), rabbit, hare, mouse, others

Duration 1 - 3 days

Note The PCR detects F. tularensis ssp. holarctica.

### 13.2.17 Helicobacter

Helicobacter (H.) spp. are helical or curved, gram-negative bacteria. At least 35 species are known; some of them colonise the gastric mucosa, while others colonise the intestine and liver of humans or animals. Transmission occurs via the oral-oral or possibly also the anal-oral route. In humans, H. pylori is correlated with gastritis and stomach ulcers and can also be transmitted to animals. However, it is not pathognomonic in dogs.

Pathogenicity of Helicobacter spp. in animals has not yet completely been clarified. Infections do not always cause a disease; prevalence is very high in both healthy as well as infected animals. H. mustelae was detected in ferrets with gastritis and stomach ulcers, H. heilmanii was found in pigs with stomach ulcers. They are also associated with gastritis, vomiting and inappetence in dogs, cats and ferrets. In cats, Helicobacter spp. are associated with progressive lymphocytic cholangitis. In Muridae, helicobacter infection is often seen in association with typhlitis or rectal prolapse. In hamsters, the infection is often subclinical. In some cases, gastritis similar to that in humans may occur.

**Gastric** Helicobacter spp. include H. heilmannii, H. felis, H. bizzozeronii, H. salomonis and others; the **intestinal** ones comprise, for example, H. canis, H. bilis, H. cinaedi as well as Flexispira rappini. **Flexispira rappini**, which is also assigned to the genus Helicobacter, is associated with **abortions in sheep**. Aborted lambs show multifocal hepatic necroses – similar to campylobacter infections.

# **Helicobacter – Pathogen Detection**

Material Vomit, gastric lavage, gastric biopsy, sheep: abortion material

Method PCR

Species Dog, cat, hamster, mouse, ferret, sheep

Duration 1-3 days

Note Positive PCR results from faecal samples do not necessarily indicate

involvement of the stomach (gastritis, stomach ulcer, etc.), as PCR also detects intestinal Helicobacter spp. For this diagnostic task, stomach biopsies or vomitus are recommended as sample

material.

# 13.2.18 Histophilus somni

Histophilus somni (formerly Haemophilus somnus) is a gram-negative bacillus of the family Pasteurellaceae. While some strains of H. somni are commensals of the mucosa of the upper respiratory and reproductive tract in cattle, sheep and other ruminants, pathogenic strains spread systemically and can cause severe diseases such as pneumonia, thrombotic meningoencephalitis, myocarditis, septicaemia, arthritis and abortions or, together with Mannheimia haemolytica, the multifactorial disease enzootic bronchopneumonia.

#### Histophilus somni - Pathogen Detection

Material (1) Swab with medium (indicate site, special culture medium

required), BAL, tissue

(2) Swab without medium, nasal lavage, BAL, tissue

Method (1) Bacteriological culture (differentiation MALDI-TOF)

(2) Realtime PCR

Species Cattle, sheep and other ruminants

Duration (1) 3 - 4 days

(2) 1 - 3 days

Note **Culture**: Take a deep swab; request detection by culture via the

service Bacteriology.

**PCR detection** is also part of the Bovine Respiratory Profile 2

(see Chapter 2.4.3, p. 64).

#### 13.2.19 Lawsonia intracellularis

#### Horse

Especially in older foals, Lawsonia intracellularis causes **proliferative enteropathy**, which is accompanied by significant hypoproteinaemia. The animals also show abdominal pain, reduced general condition and anorexia. Secondarily, oedema and a pear-shaped abdomen may occur.

# Pig

**Porcine proliferative enteropathy (PPE)** is caused by an infection with the obligate intracellular, gram-negative bacterium Lawsonia intracellularis. Infection is subclinical. It is widespread in pig herds, especially among weaners, store pigs and fattening pigs. Infected animals suffer from growth disorders and diarrhoea. Infection occurs via the oral route, the spread mainly through the purchase of infected animals. Often, the infection is subclinical.

PPE occurs in four clinically apparent forms: as acute and, if untreated, often fatal porcine haemorrhagic enteropathy (PHE) and as porcine intestinal adenomatosis (PIA), or less often as necrotic enteritis (NE) and as terminal regional ileitis (RI) with thickened and stiff ileum. While PHE mainly affects older fattening pigs and younger breeding pigs, the chronic forms PIA, NE and RI mainly occur in weaners and store pigs.

# Lawsonia intracellularis - Pathogen Detection

Material Faeces, tissue (intestine)

Method Realtime PCR

Species Horse (mainly foal), pig

Duration 1-3 days

# 13.2.20 Leptospira

Leptospira are gram-negative bacteria and zoonotic agents which belong to the spirochete group. They are very thin, flexible, spiral bacteria with a hook-shaped end. Leptospira can actively move by twisting. Within the genus Leptospira interrogans sensu lato, there are various pathogenic and saprophytic species which cannot be differentiated morphologically, but only serologically or genetically. Since 1989, more than 250 serovars have been described that are currently classified in 24 serogroups. Transmission of pathogens occurs directly through the urine or blood of infected animals or indirectly through inanimate vectors such as contaminated water, feed and sleeping places or living vectors like rodents. Leptospira best survive in a humid environment at temperatures of 0 – 25 °C.

## Dog

Clinically, leptospirosis in dogs is initially manifested by anorexia, vomiting, dehydration and fever. Later, animals are apathetic and often show difficulty breathing. The mucous membranes are icteric, anaemia with haemoglobinuria appears and, in some cases, as a complication, disseminated intravascular coagulation (DIC). Toxic degradation products lead to haemorrhagic diathesis and necroses. As a result, acute nephritis with azotaemia can arise. In some cases, hepatitis may also occur, which often has a highly acute course. Leptospira are fetotrophic.

According to our own research, there has been a shift in the types of serovars over the past years. In dogs, analysed serovars include L. Bratislava, L. Australis, L. Autumnalis, L. Icterohaemorrhagiae, L. Pomona, L. Canicola, L. Saxkoebing, L. Grippotyphosa and L. Sejroe.

#### Cat

Cats seem to show a natural resistance. However, here too, the number of cases with clinical manifestation is increasing. The predominant serovars are L. Grippotyphosa and L. Bratislava, followed by L. Icterohaemorrhagiae, L. Sejroe, L. Autumnalis, L. Australis and L. Javanica.

# Reptiles

In reptiles, leptospira antibodies can be detected quite often.

#### Horse

Leptospira infections, which are spread through the urine of rodents, are usually clinically inapparent in horses. Thus, the seroprevalence in healthy horses is high (up to approx. 75%). The pathogen is ingested with feed or water and leads to rather non-specific symptoms in horses, like fever (often intermittent), icterus, inappetence and productivity loss. Abortions have been described as well. Transmission of the pathogen between horses does practically not occur.

**Equine recurrent uveitis (ERU)** – It is likely that intraocular persistent leptospiral infection contributes to the aetiology of ERU, however, it is not the only possible aetiology. Autoimmune reactions lead to a progressive deterioration of the inner structures of the eye and may even lead to blindness.

Detection of antibodies (= most sensitive test) or pathogen detection using PCR can be carried out from aqueous humour or tissue of the vitreous body.

#### Ruminants

Leptospirosis in ruminants can cause economic losses and is, in Germany, a **notifiable animal disease in sheep**. It is predominantly cattle kept under extensive grazing conditions that get infected. In cattle, fever, anorexia, icteric mucous membranes, anaemia with haemoglobinuria and a decline in productivity are dominant. Diarrhoea and mastitis can also occur.

Predominant serovars in our own research are L. Icterohaemorrhagiae,

L. Saxkoebing and L. Bratislava. The recently emerged serovar L. Hardjo was not detected in any of the samples we examined.

## Pig

Gravid pigs are especially susceptible to leptospira. The cardinal signs are birth of weak piglets or abortions. Aborted litters normally show different sizes and degrees of decay of the foetuses as the course of the disease is usually protracted.

When testing for antibodies in pigs, we look for serovars specific to this species:

L. Canicola, L. Grippotyphosa, L. Saxkoebing, L. Bratislava, L. Sejroe, L. Pomona,

L. Copenhageni and L. Tarrasovi.

In Germany, it is **notifiable upon diagnosis** in pigs.

Leptospira – Pathoger	Detection
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Material Urine + EB 0.2 ml (bacteraemia only at the beginning of the infection!),

intraocular fluid (horse), tissue (kidney, vitreous body, abortion material)

Method Realtime PCR

Species List Dog, (cat), small mammals, horse, ruminants, pig

Duration 1 – 3 days

#### **Leptospira – Antibody Detection**

Material S, EP, HP 0.5 ml, horse: also intraocular fluid/vitreous body (if ERU

signs are present)

Method MAT

Species Dog, cat, reptiles, horse, ruminants, pig, others on request

Duration 1 da

Note Initially, antibody titres only confirm pathogen contact. Many animals

are seropositive without showing any clinical signs. Generally, titres > 1:400 or a three- to fourfold titre increase in a paired serum

Note

• Antibodies against serovars 1 and 4b are detected.

 Low titres may be non-specific (< 1:80), as there is an antigenic relatedness of L. monocytogenes with staphylococci and streptococci.

# 13.2.22 Mannheimia haemolytica

Mannheimia haemolytica is a gram-negative, facultative anaerobic bacillus of the genus Mannheimia and the family Pasteurellaceae. It is considered the primary causative agent of enzootic bronchopneumonia in cattle and sheep and, moreover, the pathogen of severe mastitis as well as septicaemia in sheep and goats. In ruminants, however, some of the serotypes described for M. haemolytica are part of the natural microflora of the upper respiratory tract.

# Mannheimia haemolytica - Pathogen Detection

Material (1) Swab with medium, tissue

(2) Swab without medium, nasal lavage, tissue

Method (1) Bacteriological culture (differentiation MALDI-TOF)

(2) Realtime PCR

Species Ruminants
Duration (1) 2 – 3 days

(2) 1 - 3 days

Note **Culture**: Take a deep swab; request detection by culture via the

service Bacteriology.

PCR detection is also part of the Bovine Respiratory Profile 2

(see Chapter 2.4.3, p. 64).

# 13.2.23 Melissococcus plutonius

The gram-positive bacterium Melissococcus plutonius is the primary pathogen of **European foulbrood (EFB)** in bees. It mainly affects so-called coiled larvae, which then die at 4 – 5 days of age. The larvae are infected through the food and the pathogen multiplies in the gut. Infected brood changes colour and becomes a semiliquid mass, which later on dries out to loose scales. Due to the partly sour smell, it is also referred to as sourbrood. After capping, the caps are sunken and perforated. The signs are very similar to those of American foulbrood, a disease which is notifiable upon suspicion in Germany, thus, a precise diagnosis is of great importance. Transmission can either occur through the bees themselves (drifting, robbing) or by the beekeeper. By forming an artificial swarm, the brood can be separated from the healthy bees and then killed.

sample at an interval of 14 days are considered positive. Peracutely infected animals only show low or even negative antibody titres. Furthermore, if animals have already been treated with antibiotics at a very early stage, the titre often does not increase as expected. For acutely affected animals, direct detection from urine and blood is recommended.

Horse: Serum antibody titres have no relevance with regard to ERU.

#### 13.2.21 Listeria

Listeriosis can affect many animal species as well as humans.

Listeria are relatively small gram-positive rods with a tendency to grow in chains. Within the genus, Listeria monocytogenes has the greatest significance. Listeria ivanovii has low virulence, but is pathogenic to humans and sheep. The pathogen has also been isolated from monkeys suffering from meningitis.

Listeriosis is predominantly a disease in sheep that contract the disease through the ingestion of spoiled silage. Cattle, chickens, pigs, rabbits and goats are much less prone to the disease. Individual cases have been described in horses, dogs and cats. In more than 80% of the cases of ovine listeriosis, the brain is affected and the characteristic clinical picture of this disease develops – the animals run in circles and show further signs up to recumbency due to a usually unilateral dysfunction of cranial nerves.

Other forms are septic listeriosis of newborn or young animals, organ listeriosis (e.g. mastitis) or gestation listeriosis with abortions.

Listeriosis (L. monocytogenes) is a **notifiable disease** in Germany.

# Listeria - Pathogen Detection

Material Faeces, swab with medium, CSF, abortion material, etc.

Method Bacteriological culture

Species Dog, cat, horse, cattle, sheep, goat

Duration 3 – 4 days

• Please clearly indicate on the submission form that listeriosis is suspected, as special culture media are required.

 If pathogenic species are detected, an antibiogram will be performed additionally (subject to a charge).

 Detection of listeria is also part of the BARF Faecal Profile (see Chapter 2.1.7, p. 45).

# Listeria - Antibody Detection

Material S, EP, HP 0.5 ml

Method IFAT

Species Dog, cat, horse, cattle, sheep, goat

Duration 1 day

# Melissococcus plutonius - Pathogen Detection

Material Bee larvae, bees

Method PCR
Species Bees
Duration 1 – 3 days

# 13.2.24 Methicillin-resistant Staphylococci: MRSA/MRSP

In human medicine, diseases caused by methicillin-resistant Staphylococcus aureus (MRSA) are known and feared as so-called "nosocomial infections". These are infections with pathogens that have developed resistance to common antibiotics. The pathogens can enter the environment through hospital visitors, personnel, equipment, etc. As most of these infections in humans are zoonoses, pathogens can also be transmitted to animals as well as vice versa, because of the close contact between humans and animals. This probably also leads to an increase in MRSA cases in veterinary medicine. MRSA is often detected in farm animals. In 2016, only about every fourth pig in Germany was MRSA-free. Approximately every fourth horse was an MRSA carrier (zoonoses monitoring 2019). In agricultural **livestock**, MRSA of a certain line are predominant, so that the term livestock-associated or laMRSA is used. laMRSA mostly belong to the clonality CC398 and were responsible for 8% of MRSA cases in humans in 2017 and for 5% in 2018. In regions with high livestock density, laMRSA cause increasing numbers of human MRSA cases. People with close animal contact, including veterinarians, are particularly at risk.

In **small animals**, we detect **MRSP**, methicillin-resistant Staphylococcus pseudinter-medius, far more frequently than MRSA. According to our own research, about 8% of all isolates of Staphylococcus pseudintermedius (formerly Staphylococcus intermedius in dogs) are already afflicted with a multidrug-resistance gene.

Material Swab with medium (skin, eye, pharynx, nose, etc.)

Method Bacteriological culture (on standard and special culture media)

Species Dog, cat, horse, farm animal, others

Duration 3 - 4 days

Note By means of PCR, detection of methicillin resistance after growing a

culture is also possible by means of PCR (detection of the resistance gene MecA or MecC). The test for MRSA is also part of the service "Analysis of Multidrug-resistant Bacteria" (see Chapter 14.4,

p. 272).

# 13.2.25 Mycobacterium avium ssp. paratuberculosis

Mycobacteria see ➤ Chapter 16.1.2, p. 287

Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of **paratuberculosis**, also called **Johne's disease**, a chronic granulomatous enteritis in ruminants. The disease is globally distributed. In addition to domesticated ruminants (cattle, sheep, goat), also wild ruminants and camelids can be affected. MAP could also be isolated from other animal species, e.g. rabbits, mice, foxes and ferrets. The pathogen is very stable and can remain infectious in the environment for up to one year. Normally, an infection already occurs orofaecally in calves through contact with faeces of infected animals, but it can also spread through the colostrum and milk, and intrauterine infections are possible as well.

The incubation period varies greatly and can take several years. The first clinical signs often tend to occur when the animals are already older than 2 years. Primary signs are continuous, profound, uncontrollable diarrhoea and progressive weight loss with regular appetite. Paratuberculosis is always lethal. Already prior to the onset of these signs, decreased milk production, reduced fertility, etc. lead to high economic losses. Not all infected animals develop clinical signs, subclinically infected carriers also (intermittently) excrete the pathogen. Animals suspected of being infected should be isolated and, in case of a positive result, should soon be eliminated from the population or slaughtered. Test results vary depending on the phase of infection, therefore, the use of repeated sampling is recommended if an infection is suspected!

In Germany, it is a **notifiable disease** in cattle, sheep and goats!

## Mycobacterium avium ssp. paratuberculosis - Pathogen Detection

Material Faeces, tissue (intestine, lymph node), milk

Method Realtime PCR

Species Cattle, sheep, goat (and others)

Duration 1 – 3 days

### Mycobacterium avium ssp. paratuberculosis - Antibody Detection

Material S. HP. milk 1 ml

Method ELISA Species Cattle Duration 3 days

# 13.2.26 Mycoplasma

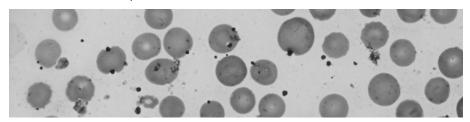
Mycoplasmas are prokaryotic pathogens that are divided into haemotropic and non-haemotropic mycoplasma. Outside the organism, mycoplasmas are very unstable.

# 13.2.26.1 Haemotropic Mycoplasma

Haemotropic mycoplasmas (formerly haemobartonella and eperythrozoon) are globally spread, gram-negative bacteria of the family Mycoplasmatacea. They attach to the surface membrane of erythrocytes and can cause anaemia.

## Dog

So far, **Mycoplasma haemocanis** and **Candidatus Mycoplasma haematoparvum** have been described in dogs. Both strains are found in Europe, especially in the Mediterranean area. Clinically, the course of the disease is often just chronic and asymptomatic. In contrast, acute infections with fever, anorexia, weight loss and lethargy are mainly seen in immunocompromised dogs, dogs that had splenectomy or those simultaneously infected with other pathogens. Deaths are also possible. Natural infection probably occurs through vectors, particularly the brown dog tick (Rhipicephalus sanguineus) is being discussed. Vertical transmission through the placenta and milk is also possible, and blood transfusions present a risk of infection as well.



Erythrocytes with mycoplasma on the membrane (dog, Diff-Quik, 1000x magnification).

## Cat

Currently, three different types of haemotropic mycoplasmas with different pathogenicity have been described in cats. In addition to the strain **Mycoplasma haemofelis**, which is known as Ohio isolate, and the most commonly found California isolate, **Candidatus Mycoplasma haemominutum**, another strain, **Candidatus Mycoplasma turicensis**, has been known for some years. The latter was first detected in cats in Switzerland, but seems to occur relatively rarely in Germany. While Mycoplasma haemofelis can cause serious illness even in immunocompetent animals, an infection with Candidatus Mycoplasma haemominutum usually progresses subclinically in healthy animals. Coinfections are possible with clinical signs typically being more distinct than in monoinfections.

Natural infection probably occurs through vectors; particularly fleas, but also ticks and stinging insects are being discussed. Vertical transmission through the placenta and milk is also possible. Blood transfusions present a risk of infection, too, as well as direct transmission between animals through bite wounds.

Clinical signs in the acute phase are anaemia (haemolytic anaemia as cardinal symptom), fever, splenomegaly, general weakness and possibly polypnoea, tachycardia

and icterus. The cause of haemolytic anaemia is damage of the erythrocyte membrane by haemotropic mycoplasmas. Because of the change in the erythrocyte surface, a secondary immune haemolytic anaemia can develop later on; in this case, the direct Coombs test will be positive. The main signs of a chronic infection include weight loss and intermittent fever. Studies have shown that a high percentage of the dog and cat population is infected without the animals showing any clinically relevant signs. These carriers present a particular risk for breeding and blood transfusions.

#### Camelids

In its acute phase, an infection with **Mycoplasma haemolamae** can cause haemolytic anaemia in affected animals. However, infections can also primarily progress silently and lead to a chronic carrier state. The disease may fully break out in these animals in situations linked to stress and/or immunosuppression.

# Pig

Porcine infectious anaemia (synonym: porcine eperythrozoonosis) is an infectious disease caused by **Mycoplasma suis** (formerly Eperythrozoon suis). The pathogens attach to the erythrocytes (adhesion, invasion) and provoke damage to and lysis of the erythrocytes due to the formation of autoantibodies. Below the normal body temperature ("cold antibodies"), they agglutinate the blood cells and result in anaemia. Once infected animals go through episodes of anaemia time and again. The disease becomes chronic. Older pigs are only latently infected and only suffer from another relapse when they are very weak. The pathogen remains in the body throughout life.

# Mycoplasma (haemotropic) - Pathogen Detection

Material EB 0.2 ml, tissue (spleen)

Method Realtime PCR
Species Dog, cat
Duration 1 – 3 days

Note PCR detection should be preferred over microscopic detection as

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the sensitivity of the microscopic detection is low (around 30%). Species differentiation is included in the detection of haemotropic mycoplasma (dog: Mycoplasma haemocanis, Candidatus Mycoplasma haematoparvum; cat: Mycoplasma haemofelis, Candidatus Mycoplasma haemominutum, Candidatus Mycoplasma turicensis).

# Mycoplasma haemolamae - Pathogen Detection

Material EB 0.2 ml
Method Realtime PCR
Species Llama, alpaca
Duration 1 – 3 days

## Mycoplasma (Eperythrozoon) suis - Pathogen Detection

Material EB 0.2 ml, tissue (spleen)

Method Realtime PCR

Species Pig

Duration 1 - 3 days

## 13.2.26.2 Non-haemotropic Mycoplasma

Non-haemotropic mycoplasmas can be found on the mucous membranes of the respiratory and the urogenital tract **(mucosa-associated mycoplasmas)**, where they can escape from the immune response of the infected animal for a very long time. Conjunctivitis and rhinitis are clinically apparent, disorders of the upper respiratory tract occur less frequently. Mycoplasmas can also be primarily pathogenic.

# Dog

Mycoplasmas often occur in dog populations and are sometimes considered as commensals in the literature. Yet, they are also associated with diseases of the urogenital region and with infertility. Clinically, an infection with canine mycoplasma can cause prostatitis and/or orchitis in male dogs, and can, amongst others, lead to endometritis in female dogs. However, mycoplasmas may also play a role in canine respiratory diseases. As it is difficult to cultivate mycoplasma, PCR detection is the method of choice.

#### Cat

In the cat common cold complex, not only viral components (FHV, FCV) play a role, but also **Mycoplasma felis**. Clinically, an infection is usually manifested by conjunctivitis and rhinitis. **Mycoplasma gatae** and **Mycoplasma feliminutum** are sometimes isolated from cats, nevertheless, their clinical relevance is questionable.

### Rat and mouse

**Mycoplasma pulmonis** is the causative agent of "murine respiratory mycoplasmosis" in rats and mice, a slowly progressing infection of the respiratory tracts associated with the formation of thick mucus. Clinical signs of infected animals are sneezing, mucopurulent nasal discharge, stertorous breathing sounds and dyspnoea. The infection can spread to the middle ear and lead to otitis media and head tilt.

In addition, especially in older female rats, Mycoplasma pulmonis can cause genital infection which leads to infertility or a small litter size. In rare cases, metritis or pyometra are also seen.

Latent infections without any clinical signs are common.

Transmission occurs through aerosols in close direct contact. Sexual or intrauterine transmission is also possible.

#### Reptiles

Several Mycoplasma spp. exist in tortoises. An infection with a virulent **Mycoplasma agassizii** strain causes the so-called upper respiratory tract disease (URTD), a disease clinically characterised by serous, mucous and purulent nasal discharge as well as ocular discharge, conjunctivitis and eyelid oedema. Furthermore, it can cause lethargy, dehydration, anorexia and fatal cachexia. An essential trait of mycoplasma infections is the fact that they can persist in the organism without triggering any symptoms. Often, the disease only breaks out if there are other microorganisms and environmental factors involved, combined with the genetic properties and immune reactions of the host. Mycoplasmas are also detected in turtles and other reptiles, especially pythons, but little is known about their clinical relevance.

#### Cattle

In the first weeks of the life of a calf, **Mycoplasma bovis** can cause mostly enzootic pneumonia and arthritis, and in cows it leads to severe mastitis.

Affected calves typically suffer from otitis with hanging earlobes and head tilt. As mastitis pathogen, Mycoplasma bovis is highly infectious. Typically, the mammary gland increases in size and hardens, and within a few weeks, the inflammation spreads to the neighbouring udder quarters.

The pathogen is also often detected in connection with chronic diseases of the respiratory tract. Reservoirs of M. bovis are the respiratory tract of clinically healthy calves and young cattle as well as the udder of cows with subclinical mastitis.

**Mycoplasma mycoides subsp. mycoides** is the causative agent of contagious bovine pleuropneumonia (**notifiable upon suspicion** in Germany).

# Pig

**Mycoplasma hyopneumoniae** is the primary causative agent of enzootic porcine pneumonia (EPP). EPP is one of the most significant causes of respiratory infectious diseases in pigs. The disease is globally distributed. However, the pathogen only causes high economic losses in pig production when combined with poor environmental conditions and secondary bacterial and/or viral infections.

# **Poultry**

Infections with **Mycoplasma gallisepticum** cause the so-called chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys. Infection occurs both horizontally through the air and direct contact as well as vertically through hatching eggs. The main symptoms include chronic inflammation of the upper respiratory tracts and air sacs, accompanied by disorders of the joints, tendon sheaths and the genital tract. Central nervous disorders can also arise. In addition, laying performance and hatching rates decrease significantly. Mixed infections with viral pathogens, such as Newcastle disease virus (NDV) or infectious bronchitis virus (IBV) are not unusual and can severely aggravate the clinical picture (also as vaccine viruses).

Duration 5 days

Note This test can be ordered individually and is also part of the serological

Porcine Respiratory Profile (see Chapter 2.5.2, p. 67).

Neoehrlichia mikurensis > see Candidatus Neoehrlichia mikurensis,

Chapter 13.2.10, p. 210

Nocardia > see Chapter 14.4, p. 273

Paenibacillus larvae > see Chapter 14.4, p. 273

#### 13.2.27 Pasteurella multocida

Pasteurella multocida is a gram-negative bacillus. Pasteurella are commensals of the mucous membrane of the upper respiratory tract. Factors that reduce resistance, such as overpopulation or a bad stable environment, provide a predisposition to infections with **toxigenic strains**. Co-infections with Bordetella bronchiseptica are common and lead to particularly severe symptoms.

Pasteurella multocida, either as a mono-infection or together with Bordetella bronchiseptica, leads to "snuffles" in rabbits. Normally, this disease is a stock problem and is often recurrent.

In **pigs**, Pasteurella multocida toxin is the aetiological agent that causes progressive **atrophic rhinitis**, with especially the toxigenic pasteurella types A and D being involved. The cytotoxic toxin (PMT) inhibits the osteoblasts. With the activity of the osteoclasts being maintained, it leads to atrophy of the nasal conchae and deformation of the nasal septum. The importance of the toxin in pneumonia in cattle and pigs has not yet been clarified.

# Pasteurella multocida (toxin producing) - Pathogen Detection

Material Swab without medium (nose, pharynx), BAL, tissue (lung)

Method Realtime PCR
Species Rabbit, pig
Duration 1 – 3 days

Note

- The serogroups/types (A F)/serotypes cannot be differentiated by PCR, as there is no correlation between the serotype and genotype characteristics.
- Detection by culture of solely P. multocida is also possible; please indicate on the submission form that pasteurella is suspected. A possible toxin formation cannot be detected by culture.

tis, which is clinically manifested by joint swellings and lameness. Inflammations of the air sacs, myocardium and pericardium also occur. Especially after mixed infections, respiratory symptoms can be seen as well. Stunted growth, reduced laying performance and greenish diarrhoea are also due to the infection. Besides game birds, geese, too, are susceptible to this pathogen.

In chickens and turkeys, Mycoplasma synoviae causes infectious synovitis and arthri-

## Mycoplasma - Pathogen Detection

Material

**Dog:** swab without medium (eye, nose, pharynx, genital tract),

BAL, abortion material

Cat: swab without medium (eye, nose, pharynx, genital tract),

BAL, abortion material

Rat, mouse: swab without medium (nose, pharynx), tissue (lung) Chelonians, snake: swab without medium (conjunctiva, mouth),

nasal flush

Cattle: swab without medium (nose, pharynx), nasal flush, BAL,

milk, synovia, sperm, tissue (lung)

**Pig:** swab without medium (trachea, nose), BAL, tissue (lung) **Poultry:** swab without medium (pharynx, cloaca), faeces, tissue

(lung)

Method PCR/realtime PCR

Species Dog, cat, rat, mouse, tortoise/turtle, snake, cattle, pig, poultry

Duration 1 - 3 days

Note • Mycoplasma PCR in **dogs** detects at least the following species:

M. arginii, M. gateae, M. spumans, M. cynos, M. molare, M. canis, M. edwardii, M. bovigenitalum, M. maculosum, M. opalescens, M.

feliminutum.

• Mycoplasma PCR in cats detects M. felis.

# Mycoplasma bovis - Antibody Detection\*

Material S 1 ml
Method ELISA
Species Cattle
Duration 5 days

Note

This test can be requested as part of the serological Bovine Respira-

tory Profile (see Chapter 2.4.2, p. 63).

# Mycoplasma hyopneumoniae – Antibody Detection\*

Material S 1 ml Method ELISA Species Pig

# 13.2.28 Rhodococcus hoagii (formerly Rhodococcus equi)

Rhodococcus hoagii is a facultative pathogenic organism in soil or horse faeces. R. hoagii is the most frequent causative agent of severe pneumonia with high fatility rates in foals aged 3 weeks to 6 months. Entry and predilection site is the lung (abscessation!), from where haematogenous spread to other organs and, through swallowing, a dissemination into the gastrointestinal tract (ulcera, diarrhoea, source of infection!) are possible. Umbilical infections occur as well. Moreover, R. hoagii shows an affinity for bones and joints.

### Rhodococcus hoagii (Rhodococcus equi) - Pathogen Detection

Material (1) Swab with medium (nose, navel), BAL, tracheal lavage (preferred),

faeces

(2) Swab without medium (nose, navel), BAL, tracheal lavage, faeces

Method (1) Culture

(2) Realtime PCR

Species Horse

Duration (1) 2 - 3 days

(2) 1 - 3 days

Note

• Because of the sensitivity of PCR, it is possible to also identify

clinically healthy carriers.

• If PCR detection is positive, the virulence factor VapA will be

automatically detected at no additional cost.

#### 13.2.29 Rickettsia

Rickettsia are obligate intracellular coccoid, rod-shaped or pleomorphic gram-negative bacteria that parasitise in reticuloendothelial cells or erythrocytes. They are usually transmitted by arthropods.

Rickettsia are divided into the categories "spotted fever group", thyphus group and "others", which includes Coxiella burnetii.

In the USA, Rickettsia rickettsii, the causative agent of **Rocky Mountain spotted fever**, and in the Mediterranean area, Rickettsia conorii, the causative agent of **Mediterranean spotted fever**, are of central importance in animal infections. Infected dogs may remain asymptomatic or show symptoms ranging from lymphadenopathies, fever, hyperesthesia, peripheral oedema up to lameness.

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# Rickettsia spp. - Pathogen Detection

Material Tick, EB, tissue (skin)
Method Realtime PCR
Species Dog, cat, others

Duration 1 – 3 days

#### Rickettsia conorii - Antibody Detection

Material S, EP, HP 0.5 ml

Method IFAT
Species Dog, cat
Duration 1 day

Note R. conorii is found in the Mediterranean, Africa, South West

Asia, India. Serological studies suggest a high prevalence in

asymptomatic dogs.

#### Rickettsia rickettsii – Antibody Detection

Material S, EP, HP 0.5 ml

Method IFAT Species Dog Duration 1 day

Note Rickettsia ricketsii infection causes Rocky Mountain spotted fever. It

is found in North and South America.

#### 13.2.30 Salmonella

Salmonella belong to the family Enterobacteriaceae and are found in the intestines of animals and humans. In most cases, infection occurs faecal-orally or by feeding raw meat.

Salmonella infections affect almost all animal species. Compared to herbivorous pets, dogs and cats are more resistant to salmonella infections. Under favourable conditions, salmonellosis causes diarrhoea with vomiting and fever; in young animals, the disease can also become septicaemic.

In reptiles and amphibians, salmonella can be part of the normal intestinal flora. In these animals, clinically relevant salmonellosis is associated with immune deficiency.

According to the Robert Koch Institute (RKI), about 10% of all human salmonella infections, which cause diarrhoea, are related to direct contact with excreting dogs, cats and particularly reptiles.

For some time now, ESBL producers have also been detected among salmonella, especially in livestock. Because of the **ESBL problem, creating an antibiogram** is essential.

In Germany, it is an **epizootic disease** in cattle that is **notifiable upon suspicion**. In other species, it is **notifiable upon diagnosis**. For commercial poultry in Germany, there is also an obligation to notify and inform the authorities, but this is strictly monitored and can result in official measures being taken in the flock.

Salmonella - Pathogen Detection

(2) Faeces; in birds also swab without medium (cloaca), eggs,

tissue

Method (1) Bacteriological culture including enrichment

(1) Faeces (swab with medium)

(2) Realtime PCR

Species All

Material

Duration (1) 2-3 days

(2) 1 - 3 days

Note Culture with enrichment is the most sensitive test method.

After successful culture growth, a serological pathogen differentiation

is carried out (subject to a charge).

Salmonella - Antibody Detection

Material S 1 ml Method MAT

Species Dog, cat, birds, horse

Duration 2 days

Note Direct detection by culturing salmonella from faecal samples is high-

ly preferable to the indirect antibody detection.

Salmonella Abortusequi - Antibody Detection\*

Material S 1 ml

Method Slow agglutination

Species Horse
Duration 5 days

Note • Export-relevant test.

 In the host-adapted serotype Abortusequi, pathogen transmission occurs orally; rarely through mating. With regard to miscarriages, this pathogen does not currently play a role in Germany anymore.

# 13.2.31 Staphylococcus

Staphylococci are gram-positive and extremely resistant bacteria. They normally reside on the skin and the mucous membranes, where they are part of the physiological microbial flora.

Inflammations caused by staphylococci are usually locally limited. Only in cases of decreased resistance, septicaemia and pyaemia can occur. In ruminants, staphylococci are of major importance as causative agents of mastitis.

Nowadays, special attention should be paid to whether methicillin-resistant strains of Staphylococcus aureus (MRSA) or, in the small animal practice, of Staphylococcus pseudintermedius (MRSP) are present (see Chapter 13.2.24, p. 224). In case of repeated wound healing problems in patients visiting the practice, which are caused by MRSA or MRSP, it should be considered testing the staff of the practice, too, whether they carry this type of pathogen on their nasal mucosa.

Detection can be done through culture examination of clinical samples, e.g. swabs of pustules, mucosal swabs and other body secretions and excretions.

#### Staphylococcus - Pathogen Detection

Material Swab with medium, milk (ruminants)

Method Culture including enrichment

Species All

Duration 2-3 days

Note Further differentiation can be made if MRSA is suspected.

### Staphylococcus - Antibody Detection

Material S 0.5 ml
Method MAT
Species Dog, cat
Duration 1 day

Note To recognise animals sensitive to Staphylococcus in cases of

pyoderma.

# 13.2.32 Streptococcus equi

The globally spread and highly infectious equine disease **strangles** is caused by an infection with **Streptococcus equi subsp. equi** and is characterised by purulent lymphadenitis and pharyngitis. It used to be a typical disease in young animals which induces long-lasting immunity. Over the past years, however, an increasing number of affected adult horses has been described, with the disease showing a rather atypical progression (mainly fever, respiratory disorders).

Compared to culture, PCR has the advantage of delivering faster results while at the same time offering a comparably higher sensitivity and specificity. This way, the identification of clinically healthy carriers, which play a major role in pathogen epidemiology, is also more reliable.

As PCR does not differentiate between dead or living organisms, a positive pathogen detection should always be formulated as a suspected diagnosis and be confirmed by culture examination.

Clinically, an infection with Streptococcus equi subsp. equi cannot always be distinguished from an infection with **Streptococcus equi subsp. zooepidemicus**. Streptococcus equi subsp. zooepidemicus can be found in all domestic animals and in humans. In horses, it is a facultative pathogenic commensal; infections can cause, amongst others, respiratory disorders and purulent bronchopneumonia. As with strangles, especially foals and young horses are affected.

#### Streptococcus equi - Pathogen Detection

Material

- (1) Swab with medium (nose, abscess, lymph node), lavage sample (guttural pouch, pharynx, BAL), TBS
- (2) Swab without medium (nose), lavage sample (guttural pouch, BAL), TBS, tissue (lymph node)

Method

(1) Culture

(2) Realtime PCR

Species

Horse

Duration

(1) 2 - 3 days

(2) 1 - 3 days

Note

- In culture, both subspecies (Streptococcus equi equi and Streptococcus equi zooepidemicus) are determined and differentiated by MAI DI-TOF
- If detection is to be done by means of PCR, it can be chosen between the single detection of Streptococcus equi equi or the detection of both subspecies mentioned above.

#### Streptococcus equi - Antibody Detection

Material Method S 0.5 ml ELISA Horse

1 – 3 days

Species Duration

Note

- Generally, this test determines both Streptococcus equi equi and Streptococcus equi zooepidemicus. However, the detected surface antigen SeM is considered a virulence factor which mainly occurs in Streptococcus equi equi.
- In case of a positive result, the titre level will additionally be determined on request until 30<sup>th</sup> June 2022. From 1<sup>st</sup> July 2022 onwards, antibodies will always be quantified. Knowing the titre level can be particularly helpful if purpura haemorrhagica or metastasising abscesses are suspected; these animals have high titres.
- The level of the titre does not give any information on the immunisation or the carrier status of a horse.

# 13.2.33 Taylorella equigenitalis

**Contagious equine metritis (CEM)** is caused by the gram-negative bacillus Taylorella equigenitalis. Transmission particularly occurs during mating; stallions latently carry the pathogen on the mucous membrane of the penis, especially in the Fossa urethralis and in the smegma of the prepuce. Transmission from infected mares to stallions is also possible. In mares, an infection leads to endometritis/cervicitis with mucopurulent vaginal discharge and to reduced fertility. Stallions show no clinical signs of the disease.

For exports, a bacteriological examination is required; within the EU, however, detection by PCR is now also recognised as a suitable test method. In Germany, there is an **obligation to notify the authorities**, if Taylorella equigenitalis is detected.

#### Taylorella equigenitalis/CEM - Pathogen Detection

Material

- (1) Swab with medium (Amies with charcoal, not older than 48 hours)
- (2) Swab with medium with charcoal (e.g. Amies), sperm Stallion: penile sheath, urethra, fossa glandis Mare: fossa clitoridis, sinus clitoridis, cervix

Method (1) Culture. MALDI-TOF

Horse

(2) Realtime PCR

Species

Duration (1) Culture: 1 week

Export to the USA: 1 week Export to Canada: 2 weeks Export to Norway: 3 weeks

(2) PCR: 1 - 3 days

Note

- Detection by PCR is offered as an individual service for one sample or as a CEM profile for the examination of several sites (see Chapter 2.3.4, p. 58). The CEM Profiles stallion 1 and mare 1 are suitable as PCR detection before export to another EU country.
- Even after successful bacteriological cultivation, it is not possible to create an antibiogram for Taylorella equigenitalis.

# 13.2.34 Treponema paraluiscuniculi

**Rabbit syphilis** (Spirochaetosis cuniculi) is caused by the highly contagious bacterium Treponema paraluiscuniculi. Only rabbits and hares are susceptible, human infections are not possible. Transmission occurs directly, usually during mating. However, the animals can also become infected through other mucosal contacts as well as bedding and feed. Rabbit syphilis typically occurs as a chronic disease, but latent infections are possible, too. The incubation period is weeks to months. First clinical signs can be seen as oedematous swellings and formation of nodules on the external genital organs. In

the further course of the disease, these nodules erode into ulcers and become purulent and encrusted. By licking the affected anogenital region, other skin areas, such as lips, eyelids or the edge of the ears often become infected as well.

Material Tissue (mainly skin lesions/scurfs; possibly regional lymph nodes),

swab without medium (vagina, prepuce)

Method Realtime PCR Species Rabbit, hare Duration 1 – 3 days

### Treponema paraluiscuniculi - Antibody Detection\*

Material S 0.5 ml

Method Treponema pallidum haemagglutination test

Species Rabbit Duration 3 days

#### 13.2.35 Yersinia

Yersinia belong to the order Enterobacteriales. Yersinia (Y.) pseudotuberculosis is the causative agent of **pseudotuberculosis/rodentiosis**, an infectious disease all mammalian and bird species can contract. For instance, rodents and cats are predisposed. The pathogen has a high tenacity. In the soil, the pathogen remains infectious for months.

Y. enterocolitica causes enterocolitis in humans and animals. Immunopathological reactions can lead to arthritis, arthrosis and skin diseases. Animals, especially pigs, sheep and poultry, often act as pathogen reservoir. Dogs rarely become ill, if at all, puppies are mainly affected. The infection manifests itself as enteritis, resulting in mucous to bloody diarrhoea. Especially in Y. pseudotuberculosis infections, abscesses can occur in various organs. Y. pseudotuberculosis can also play a role in wild ruminants (game enclosures!).

Just like Y. enterocolitica, Y. pseudotuberculosis is a zoonotic agent!

Material Faeces

Method (1) Culture including enrichment, MALDI-TOF identification

(2) Realtime PCR (only Y. enterocolitica)

Species All

Duration (1) Up to 4 weeks

(2) 1 - 3 days

Note

- A faecal sample of at least the size of a cherry is required.
- In exceptional cases, a swab with transport medium can also be used for culture.
- Detection by culture is offered as a combined service together with the detection of Campylobacter.
- After successful culture growth, a serological pathogen differentiation is carried out (subject to a charge).

# Yersinia – Antibody Detection

Material S 0.5 ml
Method MAT
Species Dog, cat
Duration 1 – 3 days

Note Detection of antibodies against Yersinia enterocolitica and Yersinia

pseudotuberculosis.

# 13.3 Fungi

# 13.3.1 Aspergillus

Aspergillus is a genus of moulds with approximately 200 species found worldwide. In the environment, Aspergillus is particularly found in soil, organic waste, but also in animal feed. Parrots often get infected through unpeeled peanuts.

Aspergillosis is often caused by the species Aspergillus fumigatus and preferably affects the skin, nose, paranasal sinuses and the lung. Aspergillosis frequently occurs in birds, especially if the animal is predisposed by improper keeping, the administration of antibiotics or stress. This very often results in severe respiratory disorders. Other organs (e.g. CNS) can also be affected.

### Aspergillus - Pathogen Detection

Material Swab with medium, BAL, nasal lavage, tracheal lavage, faeces

Method Culture Species All

Duration Up to 7 days

Note Order via the Mycology service.

# Aspergillus-Galactomannan - Antigen Detection\*

Material S 0.5 ml Method ELISA

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Species Birds
Duration 5 days

Note Galactomannan is a polysaccharide found in the cell wall of Asper-

gillus spp.

In animals affected by aspergillosis, it can be detected in the blood. For birds that only develop low antibody titres against Aspergillus (often in parrots), the detection of galactomannan in the blood can help to diagnose aspergillosis. However, galactomannan is only released during active infection, i.e. during growth and spread of the fungus. In inactive older granulomas, the polysaccharide cannot be

detected in the blood.

Animals that display high antibody titres against Aspergillus normally do not have detectable galactomannan in the blood, so that antibody and antigen detection in combination can complement each

other when making a diagnosis.

## Aspergillus sp. - Antibody Detection

Material S 0.5 ml Method MAT

Species Dog, cat, birds, cattle, others on request

Duration 1 day

Note Culture detection of Asperaillus is often very difficult due to the site of

infection. Antibody detection may be used to support the diagnosis.

# 13.3.2 Batrachochytrium

Batrachochytrium spp. are fungi which are being held responsible for large losses in **amphibians**.

# Batrachochytrium dendrobatidis

The chytrid fungus Batrachochytrium (B.) dendrobatidis was first identified in Australia in 1998 and named in 1999. This fungus causes chytridiomycosis in anurans and salamanders and is thought to be partly responsible for the population decline and the global extinction of > 200 amphibian species.

Infections with B. dendrobatidis are often associated with very high mortality rates (in lab up to 100%), but the fungus is not necessarily lethal. Other factors such as stress or co-infections with other pathogens also seem to play a role.

B. dendrobatidis multiplies in keratinised tissue and therefore affects primarily the outer skin of adult animals (stratum corneum to stratum granulosum). In larvae, the keratinised mouthparts are affected. During metamorphosis, the infections can lead to dramatically high mortality rates. The clinical signs are often non-specific and may affect the skin (often appears macroscopically unchanged or "dull" or depigmented;

hyperkeratosis and excessive episodes of skin shedding; mixed infections with severe erosions of the skin) as well as the behaviour (atypical behaviour, such as prolonged stay in the water, ataxia and CNS problems). Spontaneous deaths without previous clinical manifestation are also observed.

#### Batrachochytrium dendrobatidis – Pathogen Detection

Material Swab without medium: skin swabs of the ventral body surface (adult

animals) or of the keratinized skin at the mouth (tadpoles), tissue

(sloughed skin of infected animals)

Method Realtime PCR
Species Amphibians
Duration 1 – 3 days

## Batrachochytrium salamandrivorans

Batrachochytrium salamandrivorans is a recently described highly-contagious and deadly chytrid fungus that has massively infested and killed fire salamanders especially in North-West Europe. Infected animals show anorexia, apathy and ataxia as well as skin lesions with superficial erosions and deep ulcerations all over the body. Batrachochytrium salamandrivorans can also infect other salamanders, but has not yet been detected in anurans.

## Batrachochytrium salamandrivorans - Pathogen Detection

Material Swab without medium (skin), tissue (skin)

Method Realtime PCR

Species Amphibians (mainly salamander)

Duration 1 – 3 days

# 13.3.3 Cryptococcus

Cryptococcus is a yeast and mainly found in bird faeces and contaminated soil and dust. Cryptococci are potential causative agents of systemic infections in humans, domestic and wild animals. Direct transmission from vertebrates to humans has not yet been observed. In dogs, cryptococcosis can manifest itself in different ways, for example as gastrointestinal signs, arthritis, neurological deficits, skin changes and especially respiratory symptoms. As Cryptococcus neoformans infection can lead to serious diseases, early detection is relevant.

# Cryptococcus - Pathogen Detection (Antigen)

Material S 0.5 ml Method Agglutination

Species Dog, cat, others on request

Duration 5 days

# 13.3.4 Dermatophytes

Dermatophytes are filamentous fungi that can cause skin lesions in humans and animals. The disease is called dermatophytosis. The fungi use keratin as a carbon source and colonise keratinised tissue, such as hair, skin or claws.

Dermatophytes are highly contagious. Infection is direct or indirect. Favourable factors are, for example, immunosuppression, reduced immune response (e.g. high age) or previous damage of the skin (e.g. by ectoparasites). Moreover, spores as form of propagation can remain infectious in the environment for years.

The clinical signs are diverse and dependent on the virulence of the fungal strain, the infection period and the immune status of the host. Typical patchy alopecia may appear on the face, ears and front legs. Pruritus may be missing or may range from mild to severe. In case of skin diseases, dermatophytosis must always be considered in the differential diagnosis.

Especially guinea pigs from pet shops are (asymptomatic) carriers of Trichophyton benhamiae in 90% of the cases. Most zoonotically transmitted dermatophytoses in humans are now caused by this pathogen. Before introducing guinea pigs into a household, particularly if there are children or immunocompromised persons, the animals should be examined for dermatophytes.

#### **Dermatophytes - Pathogen Detection**

Material Hair with roots, deep skin scraping, scales, scabs, claws

Method (1) Culture

(2) Realtime PCR

Species Dog, cat, rabbit, guinea pig, horse, cattle (and other species)

Duration (1) 3 days to 4 weeks

(2) 2 - 4 days

Note

Zoonosis!

conducted on request.

- Culture also detects Malassezia; a false negative result is possible with previous treatment.
- PCR is validated for the detection of the following dermatophyte species: Microsporum canis, Nannizzia gypsea (formerly: Microsporum gypseum), Nannizzia persicolor (formerly: Microsporum persicolor), Trichophyton (T.) mentagrophytes, T. benhamiae, T. equinum, T. verrucosum, T. erinacei.
   Further types of skin fungus might also be detected by the PCR.
   Differentiation of the most common dermatophyte species can be
- PCR is not suited for therapy monitoring (dead dermatophytes are detected as well).

# 13.3.5 Encephalitozoon

# Encephalitozoon cuniculi

The pathogen Encephalitozoon cuniculi causes **encephalitozoonosis** (also called **torticollis, wry neck, head tilt**) in rabbits. Approximately 80% of healthy rabbits carry the pathogen without showing any clinical signs. Mature infectious spores are mainly excreted intermittently in the urine, so that transmission takes place orally and nasally by eating infected food or sniffing at food and litter. However, infected pregnant female hares can also transmit the pathogen to their young in the womb. Faecal excretion of pathogens was detected but seems to be of little importance.

The pathogen has also been found in many other animal species such as dogs, foxes, rodents and some bird species and even in humans. Especially in immunocompromised persons, infection can be relevant.

Apart from head tilt, the clinical picture in rabbits is mainly characterised by ataxia, nystagmus, seizures or cramps. As the disease can also take a milder course, it is recommended to test for E. cuniculi in case of any neurological sign.

# Encephalitozoon cuniculi - Pathogen Detection

Material Urine, CSF 0.2 ml, (faeces), tissue (e.g. kidney, brain or eye/lens)

Method PCR

Species Rabbit, guinea pig and others

Duration 1 - 3 days

#### **Encephalitozoon cuniculi - Antibody Detection**

Material S. EP. HP 0.5 ml

Method IFAT

Species Dog, cat, rabbit, guinea pig, others on request

Duration 1 - 2 days

Note Positive titres can be expected from day 14 p.i. onwards. Subclinical

infections are possible.

IgG are detected. On request, there is an additional test available for the detection of IgM and IgG in rabbits and possibly in other animal

species.

Antibody detection is also part of the E. cuniculi Profile for rabbits

(see Chapter 2.2.1, p. 47).

# Encephalitozoon pogonae

Encephalitozoon pogonae has been described in bearded dragons (Pogona spp.; Agamidae) and belongs to the microsporidia. Microsporidia are unicellular, intracellular, spore-forming fungi reclassified from the group of protozoa. Due to similar morphology

and genetic similarities, the pathogen was first identified as Encephalitozoon cuniculi. In 2016, it was then classified as an independent species.

Infections can be associated with non-specific signs such as lethargy, anorexia, weight loss and polydipsia. Multiplication takes place in macrophages of different organs, especially the kidneys, but also the gastrointestinal tract, liver, ovaries, spleen, lungs, the vascular endothelium and ventricular ependymal cells of the brain are affected, and granulomas are formed. Co-infections with agamid adenovirus 1 and coccidia have been described and may lead to a more severe clinical picture.

Faecal excretion can occur and transmission is likely via the faecal-oral route. Diagnosis is made by PCR and/or histopathology of the affected tissue or by PCR from a cloacal swab or faeces.

#### Encephalitozoon pogonae - Pathogen Detection

Material Swab without medium (cloaca), faeces, tissue

Method PCR

Species Bearded dragon
Duration 1 – 3 days

# 13.3.6 Macrorhabdus ornithogaster

Macrorhabdus ornithogaster is a yeast which is found in many different **avian** species, especially in budgerigars. Macrorhabdiosis is also called **megabacteriosis** or **going light syndrome.** Infected animals may develop maldigestion and lose weight even though there is no change in appetite. Undigested seeds can be excreted with the faeces, sometimes there is also blood in the faeces. Choking and vomiting as well as a general weakness can also be found in macrorhabdiosis. Inapparent carriers are frequent. Transmission presumably occurs through billing and feeding as well as through contaminated feed and water.

# Macrorhabdus ornithogaster - Pathogen Detection

Material Faeces, crop lavage, proventriculus, smear on slide

Method Stain, microscopic

Species Birds
Duration 1 day

# 13.3.7 Ophidiomyces ophidiicola

Ophidiomyces ophiodiicola is a dermatophyte found in snakes. Infections with O. ophidiicola are associated with skin lesions, pustules, nodules and swelling of the skin. Lesions primarily occur on the head, but can also spread to the whole body.

## Ophidiomyces ophidiicola – Pathogen Detection

Material Swab without medium (skin), tissue (skin)

Method Realtime PCR

Species Snake
Duration 1 – 3 days

# 13.4 Parasites

# 13.4.1 Angiostrongylus vasorum

Angiostrongylus vasorum is a globally distributed nematode that parasitises the pulmonary arteries and, less frequently, the right heart of dogs and wild canids. Infections with A. vasorum occur more often in Germany than normally expected (a prevalence of 7.4% according to Barutzki and Schaper, 2009). Thus, an infection with this lungworm should always be considered in differential diagnosis if respiratory and/or cardiovascular symptoms are present.

Dogs as definitive hosts get infected by ingesting L3 larvae when eating infected snails or slugs (intermediate hosts). L3 invade the lymphatic and blood system through the wall of the small intestine of the dog and enter the pulmonary arteries. Six to eight weeks p.i., the females begin to lay eggs. Via the blood, the eggs reach the fine pulmonary capillaries where they develop into L1 larvae and enter the pulmonary alveoli. From here, they are carried up by the ciliated epithelium or are coughed up, swallowed again and finally excreted with the faeces. L1 are taken up with the faeces by intermediate hosts and the infectious L3 then develop within them.

Especially young dogs between the age of one and two years are affected by canine angiostrongylosis. Besides clinically inapparent infections, the course of the disease may be mild to life-threatening. Clinical signs are highly variable, however, the main symptoms include cardiopulmonary signs such as dyspnoea and cough. The second most typical symptoms are coagulopathy with epistaxis, haemoptysis, haematoma and anaemia. Subsequently, DIC, circulatory insufficiency and death can occur. Vomiting or neurological symptoms like muscle tremor, ataxia, dizziness and epileptiform seizures are also possible.

# Angiostrongylus vasorum - Pathogen Detection

Material (1) Faeces (3-day pooled sample)

(2) EB, BAL, faeces (3-day pooled sample), tissue (lung, brain)

Method (1) Baermann-Wetzel method

(2) Realtime PCR

Species Dog

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Duration (1) 2 days

(2) 1 - 3 days

Note If blood should be examined, a combination of PCR and ICA is

recommended as it increases sensitivity.

# Angiostrongylus vasorum - Antigen Detection

S 0.5 ml Material Method ICA Species Dog Duration 1 day

# 13.4.2 Anoplocephala

Anoplocephala perfoliata is the most common type of tapeworm in horses. It is globally distributed; in Germany, focal prevalences of up to 30% are described. The moss mite acts as intermediate host; mites infected with tapeworm larvae are ingested by the horse while grazing. Within 6 – 10 weeks, the larvae develop into adult tapeworms. The adult worms colonise the mucosa of the small and large intestine, mainly the ileocecal valve, and cause local erosion and ulceration. Colic-like symptoms may occur.

# Anoplocephala - Antibody Detection

Material S, EP, HP 0.5 ml

Method ELISA Species Horse

Test frequency 1 x per week

• The test is suitable for stock screening and a targeted therapy.

· As Anoplocephala only releases eggs at intervals of several weeks, antibody detection is superior to pathogen detection from faeces (flotation/SAFC, microscopic detection of eggs) because

of the higher sensitivity and specificity.

#### 13.4.3 Babesia

Note

Babesiosis in mammals has become one of the most important parasitic diseases. The pathogens, which belong to the order Piroplasmida, are transmitted by ticks. In peracute or acute infections, non-specific clinical signs such as fever, apathy and loss of appetite appear between the 5th and 28th day p.i. Anaemia, icterus and massive haemoglobinuria occur. A chronic infection, especially with B. vulpes (= B. microti-like = B. annae), is characterised by fatigue and emaciation of the animals over months, anaemia and intermittent periods of fever.

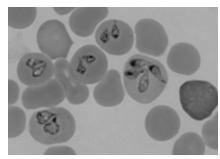
Without treatment, dogs can also develop a subclinical form, especially when infected with B. canis and B. vogeli, with the blood count being normal again. Many dogs imported from Eastern Europe are subclinically infected with B. canis and thus pose a risk of infection for other dogs. In addition, the infection can be reactivated in these dogs by various factors. Cattle and horses can also remain carriers of Babesia for many years.

# Dog

# Babesia canis

B. canis is transmitted by Dermacentor reticulatus (ornate dog tick) and is more virulent than B. vogeli. A distinction is made between the French and the Hungarian strain. French strain:

Distribution: north and east Mediterranean area, locally in Holland (The Hague, Arnhem) and England, focuses in western Germany (Saarland, Rhineland-Palatinate, Baden-Württemberg). What is often noticed about the French strain is its low antibody production.



Erythrocytes with 2 – 4 Babesia (B. canis) (dog, Diff-Quik, 1000x magnification)

Hungarian strain:

Distribution: Hungary, Ukraine, Russia (beyond the north of Moscow), Romania, eastern Germany.

What is often noticed about the Hungarian strain is its high antibody production. In 80% of the animals, new infections with the Hungarian strain lead to death if untreated.

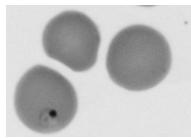
# Babesia voqeli

Distribution: North Africa, the whole Mediterranean area, Portugal,

B. vogeli is transmitted by Rhipicephalus sanguineus (brown dog tick) and often only leads to low antibody titres.

# Babesia gibsoni

Distribution: Asia. USA. Europe (imported) Transmitted by Rhipicephalus sanguineus; distribution in Europe is considered questionable. The cases of Babesia gibsoni described for Portugal and Spain were corrected later, partly into the pathogen Theileria annae (now: B. vulpes).



Erythrocytes with small Babesia (B. gibsoni) (dog, Diff-Quik, 1000x magnification)

#### Babesia vulpes (formerly Babesia microti-like, B. annae)

Distribution: north west Spain, Central Europa including England.

Vector unknown, assumed are: Ixodes hexagonus (hedgehog tick), I. ricinus (European castor bean tick), I. canisuga (dog tick) und Dermacentor reticulatus (ornate dog tick).

#### Cat

#### Babesia canis

Distribution: Thailand, Brazil, France, Poland, Germany

Very rare, only known in combination with other chronic underlying disease.

#### Babesia felis

Distribution: in parts of Africa

# Babesia cati

Distribution: India

#### Horse

#### Babesia caballi and Theileria equi (formerly Babesia equi)

Distribution: from the tropics and subtropics to the temperate zones. Ticks are vectors. Equine babesiosis (piroplasmosis) is also expected to occur in Germany.

#### Cattle

# Babesia divergens

Distribution: in Europe from Finland down to the Mediterranean. Vectors are Ixodes ricinus (European castor bean tick) and Ixodes persulcatus (taiga tick). Babesia divergens is also pathogenic to humans.

#### Babesia major

Distribution: Central Europe in small endemic areas. In Germany, only on the North Sea islands Amrum, Norderney and Juist. Haemaphysalis punctata (red sheep tick) is the vector.

#### Babesia bigemina

Distribution: tropics and subtropics; in Europe: the Balkans, coastal areas in the Mediterranean, Portugal.

# Babesia - Pathogen Detection

Material (1) EB 1 ml + blood smear

(2) EB 0.2 ml. tick

Method (1) Microscopic

(2) Realtime PCR

Species Dog, cat, horse, cattle

Duration (1) 1 day

(2) 1 - 3 days

Note

Material

Note

(1) Microscopic detection is possible from the 5<sup>th</sup> day p.i. onwards. It is preferable to collect capillary blood (edge of the ear) and spread it onto a glass slide. The detection from capillary blood significantly increases sensitivity!

(2) PCR detection is far more sensitive than the detection from a blood smear. In case of a chronic infection, it can be assumed that pathogens have spread to many sites. However, the concentration of pathogen DNA in the blood may be very low and thus lead to a negative result in the PCR. While a positive PCR is proof of an infection, a negative PCR never rules out an infection.

PCR horse: In case of a positive result, a differentiation between Theileria equi/Babesia caballi can subsequently be made on request.

#### Babesia – Antibody Detection

Matchai	0, E1, 111 0.0 1111, B. 91030111. 0 0.0 1111
Method	(1) IFAT (cat; dog and horse only when leaving the country)
	(2) ELISA (dog)
	(3) cELISA (horse – export to the USA, most sensitive test)
	(4) CFT (horse – often required for export)*
Species	Dog, cat, horse
Duration	(1 and 2) 1 day

S FP HP 0.5 ml· B gibsoni· S 0.5 ml

(3) 2 – 3 days (4) 5 days

Seroconversion from the 2<sup>nd</sup> week p. i., maximum titre after 4 weeks.

False negative results can occur in young dogs under 6 months and

in the early phase of infection.

#### 13.4.4 Coccidia

Coccidia are unicellular intestinal parasites found in a variety of domestic and farm animals. In many animal species, different species of coccidia occur with varying pathogenicity. They range from apathogenic species to highly pathogenic ones, which can lead to watery and haemorrhagic diarrhoea if there is a heavy infestation. Young animals are particularly affected here. In dogs and cats, especially puppies and kittens at 3 to 4 weeks of age can fall ill.

Coccidia have different predilection sites in the intestine, so that dissection may also provide an indication of coccidiosis and the respective coccidia species. Eimeria species are found in **unqulates**, **poultry and rabbits**. Isospora is a parasite in **dogs and** 

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cats, and in pigs, both Eimeria and Isospora occur, with Isospora suis often causing diarrhoea in piglets.

Tortoise intranuclear coccidiosis (TINC) is a severe disease in tortoises with high morbidity and mortality rates. TINC has already been detected in different tortoises and box turtles in North America and Europe. Clinical signs include lethargy, significant weight loss, erosive rhinitis, dyspnoea and occasionally skin lesions. Infections are generally systemic. These coccidia are most frequently detected in the intestine, pancreas, liver and kidney. However, they can also be found in the Eustachian tube, in macrophages of the spleen, in the middle ear, lungs and stomach. In live animals with rhinitis, they can also be detected in nasal lavage samples.

#### Coccidia - Pathogen Detection

Faeces Material Method Flotation **Species** ΑII

Note Testing for coccidia is part of the service "Endoparasites"

(see Chapter 15.1, p. 277) and should be ordered via this service.

### Intranuclear Coccidians (TINC) - Pathogen Detection

Material Swab without medium (nose, possibly cloaca), nasal lavage, tissue

(nasal mucosa, intestine, pancreas, kidney, liver)

Realtime PCR Method Species Tortoise, turtle Duration 1 – 3 days

# 13.4.5 Cryptosporidia

Cryptosporidia are very small, unicellular parasites of the gastrointestinal tract. They are classified as coccidia. Different species are described with very similar morphology. Some of these are host-specific; others (e.g. Cryptosporidium parvum) can infect various animal species and humans (zoonosis).

Infections occur after intake of sporulated oocysts. The infectious dose is very low (approx. 100 oocysts). Subsequently the liberated sporozoites infect the intestinal epithelial cells, followed by a development cycle over trophozoites, meronts, merozoites, gamonts, zygotes and in the end again oocysts are formed. The oocysts excreted in the faeces show a high tenacity, are resistant to many disinfectants and can remain infectious for months. Therefore, e.g. contaminated pens or terrariums are frequent sources of infection.

In cattle, cryptosporidiosis is a very common endoparasitosis. A large proportion of calves go through an infection with C. parvum. Clinically apparent courses with enteritis and diarrhoea occur especially in calves up to 3 weeks of life, often related to coinfections. Quite often lambs, piglets and foals are also affected. A much lower prevalence is seen in **dogs and cats**, with usually asymptomatic

infections. However, oocysts are excreted in the faeces here, too, for about 2 weeks. Manifest infections can be seen in puppies or immunocompromised animals (e.g. FeLV, FIV. distemper, neoplasia, etc.).

In **reptiles**, cryptosporidiosis is a serious disease that can cause severe losses, especially in snake and lizard stocks. C. serpentis is an important parasite in snakes and infects the gastric mucosa. Due to the chronic inflammation, a subsequent swelling and hardening of the connective tissue in the gastric area can occur. A typical sign is the regurgitation of food days after digestion. C. saurophilum (also called C. varanii) destroys the lining of the intestinal walls of affected lizards and snakes. Clinically, malabsorption with excretion of undigested food, profound weight and fluid loss are observed. Both pathogens are not pathogenic to humans. Quite often, C. muris and C. parvum are found in reptile faeces as intestinal passengers (origin: infected feeder animals). Therefore, further differentiation is absolutely necessary if the result is positive.

In laboratory diagnostics, several **methods** are available for detection. Already during the microscopic examination after specific enrichment (SAFC) oocvsts can be found. As with all parasitological faecal examinations, sensitivity is relatively limited at approximately 60%.

In cattle, ELISA testing, which detects C. parvum, is recommended. The immunofluorescence test includes a wider range of Cryptosporidium species and is therefore suitable for dogs, cats, but also small rodents (guinea pig: C. wrairi). In case of positive test results in **reptiles**, differentiation between pathogenic agents and intestinal passengers is of interest. For this, PCR with subsequent differentiation is recommended. In addition to PCR, detection by IFAT and microscopy are also available. Yet, these methods do not allow for a differentiation of the individual species. If reptile faeces are examined microscopically, the preparations will be stained additionally (modified Ziehl-Neelsen stain) to increase the detection rate. It should be noted that a single negative result does not completely rule out a Cryptosporidia infection, as the pathogen can be excreted intermittently. So far, no successful treatment is available. The emphasis in the control of cryptosporidiosis is on symptomatic treatment and hygiene management.

# Cryptosporidia - Pathogen Detection

Material Faeces, in snakes also: regurgitated material, gastric lavage,

stomach biopsy

(1) Antigen detection: EIA, reptiles: IFAT Method

(2) PCR

(3) Modified Ziehl-Neelsen staining

Species Dog, cat, small mammals, reptiles, cattle, others

Duration (1) IFAT: 1 day, EIA: 2 days

> (2) 1 - 3 days (3) 1 day

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Note If the PCR yields positive results in reptiles, it is possible to perform a

differentiation of the Cryptosporidium species to distinguish between harmless intestinal passengers (origin: infected feeder animals) and

pathogenic agents.

#### 13.4.6 Demodex

Demodex mites are strictly host-specific ectoparasites of numerous mammals and of humans. So far, there have been three species each described in dogs and cats (dogs: particularly Demodex canis, rarely D. injai and D. cornei; cats: especially D. cati, but also D. gatoi and D. felis).

The entire development of demodex mites takes place in the hair follicles, the sebaceous and apocrine glands of the host. They cannot survive very long in the environment. Transmission mainly occurs postpartum while nursing. Demodex mites belong to the physiological skin fauna, but are facultative pathogenic. In dogs, there is often a low number of mites present without any clinical symptoms (prevalence up to 85%), but demodicosis is rare. Nevertheless, it is one of the most frequent dermatoses in dogs (especially young dogs), in cats, however, it is very rare.

In **dogs**, lesions generally start in the face or on the forelegs and spread from there. The *localised form* affects a few well-defined skin areas and most notably occurs in young dogs. The skin areas are often hairless and may also be scaly. Comedones are typical as well. In general, itching only occurs in case of secondary bacterial infections. If more than four lesions are present, an entire body region or at least two paws are affected and if it continuously worsens without treatment, it is referred to as *generalised demodicosis*. There are usually secondary bacterial infections present and alopecia appears with follicular papules up to furunculosis, focal ulcerations and fistula tracts. Most of the time, there is no itching, but sometimes intense pain. Fever, anorexia, lethargy, lymphadenopathy and sepsis may occur and it might be fatal if not treated. Special forms are *podo-* and *otodemodicosis*.

Favourable factors for mass reproduction of mites include, e.g., endoparasitosis, malnutrition, cortisone treatment, neoplasia, hypothyroidism or hyperadrenocorticism. There is a genetic predisposition in young dogs (juvenile generalised demodicosis). These dogs should be excluded from breeding.

In **cats**, demodicosis particularly occurs if systemic diseases, such as diabetes mellitus, FIV, FeLV or neoplasia, are present, and most notably causes alopecia and crusts on the head and neck. Itching is also possible.

# **Demodex – Pathogen Detection**

Material Deep skin scraping Method (1) Microscopic

(2) Realtime PCR (semi-quantitative)

Species Dog, cat

Duration (1) 1 day

(2) 1 - 3 days

Note

- Since demodex mites belong to the normal skin fauna and only an excessive increase leads to demodicosis, a positive PCR result should always be interpreted in connection with clinical and epidemiological data. A negative PCR result cannot completely rule out an infection.
- If reduced immunocompetence or immunodeficiency is suspected, examination of the lymphocyte subpopulation by flow cytometry (see Immune Status, Chapter 7, p. 115) may be helpful.

#### 13.4.7 Echinococcus

Echinococcus (E.) multilocularis does not only infect foxes as definitive hosts, but also dogs and cats; it is present in Central Europe (particularly southern Germany, northern Switzerland and western Austria), West and East Europe and focally in Scandinavia. Definitive hosts of E. granulosus are dogs and other canids. E. granulosus is mainly detected in the Baltic States, East and South Europe, including the Mediterranean, and is very rare in other places.

For the definitive hosts, echinococci are harmless intestinal parasites, whereas in intermediate hosts (herbivores and omnivores), metacestode cysts are mainly formed in the liver and lungs, even in humans as accidental hosts. In cystic echinococcosis caused by E. granulosus, encapsulated lesions are formed. In alveolar echinococcosis caused by E. multilocularis, in contrast, cysts show invasive growth with metastasis, so that the disease will lead to death if untreated.

There is a higher risk of echinococcus infestation and excretion of tapeworm eggs in dogs that eat rodents or that are used for fox hunting in dens. In Germany, echinococcosis is a **notifiable disease**.

# **Echinococci – Pathogen Detection**

Material Faeces, tissue

Method PCR
Species Dog, cat, fox
Duration 1 – 3 days

Note PCR can detect infections with E. granulosus and E. multilocularis,

while microscopy after enrichment often only allows for the detection

of non-differentiable Taenia eggs.

#### **Echinococcus – Antibody Detection**

Material S, HP 0.5 ml

Methode ELISA
Species Dog
Duration 5 days

Note Antibodies against E. multilocularis are detected.

Encephalitozoon > see Chapter 13.3.5, p. 243

Fasciola hepatica > see Chapter 15.2, p. 280

### 13.4.8 Filaria

In Europe alone, five different filarial species are known to cause filariasis in dogs: Dirofilaria immitis, Dirofilaria repens as well as Acanthocheilonema (Dipetalonema) reconditum, Acanthocheilonema (Dipetalonema) dracunculoides and Cercopithifilaria grassi. Dirofilaria immitis causes cardiovascular dirofilariasis (heartworm disease), Dirofilaria repens causes cutaneous dirofilariasis. Both types of dirofilariasis are zoonoses and are transmitted by mosquitoes, including the common house mosquito (Culex pipiens) which is very common in Germany. The mosquito genera Aedes and Anopheles are also competent intermediate hosts and vectors in Europe.

#### Dirofilaria immitis - Pathogen Detection (Dirofilaria Antigen)

Material S, EP, HP 0.5 ml

Method ELISA

Species Dog, cat, ferret, others on request

Duration 1 day

Note The serological examination is the most sensitive detection method

for Dirofilaria immitis and detects the surface proteins of female, parturient filariae (macrofilariae), which are parasites in the heart or in larger vessels. The earliest time for a positive result is half a year p.i., but it can be delayed up to nine months if infected dogs receive heartworm prevention. Examination of puppies under the age of six months is therefore not appropriate. If in doubt, it is recommended to test for microfilariae or to take the test at a later point in time. Therapy monitoring should be done at the earliest 4 – 5 months after completing therapy. For the detection of microfilariae see below.

#### Microfilaria – Pathogen Detection

Material EB 0.5 ml

Method Microscopic, Knott test, filtration test

Realtime PCR; quantitative PCR (dog): droplet digital PCR

Species Dog, cat

Ferret (PCR)

Duration 1 - 3 days

Note

- Accumulation of the microfilariae of Dirofilaria immitis in the
  peripheral blood takes place in the evenings (adaptation to the
  piercing behaviour of vector mosquitoes). This behaviour has not
  yet been documented for other filarial species, but it is advisable
  to possibly take the blood sample in the evening hours.
- Before departing to South Africa, it is mandatory to do a filtration test.
- In case of a positive PCR result, differentiation of the filarial species can be done on request and is recommended in order to initiate treatment adapted to the type of filaria.
- Dog: Quantitative PCR is used for dose adjustment (in case of high pathogen loads, reduction of dose to reduce the risk of thromboembolism) and, as therapy monitoring, to exclude resistances. Quantitative PCR can be requested directly or following qualitative PCR.

#### 13.4.9 Giardia

Giardia is a flagellate that can be found in the intestine of mammals, birds, reptiles, amphibians and humans. There are some well-differentiated species, such as G. intestinalis (lamblia, duodenalis). Giardia is ingested orally (food, water) or through smear infection as cysts, excystates in the intestine and attaches as trophozoites to the intestinal wall where it replicates. Damage to and detachment of the intestinal epithelium cause chronic intermittent catarrhal to mucous-bloody diarrhoea. The cysts that are excreted with the faeces remain infectious for many months in cold water and a humid environment.

Except for Giardia in birds and amphibians, Giardia is partially of zoonotic nature. Seven variants have been identified through genetic characterisation of which variants (assemblages) A and B mainly occur in humans, variants C and D are primarily detected in dogs and variant F can mostly be found in cats. Across species, however, isolates of different subtypes of A as well as those of B can be detected in different animal species, so that a transmission from humans to animals and from animals to humans cannot be excluded. In dogs and cats, Giardia is the predominant type of intestinal parasites. In our own examinations, Giardia infections were detected in 15% of cats; 3.5% of these animals contained the human-pathogenic assemblage A.

Giardia - Pathogen Detection

Material	Faeces
Method	(1) Microscopic after enrichment
	(2) EIA (antigen detection)

(3) Realtime PCR

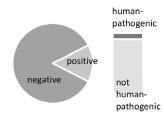
Dog, cat, small mammals, reptiles, large animals

Duration (1 and 2) 1 day (3) 1 - 3 days

Note

Species

- · Giardia infections lead to a decrease in vitamin B 12.
- · If treatment of Giardia fails in cats. Tritrichomonas foetus should also be considered.
- In case of a positive PCR result, testing for the presence of humanpathogenic assemblages A and B can subsequently be conducted.



#### Giardia in cats:

15% of the animals are positive. 3.5% of the carriers are infected with human-pathogenic assemblage A.

# 13.4.10 Hepatozoon

Hepatozoon canis belongs to the protozoa and goes through a typical coccidial life cycle with the dog as intermediate host. Asexual reproduction, schizogony. takes place in several generations in the endothelial cells of the spleen, liver and bone marrow. The merozoites formed here penetrate the leukocytes and differentiate into gamonts.

The definitive host, the tick, ingests the gamonts during the blood meal. Gamogony and sporogony take place in the tick and oocysts with 16 infectious sporozoites each are formed.

Infection with H. canis occurs by biting or swallowing an infected tick, primarily the brown dog tick (R. sanguineus), which is found in warm countries (mainly Southern Europe, South America, Africa and Asia). By now, the pathogen has also become endemic in several regions of Germany. Vertical intrauterine transmission is possible as well.



Neutrophil granulocyte with Hepatozoon canis (acidophilic capsule) (Diff-Quik. 1000x magnification)

Acute infections are characterised by fever, lymphadenitis, anorexia, apathy, myositis and epileptiform seizures (bleeding in meninges). Massive lesions up to necrosis occur in the affected organs (spleen, liver, lung, brain). Chronic infections cause intermittent fever, lymphadenopathy, anaemia, diarrhoea and vomiting. Hyperaesthesia and muscular pain with stiffening of the neck muscles and the trunk muscles occur.

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Periosteal bone proliferation can be seen and epileptiform seizures may also occur in chronic diseases. In case of low parasitaemia, the infection may be clinically inapparent

## **Hepatozoon - Pathogen Detection**

or may only have mild clinical signs.

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EB 0.2 ml, tissue (liver), tick Material

Method (1) Microscopic buffy coat smear (semi-quantitative)

(2) Realtime PCR (Hepatozoon canis/felis)

Species Dog. cat Duration 1 – 3 days

Note Occurrence of the disease is mainly linked to the presence of the

vector (preferably subtropical and tropical countries), but can also take place in animal shelters, where rhipicephalus is often able to

survive the winter.

#### 13.4.11 Leishmania

**Leishmaniosis** is an infectious disease transmitted by insects. The vectors of Leishmania are sand flies (Phlebotominae). Leishmania is taken up during the bloodsucking process. The promastigate stages, which are infectious 6 – 12 days after the blood-sucking process, multiply in the sand fly. In Europe, the pathogen is Leishmania infantum. South of the Bosporus and especially in North Africa, Leishmania tropica occurs additionally. Other species of Leishmania have been described worldwide. The main infection areas in Europe are Spain. Portugal, Italy and Greece, Foxes and possibly also small rodents are considered as pathogen reservoirs.

In Germany, naturally occurring sand flies (mainly Phlebotomus mascittii; no transmission of Leishmania is known so far) have been found along the Rhine rift in Baden-Württemberg, in Rhineland-Palatinate in the Kaiserslautern region, and in Saarbrücken in Saarland.

Infected animals can be asymptomatic for up to 7 years. The beginning of the disease is mostly characterised by lymphadenopathy, anaemia; in the cutaneous form of leishmaniosis, skin changes at the edges of the ears, the rhinarium and periorbital lesions are visible.

In chronic infections, the animals show reduced resilience, weight loss, lymphadenopathy, scaly, non-itchy skin change and eye changes.

# Leishmania - Pathogen Detection

Qualitative PCR: swab without medium (conjunctiva), bone marrow. Material

tissue (skin, lymph node, spleen), possibly EB

Quantitative PCR (dog): EB or bone marrow

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Method Realtime PCR, quantitative PCR: droplet digital PCR,

cytology, histology



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Species Dog, others on request

Duration 1 – 3 days

Note

• PCR detection is much more sensitive than microscopic detection.

• Quantitative PCR in dogs has a high predictive value and is recommended in combination with serology, especially if titres are low or questionable. Quantitative PCR is also suitable for monitoring the course of infection and the treatment (mostly if titres are high); as such, the same sample material needs to be used und tested by the same laboratory to ensure that the results are comparable. Sensitivity is much higher in bone marrow than in EB.

## Leishmania - Antibody Detection

S 0.5 ml: IFAT also EP. HP 0.5 ml Material

Method IFAT, ELISA (only dog) Species Dog, cat, horse

Duration 1 day

Note Positive antibody titres appear at the earliest 2 – 3 weeks p.i.

In asymptomatic dogs, ELISA is significantly more sensitive (approx.

90%) than IFAT (approx. 50 - 70%).

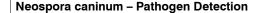
Antibody detection is not suitable for therapy monitoring. Instead. serum protein electrophoresis and determination of C-reactive

protein are recommended.

# 13.4.12 Neospora caninum

A neurological disease in dogs whose pathogens were similar to toxoplasma but could not be classified was first described in Norway in 1984. In 1988, a similar pathogen was found in dogs in the USA and was named Neospora caninum. It was later determined that Neospora caninum was identical to the Norwegian pathogen. Neosporosis has already been detected in many countries, it must therefore be assumed that it is spread worldwide. Natural infections have been found in dogs, cattle, horses, sheep, goats, red deer and cats. Numerous other animals can be experimentally infected.

Clinically, dogs and cattle are particularly severely affected. In the latter, at every stage of gestation, the clinical picture is determined by abortions. In dogs, neurological signs are prominent: ascending paralysis of the hind legs with hyperextension are a typical finding, but all limbs might be affected as well (tetraplegia). Other possible findings are dysphagia, paralysis of the jaw, head tilt, muscle weakness, cardiac insufficiency and pneumonia. Young, congenitally infected dogs show more severe signs, sometimes with sudden deaths. Older dogs often show signs of disseminated infection with polyradiculitis, polymyositis and possibly multiple organ involvement. Thus, in older dogs with neurological signs, neosporosis should always be included in the differential diagnosis. However, due to the often high antibody prevalence in certain regions, it is assumed that only a small percentage of infected dogs actually develops a clinical disease.



Material Dog: faeces, CSF

Cattle: abortion material, foetal tissue (brain, lung, liver, kidney)

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Method Realtime PCR **Species** Dog, cattle Duration 1 – 3 days

#### Neospora caninum - Antibody Detection

Material S. EP. HP 0.5 ml IFAT, ELISA (cattle) Method Dog, cat, horse, cattle Species Duration 1 day, cattle: 3 days

#### 13.4.13 Nosema

Nosemosis is the most common disease of adult honey bees. It is caused by unicellular, intracellular parasites, so-called microsporidia, which are closely related to fungi. It is spread through spores that are viable for several years. Two species can be differentiated: Nosema apis and Nosema ceranae, which can only be distinguished by PCR, but differ in pathogenicity. The pathogens infect the midgut cells and thus lead to yellowish diarrhoea. Affected animals are often unable to fly and the abdomen is bloated. Many times, symptoms are rather non-specific, the bees are weak. Nosemosis is a multifactorial disorder, which means outbreaks of the disease only occur when there are other adverse conditions involved, such as cold, other illnesses, etc. Hence, nosemosis is potentially curable by resolving the other factors. Due to the resistance of the spores, it is often difficult to completely eliminate the pathogens. As with many bee diseases, transmission occurs through the bees themselves (drifting or robbing) or through the beekeeper.

#### Nosema - Pathogen Detection

Material 30 - 40 dead bees Method (1) Microscopic

(2) PCR (differentiation)

Species Bees Duration 1 – 2 days

If the result of the microscopic examination is positive, we recom-Note

mend PCR differentiation between Nosema apis and Nosema

ceranae.

Ostertagia > see Chapter 15.2, p. 280

# 13.4.14 Sarcoptes

Sarcoptes scabiei is the only species of the genus Sarcoptes. The Sarcoptes mites that are found in the different hosts are considered varieties of S. scabiei. The varieties are mostly host-specific, yet these itch mites are able to spread to other hosts, but usually do not settle there permanently.

Sarcoptes scabiei varietas canis causes sarcoptic mange in dogs. Red foxes are considered reservoir animals. Occasionally, the mite is also transmitted to ferrets, rabbits, guinea pigs, cats and humans.

Transmission occurs by direct contact between animals, but also indirectly via the contaminated environment. In dogs, indirect transmission seems to be gaining more and more importance. The whole developmental cycle of itch mites takes place on the host animal. In abraded skin material, the mites can survive up to 3 weeks, if the environment is damp and cool.

The mites burrow their tunnels into the horny layer of the skin. They prefer skin areas that are only sparsely haired, so they are often found on ears, elbows, lower abdomen and ankles. If the disease spreads, larger areas of the body may be colonised. The main clinical sign is massive pruritus, which is often intensified by heat.

In pigs, the mites spread beginning from the inside of the pinna. Bovine sarcoptic mange especially affects the head and neck, but can also spread to the udder. Mange causes loss of performance.

## Sarcoptes - Pathogen Detection

Material Skin scraping (superficial, large-scale)

Method (1) Microscopic

(2) Realtime PCR (Sarcoptes scabei var. canis)

Species (1) Dog, cat, farm animal, others

(2) Dog, cat, rabbit, guinea pig, ferret, other canids and mustelidae

Duration (1) 1 day

Note

(2) 1 - 3 days

(Z) 1 0 dd

 Often, the infestation in dogs cannot be diagnosed by performing a skin scraping. In this case, the diagnosis can only be made by antibody detection.

- In cats, localised infections are found in the head and neck area.
- Zoonosis (pseudoscabies)
- Microscopic detection can be ordered via the service Ectoparasites.
   This also detects, for example, Notoedres.

## Sarcoptes - Antibody Detection

Material S 0.5 ml
Method ELISA
Species Dog
Duration 1 – 2 days

Note Seroconversion only begins after 2 – 3 weeks p.i. and persists for

weeks or months after successful treatment.

# 13.4.15 Toxoplasma

Toxoplasma gondii is an obligate intracellular parasite which belongs to the class Coccidia. It is ubiquitous and causes clinical signs in all warm-blooded animals, including humans.

More than 1 billion people worldwide have antibodies against toxoplasma. In addition to fever and cold-like symptoms, congenital infection during pregnancy is feared. The intrauterine infection of the foetus occurs approximately 3 – 4 weeks after the first infection of a seronegative mother, when the placental barrier is crossed and placentitis occurs. Miscarriages and severe neurological or ophthalmological diseases can occur in the newborn. The cat as the definitive host excretes oocysts for approx. 3 weeks, which sporulate and become infectious after approx. 2 – 4 days, depending on temperature (daily cleaning of the cat litter box!).

Another source of infection is meat contaminated with tissue cysts that has not been sufficiently cooked before consumption. However, the main source of infection is gardening, where oocysts may be absorbed via contaminated soil (aerosols). Cats can also be intermediate hosts at the same time; they rarely fall ill, but the clinical signs depend on where the tissue cysts are located. For example, hepatitis, cholangitis, dyspnoea may occur, and in case of CNS involvement, there may be ataxia, motor deficits and epileptic seizures. Additionally, uveitis and chorioretinitis can occur. The same signs can also be seen in dogs.

In sheep and goats, about 10% of the abortions worldwide are attributed to T. gondii.

In Germany, the **authorities must be notified** if Toxoplasma gondii is detected in cats, hares, rabbits, equids, ruminants, pigs and other mammals, especially those supplying food.

## Toxoplasma gondii - Pathogen Detection

Material Cat: faeces (detection of excretion), CSF

Dog, rabbit, guinea pig: CSF, tissue (e.g. brain)

**Farm animals:** abortion material, tissue (brain, heart, lung and others)

Method Realtime PCR

Species Dog, cat, rabbit, guinea pig, farm animal, others on request

Duration 1 - 3 days



Toxoplasma - Antibody Detection

Material S, EP, HP 0.5 ml

Method ELISA

Species Dog, cat, rabbit, guinea pig, farm animal, others on request

Duration 1 day

Note Detection of IgG (all species) and IgM (all species except for goats)

**Cat:** Usually, seronegative animals do not excrete oocysts. Increased titres of IgM may indicate the excretion of oocysts. IgG antibodies are indicative of exposure and may also indicate clinical

symptoms in cats.

# 13.4.16 Trichomonads

Trichomoniasis in birds (also called **canker** or **frounce**) is a disease of the gastrointestinal tract, especially of the crop, which is caused by protozoa of the order Trichomonadida. In particular, the flagellates are transmitted through the crop milk or through contaminated drinking water. It is most notably pigeons and finches, but also budgerigars, cockatiels and sometimes other parrots and canary birds that become infected. In pigeons, older animals are often persistently infected, clinically inapparent carriers. Trichomonas gallinae is a pear-shaped flagellate of 5 to 18  $\mu$ m in size that uses small lesions in the mucous membrane to penetrate into the tissue and triggers the characteristic focal, yellowish tumours there. Occurrence of the disease is often associated with stress, vitamin deficiency or other illnesses and in some cases it can lead to the colonisation of inner organs such as the liver and the heart. Clinical signs often include regurgitation of undigested food, but diarrhoea can be an indicator, too. In case of a longer duration of the disease, the animals lose weight and become apathetic. In young birds, the mortality rate can be up to 40%.

#### Trichomonads - Pathogen Detection

Material Swab without medium (crop), lavage sample (crop)

Method PCR
Species Birds
Duration 1 – 3 days

### 13.4.17 Tritrichomonas foetus

Tritrichomonas foetus is a protozoon belonging to the order Trichomonadida. The trophozoite is characterised by three anterior flagella and one posterior flagellum. However, similar to Giardia, these are only microscopically visible in fresh faecal samples.

Transmission between cats occurs by faecal-oral route. Transmission between cattle or pig to cat is not documented.

Affected animals show typical large intestinal diarrhoea with frequent defaecation in small portions; admixtures of mucus and blood may occur. Tenesmus and uncontrolled defaecation are frequently observed. The general condition usually remains unaffected, increases in temperature are rare. T. foetus should always be considered as differential diagnosis in cats suffering from chronic, intermittent diarrhoea.

In cattle, Tritrichomonas foetus causes bovine trichomoniasis, which is characterised by inflammation of the reproductive tract in cows leading to repeat breeding and abortion. Bulls transmit the disease but display no clinical signs. In Germany, **bovine trichomoniasis is notifiable upon suspicion**.

## Tritrichomonas foetus - Pathogen Detection

Material Cat: faeces

Cattle: swab without medium (cervix), preputial wash

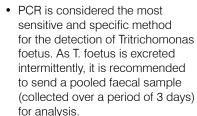
Method Realtime PCR

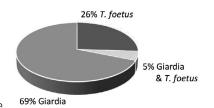
Species Cat, cattle, others on request

Duration 1-3 days

Note

• Especially in cases of faecal incontinence in cats, an infection with Tritrichomonas foetus should be considered. If anamnesis includes the following information "patient responded to therapy against Giardia, afterwards immediate recurrence", it indicates Tritrichomonas foetus.





Tritrichomonas foetus and Giardia: mono an co-infections in infected cats

# 13.4.18 Trypanosoma

# Trypanosoma equiperdum

Infection with Trypanosoma equiperdum, also known as **dourine**, is a chronic or acute infectious disease in equids, which is transmitted directly between animals during mating. Infected equids are the only natural reservoir; the pathogens are present in the genital secretions of both mares and stallions. Incubation period, severity and duration of the disease vary considerably. Subclinical infections are possible; donkeys and mules are more resistant to the pathogen. Clinically, affected animals show inflammation of the outer genitals with depigmentation of the mucosa up to peripheral-neurological disorders/paralysis. Particularly in Asia and Africa, Trypanosoma is still widespread; Central Europe is currently considered free from Trypanosoma equiperdum. Export-relevant test.

## Trypanosma equiperdum (dourine) - Antibody Detection\*

Material S 1 ml
Method CFT
Species Horse
Duration 5 days

Note In Germany, dourine is an epizootic disease that is **notifiable upon** 

suspicion.

# Trypanosoma evansi

Trypansoma evansi is present in North Africa, the Middle East, Latin America and Asia. Transmission mainly occurs mechanically through blood-sucking insects. Infections have been described in various mammals, but especially in camels, cattle and horses. However, dogs can also be infected. In contrast to camels, clinical signs in dogs are often mild.

# Trypanosoma evansi – Pathogen Detection (Antigen)

Material EB 1 ml Method Microscopic Species Dog

Species Dog Duration 1 day

# Trypanosoma evansi – Antibody Detection

Material S 0.5 ml

Method CATT (card agglutination test for T. evansi)

Species Dog Duration 1 day

# 14 Bacteriology/Mycology

# 14.1 Smears/Aspirates/Milk/Faeces

Below, the most frequent requirements for general microbiology are listed. If indicated, special tests can be ordered separately (see Chapters 13.2, p. 201; 13.3, p. 239; 14.3, p. 270; 14.4, p. 271 and 16.1, p. 282).

The bacteriological examination detects **aerobic** pathogens. **Anaerobic** pathogens are not detected in aerobic bacteriology; their examination can either be requested as an individual service or as a combined service Bacteriology (aerobic and anaerobic).

Because of the increase in multidrug-resistant pathogens such as MRSA (methicillin-resistant Staphylococcus aureus) or MRSP (methicillin-resistant Staphylococcus pseudintermedius) as well as in Enterobacteriaceae of the ESBL strains (extended spectrum of beta-lactamases), a cultural examination and a subsequent antibiogram are almost indispensable in case of bacterial infections.

Mycology can be ordered as an individual test or in combination with the bacteriological examination for aerobic pathogens.

#### **Abscess Material**

Parameter Pathogenic agents, aerobic or anaerobic

Method Bacteriological culture (MALDI-TOF) and mycological culture Species Dog, cat, small mammals, birds, reptiles, large animals

Duration Aerobic: 2 – 3 days

Anaerobic: approx. 1 week

If mycology is included: up to 1 week

Note Split the abscess cavity and swab the inside of the abscess

membrane; requesting an anaerobic bacteriological examination is

recommended, too.

#### Aspirates (from primarily sterile body cavities)

Parameter Pathogenic agents, aerobic or anaerobic

Method Bacteriological culture (MALDI-TOF) and mycological culture Species Dog, cat, small mammals, birds, reptiles, large animals

Duration Aerobic: 2 – 3 days

Anaerobic: approx. 1 week

If mycology is included: up to 1 week

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Note

- In case of aspirates from primarily sterile body cavities, the sample material should not be cooled.
- If testing for actinomycetes and Nocardia is required, it needs to be requested separately.

#### **Blood Culture**

Parameter Method Pathogenic agents

Bacteriological culture (MALDI-TOF)

aerobic and anaerobic

Species Duration Dog, cat, small mammals, birds, reptiles, large animals

7 – 10 days

Note

- Please order blood culture bottle(s) in advance (subject to a charge).
- Different blood culture bottles are available (see Chapter 1.9, p. 22).
   If there is a single bottle, analysis of aerobic and anaerobic pathogens is carried out from this bottle. The set contains a separate bottle for the blood sample for anaerobic testing with a transport medium optimised for these pathogens. The set should be preferred if sufficient blood can be obtained from the patient.
- Inoculation is done with 1 3 ml of blood for the single bottle; in the set, each bottle must be inoculated with 5 – 10 ml of blood.
- Blood should be collected during a fever episode.
- It is recommended to send in 2 3 (sets of) blood culture bottles (blood collection at different times, intervals of at least 1 hour).
- · Storage and transport are uncooled.
- Full blood in normal blood tubes (e.g. EDTA tubes) or swabs do not lead to reliable results so we recommend always using a blood culture bottle.
- Bacteraemia may occur physiologically in reptiles.

# Bronchial Lavage, Bronchial Secretion, Tracheal Secretion

Parameter Pathogenic agents, aerobic

Method Bacteriological culture (MALDI-TOF) and mycological culture Species Dog, cat, small mammals, birds, reptiles, large animals

Duration Aerobic: 2 – 3 days

If mycology is included: up to 1 week

Note For microbiological examination, lavage fluid should be sent in using

a swab with transport medium.

## **Cerebrospinal Fluid**

Parameter Pathogenic agents, aerobic or anaerobic

Method Bacteriological culture (MALDI-TOF) and mycological culture

Species Dog, cat, small mammals and large animals

Duration Aerobic: 2 – 3 days Anaerobic: 1 week

If mycology is included: up to 1 week

#### **Ear Smear**

Parameter Method Pathogenic agents, aerobic

(1) Bacteriological culture (MALDI-TOF) and mycological culture

(2) Parasitology

Species Duration Dog, cat, small mammals, large animals

(1) 2 - 3, mycology up to 7 days

(2) 1 day

Note

 Please send in a swab with medium for culture examination. If a parasitological examination is also requested, a swab without medium must be sent in additionally.

 An antimycogram for Malassezia spp. is only performed on special request.

#### Faeces

Parameter

Aerobic facultative and obligate pathogenic bacteria incl. salmonella,

fungi

Method

Bacteriological culture (MALDI-TOF) and mycological culture Dog, cat, small mammals, birds, large animals

Species Dog, cat, sr Duration 2 – 3 days

Duration Note

Diagnostic findings and significance of bacteriological faecal

examination see Chapter 16.1, p. 282.

#### Milk

Parameter

Pathogenic agents, aerobic, including determination of bacterial

count

Method Species Bacteriological culture (MALDI-TOF) Cow, sheep, goat (others: see note)

Duration 2 – 3 days

Note

- Cow: Examination of either 1/4 or 4/4 milk samples is possible.
- Sheep and goat: examination of 2/2 milk sample
- Bacteriological examination of milk samples of other mammalian species can be requested via the standard service Bacteriology.

Swabs (nose, pharynx, urethra, vagina, etc.)

Parameter Pathogenic agents, aerobic or anaerobic

Method Bacteriological culture (MALDI-TOF) and mycological culture Species Dog. cat. small mammals, birds, reptiles, large animals

Duration Aerobic: 2 – 3 days Anaerobic: 1 week

If mycology is included: up to 1 week

Note For detection by culture, a swab with medium is required. A detec-

tion by culture from purulent material is often difficult as the bacteria

are pre-damaged and therefore difficult to breed.

**Urine Bacteriological Culture, Uricult** 

Material Pathogenic agents, aerobic, including determination of bacterial

count

Method Bacteriological culture (MALDI-TOF)
Species Dog, cat, small mammals, large animals

Duration 2-3 days

Note • Submission of urine (cystocentesis or catheter urine or clean-

catch midstream urine) and a swab of urine (swab with medium)

is ideal.

The urine culture test is also offered in combination with the exam-

ination of urinalysis/sediment. In this case, please send in at least

6 ml of urine.

**Wound Swab** 

Parameter Pathogenic agents, aerobic or anaerobic

Method Bacteriological culture (MALDI-TOF) and mycological culture

Species Dog, cat, small mammals, birds, reptiles, large animals

Duration Aerobic: 2 – 3 days

Anaerobic: approx. 1 week

If mycology is included: up to 1 week

# 14.2 Skin/Hair/Feathers

## Skin Swabs

Parameter (1) Pathogenic agents, aerobic

(2) Dermatophytes, yeasts

Method (1) Bacteriological culture (MALDI-TOF)

(2) Mycological culture

Species Dog, cat, small mammals, birds, reptiles, large animals, fish

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Duration (1) 2 - 3 days

(2) Up to 4 weeks

Note For parasitological examination (see Chapter 15.3, p. 281), hairs or

a skin scraping are required.

For fish, we also offer this test in combination with the test for fish

tuberculosis (Ziehl-Neelsen staining).

Skin, Danders, Hairs, Feathers

Parameter (1) Pathogenic agents, aerobic

(2) Dermatophytes, yeasts

(3) Parasites

(4) Pathogenic agents (aerobic), dermatophytes, yeasts, ectoparasites

Method (1) Bacteriological culture (MALDI-TOF)

(2) Mycological culture + paraffin oil preparation

(3) Parasitology: paraffin oil preparation

(4) Bacteriological culture (MALDI-TOF), mycological culture, paraffin

oil preparation

Species Dog, cat, small mammals, birds, reptiles, large animals

Duration (1) 2 - 3 days

(2, 4) Up to 4 weeks

(3) 1 day

Note In case of pathogenic yeasts an antimycogram can be performed on

special request.

It is absolutely necessary to collect samples from the edge of the

skin lesions.

Trichogram/Pennogram

Parameter Current condition of coat/plumage

Method Microscopy

Species Dog, cat, small mammals, birds, reptiles

Duration 1 da

Note In skin patients, a trichogram serves as an additional method for

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diagnostic assessment. It cannot replace histology, bacteriological, mycological and parasitological examinations, cytology and other tests, such as the determination of clinical-chemical parameters or hormone assays, but it can provide very valuable information. Trichograms are particularly suitable in the diagnosis of cats with hair loss that have no apparent itching and for which alopecia sine causa is already clinically suspected. A trichogram can also provide valuable diagnostic information in case of colour mutant alopecia.

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# 14.3 Bacteriological Examination Horse

#### BAL Profile > see Chapter 18.3, p. 296

# **Reproductive Fitness**

Material Swab with medium

Mare: cervical swab

Stallion: shaft swab, urethral swab or glans penis swab

Parameter

Pathogenic agents, aerobic

Method Bacteriological culture (MALDI-TOF) and possibly mycological culture

Species Horse

2 – 3 days, mycology: 7 days

Duration Note

• This test can be ordered with or without mycological examination.

 Culture examination for reproductive fitness is also offered in combination with CEM detection (culture). In this case, please send in an additional swab with Amies medium with charcoal.

 Furthermore, we offer a combination of bacteriological and mycological culture as well as pathological examination of 1 – 3

endometrial biopsies for mares.

### Streptococcus equi

Material (1) Swab with medium (nose, abscess, lymph node), lavage sample

(guttural pouch, pharynx, BAL), TBS

(2) Swab without medium (nose), lavage sample (guttural pouch, BAL), TBS, lymph node pus, tissue (lymph node)

Method (1) Bacteriological culture (MALDI-TOF)

(1) Dacteriological culture (IVI)

(2) Realtime PCR

Species Horse

Duration (1) 2 - 3 days

(2) 1 - 3 days

Note

In culture, both subspecies (Streptococcus equi equi and Streptococcus equi zooepidemicus) are determined and differentiated.

 If detection is to be done by means of PCR, it can be chosen between the single detection of Streptococcus equi equi or the detection of both subpression mentioned above.

detection of both subspecies mentioned above.

### Taylorella equigenitalis (CEM)

Material Mare: cervical or clitoral swab

Stallion: shaft swab, urethral swab or glans penis swab

(1) Swab with medium (Amies with charcoal, not older than 48 hours)

(2) Swab with medium (Amies with charcoal), sperm

Method (1) Bacteriological culture (MALDI-TOF)

(2) Realtime PCR

Species Horse

Note

Duration (1) 7 days, for export up to 21 days

(2) 1 - 3 days

 Culture: For exports to Canada, the incubation period is 14 days, to Norway 21 days, otherwise 7 days.

 Even after successful bacteriological cultivation, it is not possible to create an antibiogram for CEM.

 Detection by PCR is offered as an individual service for one sample or as CEM profile for the examination of several sites (see Chapter 2.3.4, p. 58). The CEM Profiles stallion 1 and mare 1 are suitable as PCR detection before export to another EU

• In Germany, there is an obligation to notify the authorities.

# 14.4 Specific Pathogen Detection

# Actinomyces, microaerophilic

Material Aspirates, swab with medium, etc.

Method Bacteriological culture

Species Dog, cat, small mammals and large animals

Duration Approx. 8 days

Note This examination is offered as service "Nocardia/Actinomyces".

## Bordetella bronchiseptica (aerobic)

Material (1) Swab with medium (Amies) (nose, pharynx), bronchial secretion

(2) Swab without medium, bronchial secretion, BAL

Method (1) Bacteriological culture (MALDI-TOF)

(2) Realtime PCR

Species Dog, cat, rabbit, cattle, sheep, goat, pig, others

Duration (1) 2 - 3 days

(2) 1 - 3 days

Note When requesting a bacteriological examination, please indicate

clearly on the submission form that Bordetella bronchiseptica should

be tested, as special culture media are required.

**Dermatophilus congolensis** 

Material Scurfs

Method Slide – quick staining Species Horse, ruminants

Duration 1 day

Note Hair alone is not sufficient as sample material.

# **ESBL – Pathogen Detection**

Material Swab with medium, faeces

Method Culture

Species Dog, cat, horse, cattle, others

Duration 3 – 4 days

Note

- Extended-spectrum β-lactamase (ESBL)-producing bacteria of the order Enterobacteriales such as E. coli, Klebsiella sp. and Proteus sp. are called ESBL. Due to the specific β-lactamase with extended spectrum of activity, the bacteria are resistant to β-lactam antibiotics including cephalosporins (also to 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins). The property of ESBL formation is encoded on easily transferable genetic segments and, during reproduction, can be transferred from one bacterial generation to the next (vertical transfer) or exchanged between bacteria (horizontal transfer).
- This test is performed in addition to the bacteriological examination.
- Testing for ESBL is also part of the service "Analysis of Multidrugresistant Bacteria" (see below).

# Analysis of Multidrug-resistant Bacteria

# (MRSA, VRE, ESBL, Carbapenemase producer)

Material

4 swabs with medium

- Swab 1: nasal-buccal
- Swab 2: skin (armpit or groin) or conjunctiva
- Swab 3: pooled swab from the animal's environment (dog basket
- + food bowl + floor)
- Swab 4: rectal swab

Method

Culture, microdilution

3 – 4 days

Duration

Note

- Phenotypic detection of resistance
- This profile is used to specifically screen for bacteria that are resistant to critically important antibiotics in order to identify asymptomatic carriers (e.g. therapy dogs in hospitals and care facilities). Particularly critical resistances occur in methicillinresistant staphylococci (mainly Staphylococcus aureus and S. pseudintermedius in dogs), vancomycin-resistant enterococci

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(mainly Enterococcus faecium and E. faecalis) and enterobacteria resistant to 3rd and 4th generation cephalosporins and to carbapenem antibiotics.

- Swabs 1 3 are used to detect MRSA; swab 4 is used to detect vancomycin-resistant enterococci and multidrug-resistant gramnegative bacteria.
- The sampling sites are recommendations. If other sites are prescribed by the hospital or the respective facility, please follow these recommendations.
- The profile provides phenotypic detection of resistance of selected bacterial isolates. It does not test for resistance genes by molecular biology.
- The test result reflects the current colonisation of the animal, so it needs to be considered whether regular screenings should be carried out.

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Ν	О	ca	ra	ıa

Material Aspirates, swab with medium, etc.

Method Bacteriological culture

Species Dog, cat, small mammals and large animals

Duration Approx. 8 days

Note This examination includes the detection of Actinomyces.

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### Paenibacillus larvae (aerobic)

Material Honey and wax sample

Method Bacteriological culture (MALDI-TOF)

Species Be

Note

- Two tablespoons of feed honey from the honey dome should be scratched from a central brood comb, packed into a freezer bag and sent in
- In Germany, American (malignant) foulbrood is an epizootic disease that is notifiable upon suspicion.

# 14.5 Susceptibility Testing

All antibiograms are performed by microdilution method according to CLSI standard.

#### Antibiogram (aerobes)

There are species-specific standard programs (see note). An antibiogram is invoiced as a fixed price per bacteriological culture, even if several antibiograms need to be performed.

Duration 2-3 days

Note The antibiograms include the following number of antibiotics (at the

time of printing)
• small animals: 31

• rabbits and rodents: 29

• birds: 25

reptiles and amphibians: 19

• large animals: 34

• fish: 19

## Antibiogram (anaerobes)

We can also perform an antibiogram if anaerobes are detected. Only antibiotics with potential efficacy against anaerobes are tested.

Duration 5-7 days

Note If the antibiogram is already ordered together with the test for anae-

robes, the duration mentioned above only applies from the time of pathogen identification (the time at which you receive the findings on

the anaerobes detected).

# **Antibiogram Extended**

The complexity of an extended antibiogram depends on the respective species.

Duration 2-3 days

Note The extended antibiograms include the following number of anti-

biotics (at the time of printing)

small animals: 10rabbits and rodents: 8

• birds: 8

• fish: 6

• reptiles and amphibians: 7

horse: 9ruminants: 5pig: 6

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#### **Antimycogram**

We can perform an antimycogram if yeasts or Malassezia have been cultured. This needs to be requested, though. We store the cultivated yeasts and Malassezia for one week.

Duration 2 – 5 days

Analysis of Multidrug-resistant Bacteria > see Chapter 14.4, p. 272

# 14.6 Additional Susceptibility Testing

#### **Aromatogram Bacteria or Yeasts**

The aromatogram is an in vitro test for testing the sensitivity of bacteria or yeasts/ Malassezia to various essential oils. The procedure is based on the principle of the agar diffusion test (disk diffusion test) or the microdilution method.

The in vitro efficacy of essential oils is classified into 4 categories: from ineffective to slightly, moderately and highly effective.

Duration Bacteria: 2 days

Yeasts: up to 7 days

# 15 Parasitology

# 15.1 Parasitological Examination – Faeces

Below, the most frequent requirements for parasitological faecal examination are listed. For the enrichment by flotation or SAFC method (sodium acetate-acetid acid-formalin concentration) we need a cherry-sized amount of faeces, if possible a 3-day pooled faecal sample. For serological detection by EIA, a pea-sized amount is usually sufficient.

## **Egg Count: Modified McMaster Method**

Counting of worm eggs is done using a counting chamber after enrichment by flotation. This method is primarily used in horses, sheep and other farm animals to carry out targeted or selective deworming to reduce the development of resistance to strongyles. In targeted or selective deworming, individual deworming is only carried out if there are > 200 eggs per gram of faeces.

If all animals of a population that have a worm infestation are dewormed, only resistant worms survive. However, if only animals that have a more severe worm infestation are dewormed, an untreated worm population is also found in the animal population, which reduces the selective advantage of resistant worms and thus counteracts the further increase in resistance.

Duration 1 day

# **Egg Count Reduction Test**

The egg count reduction test is used to check whether there is resistance to anthelmintics. To do so, the number of worm eggs in the faeces is counted before and after deworming. The number of eggs per gram of faeces is determined with the **modified McMaster method.** Within the concept of selective deworming, these results are used to carry out a targeted deworming of only those animals that have > 200 eggs per gram of faeces. 10 – 14 days after treatment, further individual samples are tested using the modified McMaster method. If high egg counts are still present, anthelmintic resistance should be considered. This method is mainly used in the large animal practice in ruminants, horses and pigs.

Even if deworming does not take place selectively at the level of the individual animal but, for example, of groups of animals, regular monitoring of the effectiveness of anthelmintics by means of an egg count reduction test is recommended.

Duration 1 day

# **Endoparasites (Protozoa and Worms)**

Material Faeces

Method Microscopy after enrichment by flotation and SAFC method

Species All Duration 1 day

Note

 As parasite eggs or protozoa are only shed intermittently, the test needs to be repeated if there is any suspicion (it is best to send in 3 consecutive samples).

• Each sample is tested by flotation and SAFC method.

 Horse: From the eggs, differentiation of large and small strongyles is not possible: a larval culture would be required here.

#### Lungworm Larvae (Baermann Test)

Material Faeces

Method Baermann technique

Species Dog, cat, small mammals, large animals

Duration 2 days

Note

A Baermann test is indicated in cases of chronic cough and dyspnoea because of possible lungworm larvae infections.

The lungworm Angiostrongylus vasorum cannot only be detected by Baermann test, but also by PCR in blood or BAL (see Chapter 13.4.1, p. 245).

# **Endoparasite Profiles**

# Canine Endoparasite Profile

Material Faeces
Method Flotation, EIA

Species Dog Duration 1 – 2 days

Note

Analysis is done for endoparasites and Giardia sp. antigen (EIA).

This profile can only be ordered for dogs. For other animal species,

the parameters can be ordered individually.

#### **Endoparasites (Reptiles)**

Material Faeces

Method Microscopy after enrichment by flotation and SAFC method,

Ziehl-Neelsen staining, fresh specimen

Species Reptiles
Duration 1 day

Note As parasite eggs or protozoa are only shed intermittently, the test

needs to be repeated if there is any suspicion.

#### Endoparasites + IFAT (starting 1st July 2022)

Material Faeces (3-day pooled faecal sample)

Method Flotation and IFAT

Species Dog, cat Duration 1 day

Note The profile Endoparasites and IFAT includes the microscopic

detection of helminths and protozoa by means of flotation and the

detection of giardia and cryptosporidia by means of IFAT.

# **Equine Endoparasite Profile**

Material Faeces

Method Flotation, SAFC method and modified McMaster method

Species Horse
Duration 1 day

# Feline Endoparasite Profile

Material Faeces

Method Flotation, EIA, realtime PCR

Species Cat
Duration 1 – 3 days

Note • Test for endoparasites, Giardia sp. antigen (EIA) and Tritrichomonas

foetus (PCR).

• This profile can only be ordered for cats. For other animal species,

the parameters can be ordered individually.

# Ferret and Chinchilla Parasite Profile (starting 1st July 2022)

Material Faeces
Method Flotation, EIA
Species Chinchilla, ferret
Duration 1 – 2 days

Note Analysis is done for endoparasites and Giardia sp antigen (EIA).

#### Hedgehog Parasite Profile (starting 1st July 2022)

Material Faeces

Method Flotation, SAFC method and Baermann test

Species Hedgehog Duration 2 days

Note Analysis is done for endoparasites and lungworm larvae.

#### **Ruminant Endoparasites**

Material Faeces

Method Flotation, SAFC method and Baermann technique

Species Cattle, sheep, goat

Duration 2 days

# 15.2 Testing for Specific Parasitic or Protozoa Infections

#### Cryptosporidia – Pathogen Detection

Material Faeces, in snakes also: regurgitated material, gastric lavage,

stomach biopsy

Method (1) Antigen detection: EIA, IFAT (reptiles)

(2) Modified Ziehl-Neelsen staining

(3) PCR

Species Dog, cat, small mammals, reptiles, cattle, others

Duration (1) IFAT: 1 day, EIA: 2 days

(2) 1 day (3) 1 – 3 days

Note If the PCR yields positive results in reptiles, differentiation of the

Cryptosporidium species is possible on request. This is done to distinguish between harmless intestinal passengers (origin: infected

feeder animals) and pathogenic Cryptosporidia.

# **Echinococci - Pathogen Detection**

Material Faeces Method PCR

Species Dog, cat, fox Duration 1 – 3 days

Note Detection by PCR can reveal infections with E. granulosus and

E. multilocularis, while microscopy after enrichment is often only

able to detect non-differentiable Taenia eggs.

Echinococcosis is a **notifiable disease** in Germany.

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**Echinococcus – Antibody Detection** 

Material S, HP 1 ml
Method ELISA
Species Dog
Duration 5 days

Note Antibodies against E. multilocularis are detected. **Notifiable** 

disease in Germany, see above.

Fasciola hepatica (Liver Fluke) - Antibody Detection

Material S, HP, milk, tank milk 0.5 ml

Method EIA
Species Cattle
Duration 3 days

Giardia - Pathogen Detection

Material Faeces

Method (1) Microscopy after enrichment

(2) EIA (antigen detection)

(3) Realtime PCR

Species Dog, cat, small mammals, reptiles, large animals

Duration (1 and 2) 1 day

(3) 1 - 3 days

Note If examination should also clarify whether human-pathogenic as-

semblages A and B are present, PCR is available as an alternative

detection method (see Chapter 13.4.9, p. 255).

Nosema - Pathogen Detection

Material 30 – 40 dead bees Method (1) Microscopy

(2) PCR (differentiation)

Species Bees
Duration 1 – 2 days

Note If the result of the microscopic examination is positive, PCR can

differentiate between Nosema apis and Nosema ceranae.

Ostertagia ostertagi - Antibody Detection

Material Milk, tank milk 0.5 ml

Method EIA
Species Cattle
Duration 3 days

Toxoplasma gondii ➤ see Chapter 13.4.15, p. 261

Tritrichomonas foetus > see Chapter 13.4.17, p. 262

# 15.3 Parasitological Examination – Skin

Material Skin scraping
Method Microscopy

Species Dog, cat, small mammals, large animals

Duration 1 day

Skin

Note The detection of ectoparasites is done by examination of skin

scrapings, which should possibly be placed into shipping containers

before they are sent in.

If mites are suspected, the depth of the scraping should be adapted

to the habitat of the respective mite.

Ectoparasite detection is also part of the service Bacteriology +

Mycology + Ectoparasites.

# 16 Tests for Indigestion and Diarrhoea

# 16.1 Bacteriological Examination

The physiological intestinal flora consists of numerous bacterial species that live together with the host in a complex symbiotic ecosystem. Shortly after birth and the suckling phase, the gastrointestinal flora is established and remains largely stable for the rest of the life.

However, within the intestinal tract, there are considerable differences in distribution. While pathogen counts in the duodenum and the jejunum are rather low due to the influence of gastric acid, bile and pancreatic enzymes as well as the present mucosal defence systems, they massively increase in the ileocecal area and reach their highest concentration in the large intestine. The number of anaerobes and facultative anaerobic pathogens is 1,000 to 10,000 times higher than the number of aerobic microflora. The highest concentrations are reached by Bacteroides spp., lactobacilli and bifidobacteria as well as Enterobacteriaceae.

#### 16.1.1 Faecal Profiles

If possible, please submit a faeces tube that is  $\frac{3}{4}$  full. When doing a culture test, an aerobic bacteriological and possibly mycological examination, including enrichment for salmonella, is performed. **Pathogen differentiation** is done by **MALDI-TOF**. Unless otherwise stated, the test duration is 2-3 days.

If required, **serological pathogen differentiation** (e.g. salmonella) and an **antibiogram**, which are subject to a charge, will be performed additionally.

# Dog and Cat

#### **Combined Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, gas producers, endoparasites as well as Giardia sp. antigen EIA and Cryptosporidia antigen EIA

#### **BARF Faecal Profile**

Salmonella including enrichment, yersinia including enrichment, campylobacter, listeria, endoparasites

Duration 3 days; yersinia: 28 days

Note See also clinical chemical BARF Profile (Chapter 2.1.1, p. 31)

# Faecal Profile Pathogenic Bacteria

Salmonella including enrichment, yersinia including enrichment, campylobacter, enteropathogenic E. coli incl. virulence factors (STa, stx1, stx2, eae)

Duration 2 – 3 days; yersinia: 28 days

#### Faecal Profile Puppy/Kitten

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, gas producers, parvovirus, endoparasites, Giardia sp. antigen EIA

## **Large Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, Clostridium perfringens enterotoxin, Clostridioides difficile toxin A and B, gas producers, endoparasites, Giardia sp. antigen EIA

#### **Small Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, gas producers

# Dog and Cat - Faecal Profiles PCR

# Diarrhoea, Human Pathogenic Causes

Salmonella, Yersinia enterocolitica, Campylobacter jejuni

# **Diarrhoea Pathogens Cat**

Coronavirus, Tritrichomonas foetus, giardia, parvovirus, cryptosporidia

# **Diarrhoea Pathogens Dog**

Coronavirus, parvovirus, circovirus, giardia, cryptosporidia

# **Dysbiosis Profile**

Key bacteria intestinal microbiome quantitative (see Chapter 16.5, p. 292), mycology, calprotectin, endoparasites;

until 30<sup>th</sup> June 2022:  $+ \alpha$ -1 antitrypsin,

from 1<sup>st</sup> July 2022 onwards: + pancreatic elastase (dog) and microscopic nutritive digestion (cat) (duration 1 week)

# Small Mammals, Birds and Reptiles

#### **Avian Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites

Note

This profile is also available without the detection of endoparasites.

#### **Ferret Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites, Giardia sp. antigen EIA

#### **Pigeon Faecal Profile**

Salmonella incl. enrichment, endoparasites (incl. coccidia)

#### **Reptile Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella

#### **Rodent and Rabbit Faecal Profile**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites

#### Horse

Dysbiosis Analysis > see Chapter 16.5, p. 292

#### **Faecal Profile Foal**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, gas producers, rotavirus, Clostridium perfringens enterotoxin, endoparasites incl. protozoa, strongyloides

# Large Faecal Profile (horse)

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, Clostridium perfringens enterotoxin, Clostridioides difficile toxin A and B, gas producers, endoparasites, equine coronavirus

# **Small Faecal Profile (horse)**

Bacteriology (aerobic) and mycology, obligate and facultative pathogenic bacteria including enrichment for salmonella, endoparasites

#### Ruminants

#### **Bovine Faecal Profile**

In addition to an aerobic bacteriological, mycological examination and the test for obligate and facultative pathogenic bacteria, including enrichment for salmonella, as well as testing for endoparasites, this Bovine Faecal Profile also includes the detection of Mycobacterium avium ssp. paratuberculosis by means of PCR.

#### Calf Faecal Profile - Large

The Calf Faecal Profile Large includes the general aerobic bacteriological and mycological examination, including enrichment for salmonella and, if E. coli is present, its serological typing (K99). Furthermore, this profile includes testing for endoparasites, cryptosporidia and coccidia as well as the virological examination for rotavirus and coronavirus. If salmonella are detected, they will be serologically typed as an additional service (subject to a charge).

#### Calf Faecal Profile (EIA)

The Calf Faecal Profile includes testing for rotavirus and coronavirus, E. coli K99 and cryptosporidia. The advantage of ELISA testing is the short test duration (1 day).

# Camelid Faecal Profile (Alpaca, Llama)

This profile includes the general aerobic bacteriological and mycological examination, including salmonella, testing for endoparasites, incl. coccidia, cryptosporidia as well as the virological examination for rotavirus and coronavirus.

# Pig

# **Piglet Faecal Profile**

The Piglet Faecal Profile includes the general aerobic bacteriological and mycological examination, including salmonella, testing for endoparasites, the virological examination for rotavirus and coronavirus as well as the test for Clostridium perfringens enterotoxin. If salmonella or E. coli (K88) are detected, they will be serologically typed as an additional service (subject to a charge).

#### **Porcine Faecal Profile**

The Porcine Faecal Profile includes the general aerobic bacteriological and mycological examination, including salmonella, as well as the detection of Lawsonia intracellularis by means of PCR.

Faeces

# **LABOKLIN**

# 16.1.2 Single Determinations

# Campylobacter

Note

Material (1) Faeces, swab with medium (intestine, cloaca)

(2) Faeces, swab without medium (intestine, cloaca)

Method (1) Bacteriological culture (MALDI-TOF)

(2) Realtime PCR (only detection of Campylobacter jejuni)

Species No limitations known

Duration (1) 2 - 3 days

(2) 1 - 3 days

 A combined detection of campylobacter and yersinia by culture is also available.

> Resistances are common; treatment should therefore only be carried out after an antibiogram has been performed. Preparation of an antibiogram is only possible after performing a culture test.

• In dogs, feeding a barf diet is a source of infection for C. jejuni.

Campylobacter of the species C. jejuni, C. coli, C. lari and C. upsaliensis are classified as thermophilic campylobacter.

 In Germany, campylobacteriosis (thermophilic campylobacter) is a notifiable disease in dogs, cats, ruminants and poultry.

• For genital infection cattle, sheep, see Chapter 13.2.9, p. 209.

#### Clostridioides difficile Toxin A and B

Material Faeces Method ELISA

Species No limitations known

Duration 1-2 days

Determination is specifically indicated if colitis-like symptoms occur.

A faecal sample at least the size of a cherry is required.

#### Clostridium perfringens Enterotoxin

Material Faeces Method ELISA

Species No limitations known

Duration 1 - 2 days

Note

Determination is specifically indicated if colitis-like symptoms occur.

A faecal sample at least the size of a cherry is required.

 In carnivores, Clostridium perfringens enterotoxin can cause diarrhoea and vomiting of varying severity; enterotoxaemia is rare.
 Toxin formation is induced by antibiotic administration, stress, coinfections or especially by an unbalanced diet rich in proteins and connective tissue. E. coli, eae Gene (Intimin)

Method PCR after prior detection of E. coli by culture

PCR detection of the eae gene

Species Calf, piglet Duration 3 days

Note The eae gene is a virulence factor of E. coli. The eae gene (E. coli

attaching and effacing) encodes the production of intimin, which

permits E. coli to attach itself to the intestinal cells.

#### Helicobacter

Note

Material

Material Vomitus, gastric lavage, gastric biopsy

Method PCR

Species Dog, cat, hamster, mouse, ferret

Duration 1-3 days

 Positive PCR results from faecal samples do not necessarily indicate involvement of the stomach (gastritis, stomach ulcer, etc.), as PCR also detects intestinal Helicobacter spp. For this

diagnostic task, stomach biopsies or vomitus are recommended
In Muridae, helicobacter can cause typhlitis and rectal prolapse, in sheep, however, it can cause abortions (see Chapter 13.2.17,

p. 218)

## Macrorhabdus ornithogaster

Material Faeces, smear on slide, crop lavage, proventriculus

Method Stain, microscopy

Species Birds Duration 1 day

Note A faecal sample possibly the size of a pea is required.

# Mycobacteria (Microscopic Detection of Acid-fast Rods)

Material Faeces, smear on slide

Method Ziehl-Neelsen staining, microscopy

Species No limitations known

Duration 1 day

Note A faecal sample with a diameter of at least 2 cm is required.

For fish, we also offer this test in combination with the bacteriological examination (service Bacteriology Fish + Fish Tuberculosis, material:

tissue or swab, see Chapter 2.6, p. 68).

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# **16.2.2 Single Determinations**

Material Faeces, in pigs also tissue (intestine)

Method Realtime PCR Ferret: PCR

Quantitative PCR (cat): droplet digital PCR

Species Dog, cat, ferret, horse, ruminants, pig

Duration 1-3 days

Note

 In small animals, a pooled faecal sample is recommended as it increases sensitivity.

 Cat: Quantitative PCR (from a 3-day pooled faecal sample) is available to evaluate the pathogen excretion, e.g. during rehabilitation of the cat population. For more information see Chapter 13.1.14, p. 166.

 Pathogen detection by means of an antigen test is part of the Virological Faecal Profile (EIA) or the Calf Faecal Profiles (see Chapter 2.4.4, p. 65).

• Bovine coronaviruses also cause respiratory diseases (see Chapter 13.1.14, p. 164).

For SARS-CoV-2 see Chapter 13.1.45, p. 197.

# Parvovirus - Pathogen Detection

Material Dog: qualitative PCR: faeces, EB 0.2 ml, tissue (e.g. intestine or heart)

quantitative PCR: faeces

Cat: faeces. EB 0.2 ml

Ferret: faeces, EB (viraemia), swab without medium (rectum), tissue

(e.g. spleen, lymph node or bone marrow)

Method Realtime PCR

Ferret: PCR

Quantitative PCR (dog): droplet digital PCR

Species Dog, cat, ferret 1 – 3 days

Note • PCR c

 PCR can be positive up to four weeks after vaccination with live vaccine.

 In dogs, differentiation between vaccine strain and field strains is possible on request (see Chapter 13.1.35, p. 190).

• In dogs, quantitative PCR is also possible from faeces (see Chapter 13.1.35, p. 190).

 Direct detection of parvovirus in the blood is possible approx. 1 – 5 days after infection.

• Equine parvovirus hepatitis virus see Chapter 13.1.35, p. 190.

 Porcine parvovirus causes fertility disorders (SMEDI, see Chapter 13.1.35, p. 190).

Salmonella Material (1) Faeces, (intestinal swab or cloacal swab) (2) Faeces; in birds also swab without medium (cloaca), eggs, tissue Method (1) Bacteriological culture including enrichment, MALDI-TOF (2) Realtime PCR No limitations known Species Duration (1) 2 - 3 days(2) 1 - 3 daysNote Culture with enrichment is the most sensitive test method. After successful culture cultivation, a serological pathogen differentiation follows (subject to a charge).

Yersinia Material Faeces Method (1) Culture (MALDI-TOF) with cold enrichment (2) Realtime PCR (only Yersinia enterocolitica) Species No limitations known Duration (1) 4 weeks (2) 1 - 3 days Note • A faecal sample at least the size of a cherry is required. In exceptional cases, a swab with transport medium can also be used for culture. • After successful culture cultivation, a serological pathogen differentiation follows (subject to a charge). A combined detection of campylobacter and versinia by culture is

# **16.2 Virological Examination**

also available.

# 16.2.1 Faecal Profiles - Virology

Diarrhoea Profiles (dog/cat) > see Chapter 2.1.6, p. 43

# Virological Faecal Profile (dog, cat)

Parvovirus, rotavirus, coronavirus

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# Parvovirus - Antigen Detection

Material Faeces
Method EIA
Species Dog, cat
Duration 1 day

Note A faecal sample at least the size of a cherry is required. The test may

yield positive results 5 – 12 days after vaccination with live vaccine.

#### Rotavirus - Antigen Detection

Material Faeces Method EIA

Species Dog, cat, horse, cattle

Duration 1-2 days

Note A faecal sample at least the size of a cherry is required.

# 16.3 Tests for Maldigestion/Malabsorption

A faecal sample the size of a cherry is required.

#### **Bile Acids**

Material Faeces
Method ELISA
Species Dog, cat
Duration Up to 7 days

Note Bacterial overgrowth of the small intestine or a shortened intestinal

passage after surgery can lead to diarrhoea which causes a loss of bile acids. The symptoms are aqueous diarrhoea or steatorrhoea.

# **Microscopical Nutritive Digestion**

Material Faeces
Method Microscopy
Species Dog, cat
Duration 1 day

Note This is a semi-quantitative detection of undigested food compo-

nents, which depends on the type and composition of the diet. Increased excretion of starch, neutral fats, fatty acids and muscle fibres can therefore only indicate a reduced digestive and absorptive

capacity (maldigestion or malabsorption).

#### Pancreatic Elastase E1

Material Faeces
Method EIA
Species Dog
Duration 2 days

Note This is a functional test for the diagnosis of exocrine pancreatic

insufficiency in dogs.

Elastase is pancreas-specific, stable in the intestine, and the test

results are not altered by a substitution therapy.

#### **Particle Size**

Material Faeces
Method Measurement
Species Horse

Duration 1 day

Note The particle size provides information about insufficient chewing of

the feed components.

Dental examination should follow. Furthermore, the ration should be checked with regard to the amount of components that are difficult to digest (e.g. excessive feeding of straw) and to structure (e.g.

sufficient fibre length).

# 16.4 Determination of an Inflammatory Exudative Process

For these tests, a faecal sample approximately the size of a cherry is required.

#### Calprotectin

Material Faeces
Method Turbidimetry
Species Dog, cat
Duration 1 day

Note Biomarker used to diagnose an acute or chronic inflammatory

intestinal disease.

Calprotectin is also part of the Dysbiosis Profile (see Chapter 2.1.6,

p. 43).

#### **Chemical Detection of Blood**

Material Faeces

Method Chemical (modified Guajak test)

Species No limitations known

Duration 1 day

Note To avoid false-positive results, carnivores should not be fed any

meat for 3 – 4 days.

# 16.5 Microbiome Analysis

The microbiome is the total of all microorganisms that colonise the body surfaces and the inside of an animal. Most of them are located in the colon ( $10^{11} - 10^{12}$  bacteria/g of faeces). 99.9% of the bacteria that grow under these conditions are anaerobes. The main function of these organisms is the nutrition and protection of the mucous membrane. If there are bacterial imbalances, these tasks can only be fulfilled insufficiently. As a result, colonisation resistance is reduced and increased colonisation with obligatory or facultative pathogens takes place. Due to the reduced barrier function of the mucosa, antigens, endotoxins and histamine, amongst others, can pass from the intestinal lumen into the bloodstream and thus initiate or aggravate pathomechanisms.

#### **Dysbiosis Analysis**

Material Faeces Method PCR

Species Dog, cat, horse Duration 3 – 5 days

Note

- Key bacteria intestinal microbiome quantitative (incl. anaerobes)
- Indications for the examination of the microbiome:
  - chronic diarrhoea, flatulence, constipation
- exocrine pancreatic insufficiency, deficiency symptoms
- disorders of the immune system (immune deficiency, feed allergies, atopic dermatitis)
- inflammatory intestinal diseases (also for therapy monitoring)
- leaky gut
- loss of performance
- diagnosis of microflora dysfunction after antibiotic therapy
- It is also possible to perform this test during treatment with synbiotics or probiotics!
- For dogs and cats, microbiome analysis is also part of the Dysbiosis Profile (see Chapter 16.1, p. 283)
- Possible treatment option autovaccines: see Chapter 17, p. 293.

# 17 Autogenous Vaccine (Autovaccine)

For chronic recurrent bacterial infections, treatment with an autogenous vaccine (autovaccine) is an alternative and promising option. Such treatment also helps to counteract the development of resistance, as it can often reduce or avoid the administration of antibiotics.

Autogenous vaccines are individually produced for each animal from their own aerobic pathogens which are relevant for the infection. It is also possible to produce an autovaccine for several animals that show the same signs. The aim of the treatment with autovaccines is to sensitise the immune system to the isolated pathogen(s) and to stimulate the formation of specific antibodies.

Pathogen concentration, application method (see Chapters 17.1 and 17.2) as well as application quantity, intervals and duration depend on the sampling site, preliminary report and species. It should be noted that an autovaccine can only reach its full potential if underlying diseases have been excluded in advance through extensive diagnostics.

## Preparation of an autovaccine

Order Must be done in writing.

We need a **prescription** from your veterinary practice/clinic for the

preparation of this product!

Species We prepare autovaccines for the following species:

Dog, cat,

Rabbit, rodents, ferret,

Parrot, budgerigar, mynah, hawk, pigeon, ostrich,

Snake, Horse,

Llama, alpaca,

Gibbon, orang-utan, gorilla, marmosets, giant panda, lion, tiger,

elephant, tapir, elk, giraffe, Bactrian camel, kangaroo

(Applies only to non-food producing animals.)

Method An aerobic microbiological examination is performed of a sample of

the affected organ system and the relevant pathogens are isolated. They are multiplied in pure culture, then inactivated and subsequent-

ly used to produce the vaccine.

Duration 3 weeks

Delivery Exclusively to the veterinary pharmacy

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# 17.1 Autovaccine

Material

Swab with medium, hairs, etc.

Note

• Indications: chronic skin/ear infections (e.g. Staphylococcus pseudintermedius), respiratory infections

• For ordering, species, method and duration, see introduction.

#### **Oral Vaccine**

Material

Faeces (possibly also faecal swab with medium)

Note

• Indications: chronic diarrhoea, faecal water syndrome horse

• For ordering, species, method and duration, see introduction.

• Chronic diarrhoea is one of the indications for microbiome analysis (see Dysbiosis Analysis/Profile in Chapter 16.5, p. 292).

#### Vaccine for Inhalation

Material

Swab with medium (respiratory tract), BAL, tracheal lavage

Note

- Indications: chronic respiratory infections (nasopharyngeal area)
- For ordering, species, method and duration, see introduction.

# 17.2 Combination Vaccine

#### **Combination Vaccine (Oral and Injection Vaccine)**

Material

Swab with medium (e.g. vagina), urine

Note

• Indications: chronic urogenital infections

• For ordering, species, method and duration, see introduction.

# 18 Pathology

# 18.1 Histopathology

#### **Endometrial Biopsy (mare)**

Material

1-3 tissue samples approx.  $1.0\,x\,1.0\,x\,0.5$  cm (1 x corpus, 2 x cornua

uteri), formalin-fixed (4% neutral buffered formaldehyde ≜ 10%

formalin)

Method Duration Microscopy (standard und special stainings)

2 – 4 days

Note

 For the following clinical issues: breeding suitability test, barren mare, abortion, etc.

 Histological diagnosis of endometritis, endometrosis, angiopathies, (pathological) inactivity, lymphatic lacunae, false differentiations, etc.

 Fertility prognosis (categorisation according to Kenney & Doig 1986, mod. according to Schoon et al. 1992)

 Endometrial biopsy can also be requested via the standard service Pathology and as a combined service together with reproductive fitness and mycological examination.

#### Histopathology

Material

 Formalin-fixed tissue samples (fixation in 4% neutral buffered formaldehyde 

10% formalin; if there is a risk of freezing, please add a maximum of 10 vol% abs. of alcohol to prevent the sample from freezing)

For dermatologic issues use skin punches (≥ 0.6 cm).
 Microscopy (standard und special stainings)

Method Duration

2 – 4 days

Note

Fill in the submission form Pathology.

# 18.2 Immunohistology

# Immunohistology

Note

Material Formalin-fixed and/or paraffin-embedded tissue samples

Method Microscopy (labelling with specific antibodies)

Duration Approx. 1 week

Examination only after histopathological diagnosis, e.g. tumour

diagnosis.

• CD3/CD20 in lymphoma diagnosis

• c-kit expression pattern in mast cell tumours

• Ki-67 antigen as proliferation marker

 Cox-2, prostaglandin synthesis enzyme, in tumours possibly an indicator of the effectiveness of inhibitors (NSAIDs)

 Differentiation between epithelial/spindle cell/round cell/ melanocytic tumours

Infection diagnosis:

• e.g. FIP virus

# 18.3 Cytology

**BAL Profile** 

Material	Bronchoalveolar lavage

Method Cytology,

culture (bacteriology, mycology).

molecular biology: PCR (dog), realtime PCR (cat)

Species Dog, cat, horse Duration 2 – 7 days

Note The profile includes cytology, bacteriology and mycology, as well as

analysis for mucous membrane-associated mycoplasma in dogs and

Mycoplasma felis in cats.

# Cytology

Material Aspirate, air-dried smears on slides after puncture, impression smears

or fine needle aspiration (stained or unstained on slides without

cover glass)

Method Microscopy (standard and special stainings)

Duration 1 day, 2 days for bone marrow cytology

Please send native **liquids** (puncture fluids, excretions, secretions) for further clinical chemical examinations in neutral tubes (also

suitable for bacteriology) and additionally in EDTA tubes (superior

cell morphology).

 We offer the following combinations of cytological and clinical chemical examinations:

**Thoraxic/abdominal cavity** (cytology, total protein, cell count, Rivalta (cat), cholesterol, triglycerides, LDH, glucose, albumin/globulin) **CSF** (cytology, total protein, cell count, glucose) **Synovia** (cytology, total protein, cell count)

Quick diagnosis of digital images: After prior contact for the
preliminary clinical report, digital images of cytological preparations
from the practice can be sent for quick diagnosis if the quality of the
image is good. If necessary, additional smears can be sent in later
for validation.

# 18.4 Clonality Analysis of Lymphocytes

#### Lymphocyte Clonality

Material Tissue, air-dried stained or unstained cytological smears on slides

without cover glass, lymphoid material

Method PARR
Species Dog, cat
Duration 3 – 5 days

Note This test provides the possibility

1) to confirm a suspected diagnosis (lymphoma/lymphatic leukaemia vs. reactive hyperplasia) and

2) if lymphoma/lymphatic leukaemia is present, to differentiate between T- or B-cell origin.

As only a cytological or a histological examination can reliably determine the presence of a lymphocyte population, the respective examinations are highly recommended. With regard to interpretation and limitations, please refer to the literature (e.g. Vet Clin Small Anim 43 (2013) 1331 – 1347). Furthermore, the results of all preexaminations as well as the clinical picture should be included in the overall diagnosis (summary probability diagnosis).

The examination can be performed on all materials containing a sufficient number of lymphocytes (fixed and unfixed cell material/tissue/smears/EDTA blood). Cell material can also be obtained directly from the cytological smear (without cover glass) or the histological paraffin block.



# 18.5 Tumour-genetic Tests

#### **BRAF Mutation Test**

Material

If urinary bladder/urethral carcinoma (transitional cell carcinoma) is

suspected:

Urinary sediment (especially early morning urine; fluid + smear)

Aspiration cytology (smears)

Biopsies (formalin-fixed)

If prostate carcinoma is suspected:

Smears rich in cells or biopsies (formalin-fixed)

Droplet digital PCR

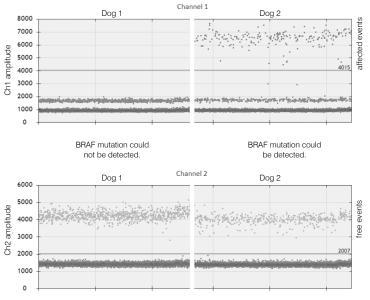
Method Species

Dog

Duration 3 days

Note

- Detection of BRAF variant V595E.
- Indications:
- It was not possible to get a reliable histological/cytological diagnosis.
- Screening for transitional cell carcinoma in certain predisposed terrier breeds (e.g. Scottish Terrier, Fox Terrier, Jack Russell Terrier, West Highland White Terrier)
- Difficult patient



#### BRAF diagnosis by droplet digital PCR

("Affected events": Detection of cells with BRAF mutation; "free events": Detection of cells without BRAF mutation)

- Only a positive result is conclusive.
- Reasons for negative results:
  - The transitional cell/prostate carcinoma is not caused by BRAF mutation (approx. 30 – 50% of these carcinomas – depending on the breed).
  - There were no mutated cells present in the sample.
  - It is not a transitional cell/prostate carcinoma.
- The test cannot be used for cats.

# 18.6 Differentiation Exsudate/Transudate

Parameter	Transudate ≘ low protein transudate	Exsudate	Modified Transudate ≘ high protein transudate
Colour	Slightly yellow	Bloody, purulent	Variable
Transparency	Clear	Mostly opaque	cloudy
Total protein	< 25 g/l	> 30 g/l	25 - 75 g/l
Cell count	< 1000/µI	> 5000/µI	1000 – 7000/μΙ

# 18.7 Publication on the Subject of Pathology

# **Book "Diagnostic Colour Atlas of Bee Pathology"**

With this bilingual book (German/English), PD Dr. Heike Aupperle and Prof. Dr. Elke Genersch have created a new standard work for all those interested in bees, bee diseases and the (functional) anatomy of bees. The atlas with more than 350 full-colour images is a reference work for pathologists, scientists, students and interested beekeepers when it comes to diagnosing diseases of all developmental stages of bees.

# 19 Sex Determination in Birds

The method we use to determine the gender is based on the principle of polymerase chain reaction (PCR). It allows for quick and reliable determination of the sex of the bird using small quantities of genome-containing samples. The test is based on the replication of two highly conserved target genes, which makes it possible to examine many different species.

The method carried out by us provides double safety: During PCR, one probe specifically binds to the "female" sequence, while the other one binds to the "male" sequence, if these are present. This way, there will be one sex confirmed and the other sex excluded.

#### Which sample material is appropriate?

Sex determination can be done by using either blood or quills. One to three drops of whole blood (preferably EB) are sufficient. They can be collected in suitable micro capillary tubes or applied dropwise to a filter card. Filter cards/blood cards should be completely dry before shipment.

Alternatively, 2 – 3 quills from freshly plucked feathers are required. Lost feathers and down feathers are not suitable for the test.

To ensure a correct analysis, the sample must not be contaminated with foreign DNA. For this purpose, please wear gloves when sampling or wash your hands after each sample you take. Please pack feathers separately for each bird. For "dry" feathers, it is sufficient to use an envelope or a small paper bag, "wet" feathers can, for example, be packed in blood or urine tubes or in standard freezer bags. Additionally, we offer so-called SampleKits for submitting feather samples or blood cards. You can request them free of charge. We recommend marking the samples with the ring or chip number of the bird.

#### Which bird species can be tested?

We have performed sex determination tests for several years and thus have already tested many different bird species. Only after we tested a female and a male bird of a species, we give clearance for routine diagnostics. In some species, PCR differentiation is not possible. We will be pleased to provide you with information on which bird species we test.

It is **essential to specify the exact bird species** when submitting the samples.

# 20 Hereditary Diseases/Phenotype/ Breed Characteristics

# 20.1 Heredities

#### Autosomal recessive inheritance

Carriers (N/mut) do not fall ill themselves, but each of them passes on the defective gene to their offspring with a probability of 50%. If two carriers are mated, there is a probability of 25% that one of the offspring will be affected (mut/mut; carriers 50%, free (N/N) 25%). Recessively inherited diseases can spread within the population without clinical manifestation.

### X-linked recessive inheritance

The defective gene is located on a sex chromosome. Heterozygous female animals  $(X_{v_i}/X_{v_{int}})$  are carriers, male carriers  $(X_{v_{int}}/Y)$  express the disease.

### Autosomal dominant inheritance with incomplete penetrance

Heterozygous carriers also show signs of the disease, although the degree of severity varies.

#### Autosomal dominant inheritance

Heterozygous carriers also show signs of the disease.

# 20.2 Dog

# 20.2.1 Hereditary Diseases

#### Acatalasaemia

Material EB 1 ml, buccal swab

Method Sequencing Breed Beagle

Inheritance Probably autosomal recessive

Duration 1 – 2 weeks

Note Acatalasaemia is caused by the absence of the enzyme catalase

which is important for cellular defence in oxidative stress. Affected dogs suffer from ulcers and tissue necrosis in the oral cavity.

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## Achromatopsia/Day Blindness (ACHM)

Material EB 1 ml, buccal swab

Method Sequencing

Breed German Shepherd, Labrador Retriever

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Achromatopsia ACHM is a disease in which the cone cells of the

retina, which are responsible for colour and daylight vision, are not developed properly. Initial symptoms of day blindness are noticeable in affected dogs from 8 – 10 weeks of age. In low light conditions,

their visual function is comparable to normal dogs.

# **Acral Mutilation Syndrome (AMS)**

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed American Cocker Spaniel, German Short-Haired Pointing Dog,

English Cocker Spaniel, English Pointer, English Springer Spaniel,

French Spaniel

Inheritance Autosomal recessive

Duration 3 – 5 days

Note AMS is characterised by a sensory neuropathy of the peripheral

parts of the body. Affected puppies show an insensitivity to pain in their distal extremities and begin to lick, bite or injure themselves on the paws and toes at around 4 months of age. Proprioception, motor

skills and spinal reflexes remain intact.

# **Acute Respiratory Distress Syndrome (ARDS)**

Material EB 1 ml, buccal swab

Method Sequencing Breed Dalmatian

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In Dalmatians, a familial juvenile respiratory disease similar to ARDS

in humans was found. The clinical signs are tachypnoea, dyspnoea and pulmonary lesions. Some affected puppies also showed renal aplasia and hydrocephalus. The first signs of the disease typically appear at 5-10 months; these puppies usually have to be euthanised

1 - 6 weeks later.

#### Alaskan Husky Encephalopathy (AHE)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Husky

Inheritance Autosomal recessive

Duration 3 – 5 days

Note AHE is a disease that is already fatal in puppies. Affected dogs

mainly show behavioural disorders and central nervous deficits such as dysphagia, lack of responsiveness and analgesia, blindness, movement and coordination disorders, ataxia and paralysis.

#### Alaskan Malamute Polyneuropathy (AMPN)

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Alaskan Malamute Inheritance Autosomal recessive

Duration 3 – 5 days

Note AMPN is a neurological disease which leads to progressive muscle

weakness and exercise intolerance as well as signs of paralysis and

respiratory problems at a later stage.

#### Alexander Disease (AxD)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Labrador Retriever
Inheritance Autosomal dominant

Duration 1 – 2 weeks

Note In Labrador Retrievers, Alexander disease causes progressively

worsening tetraparesis with a spastic position of the thoracic limbs and flattened chest. Later on, myoclonic jerks at the head and the cervical region, absent patellar reflexes, weakness on the four limbs

and mild generalised muscle atrophy can become obvious.

# Amelogenesis Imperfecta/Familial Enamel Hypoplasia (AI/FEH)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Akita, American Akita)

Sequencing (Italian Sighthound, Parson Russell Terrier, Samoyed)

Breed Akita, American Akita, Italian Sighthound, Parson Russell Terrier and

Samoved

Inheritance Autosomal recessive

Duration 3 – 5 days (Akita, American Akita)

1 – 2 weeks (Italian Sighthound, Parson Russell Terrier, Samoyed)

Note Al is a hereditary enamel hypoplasia. Affected animals have small,

pointed teeth with brown, thin enamel. Despite this change, the teeth

do not seem to be more susceptible to caries.

## **Bardet-Biedl Syndrome (BBS)**

Material EB 1 ml, buccal swab

Method Sequencing

Breed Puli

Inheritance Autosomal recessive

Duration 1-2 weeks

Note BBS is characterised by retinopathy, obesity and infertility. These

highly variable symptoms occur in different forms in affected dogs

and may already appear in puppies.

### **Behaviour Propensity**

Material EB 1 ml, buccal swab

Method Sequencing

Breed Belgian Shepherd (only Malinois)

Inheritance See note
Duration 1 – 2 weeks

Note The Malinois is a variety of the Belgian Shepherd. In addition to

the provoked form of aggression ("targeted aggression") aimed at in their training, unpredictable, episodic aggression is reported. This undesired aggression happens for no apparent reason and is completely unpredictably; these dogs no longer react to any external influences and cannot be controlled. A correlation was found between undesirable aggression and the dopamine transporter gene SLC6A3: allele A22 occurs more frequently. According to their owners, Malinois with the genotypes A0/A22 or A10/A22 more often show undesired aggression. Genotype A22/A22 was found to be particularly common

in extreme behavioural problems.

# Brachyuria (stumpy tail)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Australian Shepherd, Australian Stumpy Tail Cattle Dog, Austrian

Pinscher, Bourbonnais Pointing Dog, Bouvier des Ardennes, Brazilian Terrier, Brittany Spaniel, Croatian Sheepdog (Hrvatski Ovcar), Danish-Swedish Farmdog, Jack Russell Terrier, Karelian Bear Dog, Miniature American Shepherd, Mudi, Polish Lowland Sheepdog (PON), Pyrenean Sheepdog, Savoy Sheepdog, Schipperke, Spanish Water Dog, Swedish Vallhund, Welsh Corgi Cardigan, Welsh Corgi

Pembroke

Inheritance Autosomal dominant

Duration 3 – 5 days

Note Particularly the tail length gives many dog breeds their characteristic

look. In most countries, docking a dog's tail is forbidden. The DNA analysis now allows to confirm whether the stumpy tail is of natural

oriain.

# C3 Deficiency (C3)

Material EB 1 ml, buccal swab

MethodSequencingBreedBrittany SpanielInheritanceAutosomal recessive

Duration 1 – 2 weeks

Note The third componen of the complement system (C3) plays an

important role in the body's immune defence against microorganisms such as bacteria, fungi or parasites. The causative mutation in the C3 gene prevents the complete formation of C3 and interrupts the defence cascade. Affected dogs exhibit higher susceptibility to

bacterial infections, e.g. glomerulonephritis.

## Canine Leucocyte Adhesion Deficiency (CLAD)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Irish Red and White Setter, Irish Red Setter

Inheritance Autosomal recessive

Duration 3 – 5 days

Note CLAD is a hereditary immune deficiency that is usually fatal.

Symptoms include various inflammatory processes and unsteady

gait.

# Canine Multi-focal Retinopathy (CMR1/2/3)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay (Coton de Tuléar)

Sequencing (all the other breeds listed below)

Breed American Bulldog, Australian Shepherd, Boerboel, Bullmastiff, Coton

de Tuléar, Dogue de Bordeaux, English Bulldog, Finnish Lapphund, French Bulldog, Italian Cane Corso, Lapponian Herder, Mastiff, Miniature American Shepherd, Presa Canario, Pyrenean Mountain

Dog, Swedish Lapphund

Inheritance Autosomal recessive

Duration 3 – 5 days (Coton de Tuléar)

1 – 2 weeks (all the other breeds mentioned above)

Note CMR is an inherited disease which causes multiple lesions of the

retina. First symptoms can typically be found at the age of four months. In some cases, lesions disappear for some time and occur again at a later point in time. Impaired vision or sight disorders are

not described in affected dogs.

#### Canine Multiple System Degeneration (CMSD)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Chinese Crested Dog, Kerry Blue Terrier

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Animals affected by CMSD show normal development up to an age

of 3 – 6 months. After that, symptoms such as cerebellar ataxia and movement disorders become apparent. Most dogs must be euthanised

at 1-2 years of age.

#### Cardiomyopathy with Juvenile Mortality (CJM)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Belgian Shepherd, (all varieties: Groenendael, Laekenois, Malinois,

Tervueren)

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In the Belgian Shepherd, a genetic variant of the tyrosyl-tRNA

synthetase gene (YARS2) correlates with a form of mortality in puppies. CJM manifests itself through non-specific signs (vomiting, movement disorders, respiratory problems) at the latest at the age of 6 – 8 weeks. The animals usually die of heart failure within a few days. Carriers should only be mated dogs that do not carry this

variant.

# Centronuclear Myopathy (CNM)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (German Hunting Terrier, Great Dane)

FLP (Labrador Retriever)

Breed German Hunting Terrier, Great Dane, Labrador Retriever

Inheritance Autosomal recessive

Duration 3 – 5 days (German Hunting Terrier, Great Dane),

1 – 2 weeks (Labrador Retriever)

Note If Labrador Retrievers or Great Danes suffer from CNM, the dog's

muscles do not develop properly. Affected dogs lack tendon reflexes and gain less weight than puppies of the same age (at 4 weeks). From an age of approximately 12 to 20 weeks onwards, muscular weakness, abnormal posture, clumsy gait and feeding difficulties

appear.

In German Hunting Terriers, the disease is also called Exercise Induced Metabolic Myopathy (EIMM). EIMM is caused by a deficiency of acyl-CoA dehydrogenase (VLCAD) and therefore of the fatty acid oxidation (insufficient energy production). During or after exertion, affected dogs suffer from weakness up to collapse, severe muscle pain, muscle cell necroses and myoglobinuria from an age of 7-24 months onwards. About 30-120 minutes after physical exertion, they can develop tetraparesis or tetraplegia. Increased levels of CK,

ALT and the long-chain fatty acid C14:1 can be detected.

# Cerebral Dysfunction (CDFS)

Material EB 1 ml, buccal swab

Method TagMan SNP assay, if necessary sequencing

Breed Stabijhoun

Inheritance Autosomal recessive

Duration 3-5 days (1-2) weeks for sequencing

Note CDFS in the Stabijhoun breed is an inherited disease of the brain.

Clinically affected animals exhibit a large variety of neuronal signs such as depressive behaviour, walking circles, obsessive sniffing

and running backwards.

# **Charcot-Marie-Tooth Neuropathy (CMT)**

MaterialEB 1 ml, buccal swabMethodTaqMan SNP assayBreedMiniature SchnauzerInheritanceAutosomal recessive

Duration 3 – 5 days

Note The neuromuscular disease CMT leads to changes in the myelin

sheath of the axons of peripheral nerves due to a variant in the SBF2 gene (also called MTMR13 gene). Affected dogs show frequent regurgitation and respiratory difficulties at a young age (< 2 years) caused by megaoesophagus and laryngeal paralysis, and have a

relatively long survival of more than 3 years after diagnosis.

#### Chondrodysplasia (dwarfism)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Chinook, Karelian Bear Dog, Norwegian Elkhound

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Chondrodysplasia is a genetically determined skeletal dysplasia

which leads to malformations in the structure of the long bones and dwarfism. In addition to shortened limbs, clinical signs include a

large skull, spine changes and deformations of the legs.

#### Chondrodysplasia and -dystrophia (IVDD risk)

Material EB 1 ml, buccal swab

Method FLP

Breed All, particularly short-legged breeds

Inheritance CDPA autosomal dominant; semi-dominant for CDDY-related leg

length, dominant for IVDD risk

Duration 1-2 weeks

Note Breed-specific short limbs can be caused by chondrodystrophy

(CDDY) and/or chondrodysplasia (CDPA). Only CDDY is associated with an increased **risk of a herniated disc** (Hansen's Type I Inter-

vertebral Disc Disease. IVDD).

CDDY is semi-dominantly inherited with regard to leg length, i.e. heterozygous dogs have shorter legs than homozygous free dogs, while homozygous affected dogs have even shorter legs than the heterozygous dogs. The IVDD risk is inherited as an autosomal dominant trait, which means that already one copy of the altered

chromosome significantly increases the risk.

Cleft Lip/Palate and Syndactyly (CLPS)

Material EB 1 ml. buccal swab

Method TagMan SNP assay, if necessary sequencing

Breed Nova Scotia Duck Tolling Retriever

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

Note Cleft lip/palate and syndactyly (CLPS) describes a hereditary

disease, which so far has only been detected in Nova Scotia Duck

Tollina Retrievers.

Affected puppies develop a cleft palate, cleft lips as well as syndactyly.

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#### CNS Atrophy with Cerebellar Ataxia (CACA)

Material EB 1 ml, buccal swab

Method FLP

Breed Belgian Shepherd Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note CACA is caused by a deletion of the SELENOP gene for seleno-

protein P, which is responsible for selenium transport to the brain and tissues. Selenium deficiency in the brain causes uncoordinated movements, intention tremor, spastic fits, increased muscle tone and a reduced swallowing reflex. These neurological signs can be observed in varying intensity from the age of about 2 weeks onwards

and either result in early euthanasia or are mild.

## Collie Eye Anomaly\* (CEA)

Material EB 1 ml, buccal swab Method Partner laboratory

Breed Australian Kelpie, Australian Shepherd, Bearded Collie, Border

Collie, Boykin Spaniel, Collie (rough/smooth), Hokkaido, Lancashire Heeler, Long-haired Whippet (Silken Windsprite), Miniature American Shepherd, Nova Scotia Duck Tolling Retriever, Shetland Sheepdog

(Sheltie), Silken Windhound

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note CEA leads to changes in the retina of the eye and can manifest

itself in different degrees of severity. In the worst form of CEA, blood vessel changes cause retinal haemorrhage, which can lead to retinal

detachment and blindness in the dog.

# Colour Dilution and Neurological Defects (CDN)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Dachshund (Dackel)
Inheritance Autosomal recessive

Duration 1-2 weeks

Note In Miniature Dachshunds, a variant in the MYO5A gene has been

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found which causes CDN and resembles human Griscelli syndrome type I. Myosin VA-mediated transport plays an important role in neurons, the cerebellum and in the transport of melanosomes into

growing hair shafts.

An affected 4-week-old puppy had noticeably pale fur, was unable to hold itself in a prone position and showed rowing movements in the lateral position. It could neither maintain an upright head position nor coordinate head movements. The puppy also hardly reacted to environmental stimuli and was euthanised. The histopathological findings were a multifocal accumulation of melanin and a deposition of clumped keratin in the follicular epithelium of hairy skin.

### Congenital Hypothyroidism with Goiter (CHG)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Fox Terrier, French Bulldog, Rat Terrier, Spanish Water Dog,

Tenterfield Terrier, Toy Fox Terrier

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Affected dogs usually die a few days after birth. Administration of

hormones can prolong the life span, but the dogs still suffer from

dwarfism and goitre formation.

## **Congenital Ichthyosis**

Material EB 1 ml, buccal swab

Method Sequencing Breed Great Dane

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note So far, lamellar ichthyosis is only known in Great Danes. In the

course of the disease, the skin becomes dry and loses its elasticity, resulting in a generally wrinkled appearance particularly in the head area. In addition, affected puppies may develop severe swelling of the eyelids. The skin changes in the area of the folds enhance the

risk of secondary infections.

# Congenital Myasthenic Syndrome (CMS)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Golden Retriever, Jack Russell Terrier, Labrador Retriever, Old Danish

Pointing Dog, Parson Russell Terrier

Inheritance Autosomal recessive

Duration 1-2 weeks

Note The main sign of CMS is a generalised muscle weakness,

especially after stress or excitement. It can already be seen at just two weeks of age. Mobility of all limbs is severely limited, even the ability to carry the own body weight diminishes over time. In all areas

of the limbs, reflexes are significantly reduced.

#### **Congenital Stationary Night Blindness (CSNB)**

Material EB 1 ml, buccal swab

Method TagMan SNP assay, if necessary FLP

Breed Briard

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for FLP)

Note In affected dogs, night vision is severely impaired already at a few

weeks of age; after a few years, some dogs also suffer from reduced

daylight vision.

# Copper Storage Disease – Copper Toxicosis (CT/COMMD1) in the Bedlington Terrier

Material EB 1 ml, buccal swab

Method FLP

Breed Bedlington Terrier
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Copper toxicosis in Bedlington Terriers is caused by a disturbance of

the copper metabolism which leads to an accumulation of copper in

the liver.

# Copper Storage Disease – Copper Toxicosis\* (CT) in the Dobermann and Labrador Retriever

Material EB 1 ml, buccal swab Method Partner laboratory

Breed Dobermann and Labrador Retriever

Inheritance See note
Duration 1 – 2 weeks

Note In Labrador Retriever and Dobermann breeds, a variant in the

copper-transporting **ATP7B**-ATPase gene leads to reduced copper excretion resulting in excessive copper storage in the liver and other organs. Usually, symptoms only appear in middle-aged or older dogs. Inheritance is autosomal dominant with incomplete penetrance. Dogs with 2 mutant alleles are normally more severely affected than heterozygous animals, but may also be free of symptoms throughout

their lives.

# Dandy-Walker-like Malformation (DWLM)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Eurasian

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Dogs affected by DWLM suffer from underdevelopment of the

cerebellum, which is already noticeable in puppies. Depending on the severity, ataxia, spontaneous falling, and even severe epileptic

seizures may occur.

# Craniomandibular Osteopathy (CMO)

detected.

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Cairn Terrier, Scottish Terrier, West Highland White Terrier

Inheritance Autosomal dominant with incomplete penetrance

Duration 3 – 5 days

Note CMO is a painful proliferative disease of the jaw bones affecting

dogs in the first year of life.

Clinical signs of the disease are recurrent episodes of fever and

In Labrador Retrievers, the risk of contracting the disease may be

reduced: A second mutation – in the **ATP7A**-ATPase gene – leads to

reduced accumulations of copper. As this second variant is inherited

in an X-linked dominant manner with incomplete penetrance, female

dogs are more likely to have the disease, since the second mutation

often only affects the metabolism if it is homozygous, whereas in

This second mutation was also identified in Dobermanns, but so

far, no correlation with the hepatic copper concentration has been

male dogs, one copy of this gene variant is sufficient.

painful mandibular swelling.

# Cystinuria

Material EB 1 ml. buccal swab

Method TagMan SNP assay (Continental Bulldog, English Bulldog, French

Bulldog, Landseer, Mastiff, Newfoundland, Olde English Bulldog) Sequencing (Australian Cattle Dog, Labrador Retriever, Miniature

Pinscher)

Breed Australian Cattle Dog, Continental Bulldog, English Bulldog, French

Bulldog, Labrador Retriever, Landseer, Mastiff, Miniature Pinscher,

Newfoundland, Olde English Bulldog

Inheritance Autosomal recessive;

autosomal dominant in Australian Cattle Dog, Miniature Pinscher

Duration 3 – 5 days (Continental Bulldog, English Bulldog, French Bulldog,

Landseer, Mastiff, Newfoundland, Olde English Bulldog)

1 – 2 weeks (Australian Cattle Dog, Labrador Retriever, Miniature

'inscher)

Note A transport disorder of dibasic amino acids in the kidney leads to the

formation of cystine calculi.

#### Degenerative Myelopathy (DM) (Exon 1 and 2)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed All (exon 2)

Bernese Mountain Dog (exon 1+2)

Inheritance Autosomal recessive with age-dependent incomplete penetrance;

a major risk factor (mutation in the SOD1 gene), which is associated

with DM, is detected.

Duration 3 – 5 days

Note The disease is characterised by a degeneration of the axons and

the myelin in the thoracic and lumbar part of the spinal cord causing progressive ataxia and paresis. The test detects a mutation which is

considered the main risk factor for this disease.

DM exon 2: Laboklin owns the exclusive license to perform this

genetic test.

#### Dental-skeletal-retinal Anomaly (DSRA)

MaterialEB 1 ml, buccal swabMethodTaqMan SNP assayBreedItalian Cane CorsoInheritanceAutosomal recessive

Duration 3-5 days

Note DSRA is the result of a defect in the MIA2 gene and is characterised,

among others, by dental problems (discolouration, chips and fractures, smaller teeth than normal), skeletal problems and progressive retinal atrophy (PRA). Further manifestations and pathogenesis are

subject of current research.

## **Dermatomyositis (DMS)**

Material EB 1 ml, buccal swab

Method 2 x TaqMan SNP assay + sequencing

Breed Collie (rough/smooth), Shetland Sheepdog (Sheltie)

Inheritance Polygenic
Duration 1 – 2 weeks

Note DMS is an autoimmune disease that causes skin lesions (hair loss

and crusts) in Collies and Shetland Sheepdogs and can be detected histologically (biopsy). Only in Collies, additional muscular dysfunctions (difficulty to swallow, a high and stilted gait with muscular atrophy in the head and neck area) have been described. The complex genetic trait needs an additional external trigger like vaccination or viral infection to cause signs of the disease. Based on genotype combinations of three different loci (A, B, C), the likelihood of developing DMS can be classified. Breeding puppies with genotypes that have a high risk (especially: AABB, AABB, AABb) should be avoided wherever possible. Genotypes with moderate risk are AAbbCC, AaBbCC,

# Digital Hyperkeratosis (DH/HFH)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Dogue de Bordeaux, Irish Terrier, Kromfohrländer

Inheritance Autosomal recessive

Duration 3 – 5 days

Note This disease, which is also called "corny feet", manifests itself a few

months after birth as excessive keratin formation on the foot pads, which can lead to skin cracks and subsequently to secondary infec-

tions at these sites. Nail growth is also often accelerated.

# Dilated Cardiomyopathy (DCM) in the Schnauzer and Giant Schnauzer

Material EB 1 ml, buccal swab

Method Sequencing

Breed Giant Schnauzer, Schnauzer

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note DCM is caused by different mutations in different breeds. In the

Schnauzer, a variant in the RBM20 gene was identified which correlates very well with DCM. In this breed, the first symptoms typically appear at the age of 1-3 years. DCM can lead to sudden cardiac death

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even without any previous clinical signs.

# Dilated Cardiomyopathy (DCM) in the Welsh Springer Spaniel

Material EB 1 ml, buccal swab

Method Sequencing

Breed Welsh Springer Spaniel

Inheritance Autosomal dominant with incomplete penetrance

Duration 1 – 2 weeks

Note In the Welsh Springer Spaniel, a genetic variant in the phospho-

lamban gene was found which is associated with DCM. Phospholamban plays an important role in regulating the intracellular calcium concentration. Signs usually become apparent up to the age of 20 months and can lead to sudden cardiac death. The disease is inherited in an autosomal dominant manner with incomplete penetrance, although penetrance here is very high compared to other

cardiac diseases found in dogs.

#### Dilated Cardiomyopathy (DCM1 and DCM2) in the Dobermann

Material EB 1 ml, buccal swab

Method Sequencing Breed Dobermann

Inheritance Autosomal dominant with incomplete penetrance

Duration 1-2 weeks

Note DCM is widespread in the Dobermann. Affected dogs suffer from

heart failure or sudden cardiac death. So far, two genetic variants have been identified: the DCM1 variant in the PDK4 gene (regulates the energy supply of the heart) and the DCM2 variant located in the titin (TTN) gene (influences heart contraction). Penetrance of DCM is highly variable, so genetically affected dogs might only show very mild or even no signs at all throughout their lives. Apart from the genotype of the two variants, diet, exercise level and other genes

also seem to influence a dog's individual risk.

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Dogs carrying the DCM1 variant (heterozygous or homozygous) have a 10-fold increased risk of developing DCM; 37% of the dogs show signs. Carriers of the DCM2 variant are 21 times more likely to develop DCM, while 50% develop the disease. Dogs with both variants have a 30-fold increased risk of DCM and 60% show relevant

signs.

# **Disproportionate Dwarfism**

Material EB 1 ml. buccal swab

Method Sequencing
Breed Dogo Argentino

2022/23 Hereditary Diseases/Phenotype – Dog

**LABOKLIN** 

Inheritance Autosomal recessive

Duration 1-2 weeks

Note Disproportionate dwarfism in the Dogo Argentino is caused by a

variant in the PRKG2 gene. This gene encodes for a protein which has a signalling role in the termination of chondrocyte proliferation and initiates differentiation into bone cells. From about 2 months of age onwards, a smaller body size and length, a disproportionately large head with a pronounced vertical groove between the eyes and

possibly impaired gait due to carpus valgus can be seen.

# **Dry Eye Curly Coat Syndrome (CCS)**

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Cavalier King Charles Spaniel

Inheritance Autosomal recessive

Duration 3-5 days

Note Affected puppies have an unusual coat (rough and curly) and show

signs of keratoconjunctivitis sicca (conjunctivitis/keratitis due to a lack of lacrimal fluid). Changes of the skin, the foot pads and the nails cause pain and lameness. Teeth and hair are also affected.

# Dystrophic Epidermolysis Bullosa (DEB)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Central Asian Shepherd Dog

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In DEB. blister formation occurs beneath the lamina densa of the

cutaneous basement membrane. A severe form of DEB is found in Central Asian Shepherd Dogs. It is caused by a nonsense mutation in the COL7A1 gene, which encodes for type VII collagen. Already at an early age, affected puppies suffer from skin lesions, blisters and ulcers on the paws, ears, muzzle and oral mucosa and must be

euthanised due to the unfavourable prognosis.

# Ectodermal Dysplasia/Skin Fragility Syndrome (ED/SFS)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Chesapeake Bay Retriever Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Already at birth, the skin of affected dogs is translucent on the

ears, feet, nose and mouth. Bleeding or skin detachment occurs in these areas when slight friction is present. Affected dogs have to be

euthanised.

# Epidermolytic Hyperkeratosis (EHK)

Material EB 1 ml, buccal swab

Method Sequencing Breed Norfolk Terrier

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Because of a keratin defect, this disease leads to superficial, mild,

plantar epidermolytic hyperkeratosis with epidermal fragility. Affected

dogs show clinical signs from birth up to old age.

## **Episodic Falling (EF)**

Material EB 1 ml, buccal swab

Method FLP

Breed Cavalier King Charles Spaniel

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Episodic falling is a neurological disorder. Episodes are triggered by

stress, excitement or exertion and can range from stiffness to collapse. Laboklin owns the exclusive license to perform this genetic test.

# **Exercise Induced Collapse (EIC)**

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Bouvier des Flandres, Boykin Spaniel, Chesapeake Bay Retriever,

Clumber Spaniel, Curly Coated Retriever, German Wire-Haired Pointing Dog, Labrador Retriever, Old English Sheepdog, Welsh Corgi Pem-

broke

Inheritance Autosomal recessive

Duration 3 – 5 days

Note The first signs of EIC are a swaying or stiff gait as if the dog had stiff

legs. After only 5 – 15 minutes of exertion, affected dogs develop

muscle weakness and collapse.

Laboklin owns the exclusive license to perform this genetic test.

## **Exfoliative Cutaneous Lupus Erythematosus (ECLE)**

Material EB 1 ml, buccal swab

Method Sequencing

Breed German Short-haired Pointing Dog, Magyar Vizsla

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note The autoimmune skin disease ECLE, also known as lupoid derma-

titis, is caused by a variant in the UNC93B1 gene, which plays an important role in the immune response. Typical signs for ECLE are excessive scales – local or all over the body, hypopigmentation, erythema, hair loss, crusts, ulcers as well as secondary bacterial skin infections caused by immunodeficiency and, sometimes, short-term lameness. The first signs are seen at a juvenile or early adult age. Due to the severe symptoms and insufficient treatment options,

affected dogs are usually euthanised.

# **Factor VII Deficiency**

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Airedale Terrier, Alaskan Klee Kai, Beagle, Deerhound, Finnish

Hound, Giant Schnauzer, Papillon, Phalène, Welsh Springer Spaniel,

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Affected dogs show a mild to moderate bleeding tendency but can

also remain asymptomatic.

# Familial Nephropathy (FN)

Material EB 1 ml. buccal swab

Method Partner laboratory\* (English Cocker Spaniel, Welsh Springer Spaniel)

Sequencing (English Springer Spaniel, Samoyed)

Breed English Cocker Spaniel, English Springer Spaniel, Samoved, Welsh

Springer Spaniel

Inheritance Autosomal recessive (English Cocker Spaniel, English Springer

Spaniel, Welsh Springer Spaniel)

X-linked recessive in Samoyed

Duration 1 – 2 weeks

Note Dogs with FN typically develop chronic renal failure between 6

months and 2 years of age, which sometimes leads to a rapid

destruction of both kidneys and is ultimately fatal.

#### Fanconi Syndrome

Material EB 1 ml, buccal swab

Method Sequencing
Breed Basenji
Inheritance Unknown
Duration 1 – 2 weeks

Note Fanconi syndrome is a disease in which the kidneys are no longer

able to reabsorb electrolytes and nutritive substances from the urine into the body. Typical signs are polydipsia and polyuria. Without treatment, the disease leads to death because of muscle weakness and acidosis. In Basenjis, Fanconi syndrome is hereditary and is

usually seen between the ages of 4 – 8 years.

#### Finnish Hound Ataxia (FHA)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Finnish Hound, Norrbottenspitz

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Starting at about 4 weeks of age, this disease can lead to a progres-

sively worsening ataxia in affected animals, which initially manifests itself as slight coordination problems, but later as paralysis up to

immobility.

#### **Fucosidosis**

Material EB 1 ml, buccal swab

Method Sequencing

Breed English Springer Spaniel Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note This storage disease causes deposits in cerebral and peripheral

nervous tissue. Affected animals show a disturbed coordination of movements, behavioural abnormalities, blindness, deafness and impaired deglutition. The disease manifests itself between the age of 18 months and 4 years with a progressive course and ultimately fatal

outcome.

#### **Gallbladder Mucoceles**

Material EB 1 ml, buccal swab

Method Sequencing

Breed American Cocker Spaniel, Cairn Terrier, English Cocker Spaniel,

Pomeranian, Shetland Sheepdog (Sheltie)

Inheritance Autosomal dominant with incomplete penetrance

Duration 1 – 2 weeks

Note Undetected gallbladder mucoceles can lead to cholecystitis and

thus increase the risk of a rupture of the gallbladder. Clinical signs occur in older dogs and include vomiting, anorexia, lethargy, icterus

and abdominal pain.

# Glanzmann Thrombasthenia (GT)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Pyrenean Mountain Dog Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note GT is a bleeding disorder that occurs in two different types. They

differ in the amount of specific glycoproteins ( $\alpha$ IIb $\beta$ 3) embedded in the cell membrane of platelets (thrombocytes), which are necessary for coagulation. In the more severe form of GT, type I, the level is less than 5% of the normal value. A mutation in the  $\alpha$ IIb gene disrupts the production of one main component of these glycoproteins. Symptomatically, bleeding diathesis is usually recognised by continuous gingival bleeding after shedding of deciduous teeth. Persistent

epistaxis can also be an indication for this disorder.

#### Glaucoma and Goniodysgenesis (GG)

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Border Collie

Inheritance Presumably autosomal recessive (still in research).

Duration 3 – 5 days

Note A mutation in the olfactomedin-like 3 gene (OLFML3) leads to a pre-

disposition for severe goniodysgenesis with narrowing or occlusion of intraocular channels of the iridocorneal angle and glaucoma and blindness as possible consequences. In heterozygous carriers, goniodysgenesis without glaucoma was diagnosed. Furthermore, several dogs did not develop glaucoma despite severe goniodysgenesis for 15 years or more. It is therefore assumed that the development of glaucoma is influenced by a combination of genetic

factors as well as environmental and/or random factors.

## Globoid Cell Leukodystrophy (Krabbe Disease)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay (Cairn Terrier, West Highland White Terrier)

Sequencing (Irish Red Setter)

Breed Cairn Terrier, Irish Red Setter, West Highland White Terrier

Inheritance Autosomal recessive

Duration 3 – 5 days (Cairn Terrier, West Highland White Terrier)

1 – 2 weeks (Irish Red Setter)

Note Krabbe disease is an incurable lipid storage disorder with progres-

sive degeneration of the white matter of the CNS. It is characterised by muscle atrophy and neurological degeneration with the onset of

the signs being at 1-3 months of age.

# Glycogen Storage Disease Type Ia (GSD Ia)

Material EB 1 ml, buccal swab

Method Sequencing

Breed German Pinscher, Maltese Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note GSD la is caused by a congenital disorder of the glucose metabo-

lism which leads to organ dysfunction of varying severity. In affected puppies, an undersupply of glucose and delayed growth occur very

early after birth.

# Glycogen Storage Disease Type II (GSD II, Pompe Disease)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Finnish Lapphund, Lapponian Herder, Swedish Lapphund

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Affected dogs suffer from vomiting, progressive muscle weakness,

loss of condition and cardiac insufficiency, which leads to death at

about 1.5 years of age.

# Glycogen Storage Disease Type IIIa (GSD IIIa)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Curly Coated Retriever Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Affected animals often only show few clinical signs in the first years

of life. With advancing age, the disease manifests itself more and more

frequently as lethargy and episodic hypoglycaemia with collapse.

GM1 Gangliosidosis (GM1)

Breed

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Shiba Inu)

Sequencing (Husky, Portuguese Water Dog) Husky, Portuguese Water Dog, Shiba Inu

Inheritance Autosomal recessive
Duration 3 – 5 days (Shiba Inu)

1 – 2 weeks (Husky, Portuguese Water Dog)

Note GM1 is a lysosomal storage disease that leads to neurological

disorders. Affected dogs suffer from paralysis of the extremities and

spasticity of the muscles.

GM2 Gangliosidosis (GM2)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Japanese Chin, Poodle, Shiba Inu

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note GM2 gangliosidosis, also known as Sandhoff's disease, is a pro-

gressive neurodegenerative lysosomal storage disease. The first neurological signs appear at 9 to 12 months of age, which rapidly worsen and lead to death at 18 to 23 months. Signs include loss of vision, difficulty walking, loss of balance, tremor and vomiting.

Grey Collie Syndrome (GCS) (Canine Cyclic Neutropenia)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Collie (rough/smooth)
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Due to a dysfunction in stem cell formation in the bone marrow,

affected dogs are more susceptible to infections and tend to bleed.

## Haemophilia A (Factor VIII Deficiency)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay (German Shepherd)

Sequencing (Boxer, Old English Sheepdog) FLP (Havanese, Rhodesian Ridgeback)

Breed Boxer, German Shepherd, Havanese, Old English Sheepdog.

Rhodesian Ridgeback

Inheritance X-linked recessive

Duration 3 – 5 days (German Shepherd)

1 – 2 weeks (Boxer, Havanese, Old English Sheepdog, Rhodesian

Ridgeback)

Note Depending on the severity of factor VIII deficiency, there is slight to

severe bleeding diathesis.

# Haemophilia B (Factor IX Deficiency)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Rhodesian Ridgeback)

Sequencing (American Akita, Hovawart, Lhasa Apso)

Breed American Akita, Hovawart, Lhasa Apso, Rhodesian Ridgeback

Inheritance X-linked recessive

Duration 3 – 5 days (Rhodesian Ridgeback)

1 – 2 weeks (American Akita, Hovawart, Lhasa Apo)

Note Haemophilia B is one of the most important hereditary coagulation

disorders in the Rhodesian Ridgeback. Depending on the severity of factor IX deficiency, there is a slight to severe bleeding diathesis. Other genetic causes of haemophilia B have been found in the

American Akita, Hovawart and Lhasa Apso.

# Haemorrhagic Diathesis (Scott Syndrome)

Material EB 1 ml, buccal swab

Method Sequencina

Breed German Shepherd Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note This bleeding diathesis is caused by impaired coagulation activity;

activated platelets can be detected which are incapable of presenting anionic phospholipids, especially phosphatidylserine, and to release procoagulant microparticles. Other coagulation parameters remain unchanged, except for a reduced prothrombin consumption

during the coagulation of whole blood.

Hereditary Ataxia (HA)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Gordon Setter, Old English Sheepdog)

Sequencing (Norwegian Buhund)

Breed Gordon Setter, Norwegian Buhund, Old English Sheepdog

Inheritance Autosomal recessive

Duration 3 – 5 days (Gordon Setter, Old English Sheepdog)

1 – 2 weeks (Norwegian Buhund)

Note Affected dogs suffer from a progressively worsening dysfunction of

the musculoskeletal system, which initially manifests itself as slight coordination and balance problems and later as severe gait disturbances. The first clinical symptoms appear at the age of 6 months to

4 years.

**Hereditary Cataract (HSF4)** 

Material EB 1 ml, buccal swab

Method Partner laboratory\* (Boston Terrier, French Bulldog,

Staffordshire Bull Terrier)

Sequencing (Australian Shepherd, Miniature American Shepherd,

Wäller)

Breed Australian Shepherd, Boston Terrier, French Bulldog, Miniature

American Shepherd, Staffordshire Bull Terrier, Wäller

Inheritance Autosomal recessive (Boston Terrier, French Bulldog, Staffordshire

Bull Terrier)

Unclear (Australian Shepherd, Miniature American Shepherd, Wäller)

Duration 1 – 2 weeks

Note Cataract is one of the most frequent causes for blindness in dogs.

In the Boston Terrier, French Bulldog and Staffordshire Bull Terrier, hereditary cataract is caused by a different mutation in the HSF4 gene (heat-shock factor 4 gene) than in the Australian Shepherd,

Miniature American Shepherd and Wäller.

In the latter breeds, homozygosity results in nuclear cataract, but heterozygosity only causes posterior subcapsular cataract, which rarely impairs vision. An autosomal recessive mode of inheritance is also suspected in these breeds, but it is influenced by at least one

other genetic factor.

**Hereditary Deafness** 

Material EB 1 ml. buccal swab

Method Sequencing

Breed Dobermann, Rottweiler

Inheritance Autosomal recessive

Duration 1-2 weeks

Note In Dobermann puppies, deafness and a disorder of the vestibular

system have been known since 1992. Recently, a variant in the PTPRQ gene was found which can be associated with it. Affected animals are already deaf 3 weeks after birth and suffer from balance disorders. Pathologically, progressive cochlear degeneration with loss of auditory sensory cells in the inner ear has been reported. In

addition, otoconia may be missing or malformed.

In the Rottweiler, a variant in the LOXHD1 gene leads to early hearing loss. It has not yet been conclusively clarified whether the puppies are born deaf or if they are initially hard of hearing and then become completely deaf within a few weeks. The LOXHD1 gene is probably involved in maintaining the function of the hair cells in the cochlea.

Hereditary Nasal Parakeratosis (HNPK)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Labrador Retriever)

Sequencing (Greyhound)

Breed Greyhound, Labrador Retriever

Inheritance Autosomal recessive

Duration 3 – 5 days (Labrador Retriever)

1 – 2 weeks (Grevhound)

Note Affected dogs suffer from crust formation on the nose. Treatment

can only be symptomatic.

Laboklin owns the exclusive license to perform this genetic test in

Labrador Retrievers.

**Hereditary Neuropathy (GHN)** 

Material EB 1 ml, buccal swab

Method FLP

Breed Greyhound

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Signs particularly include progressive amyosthenia, exercise intole-

rance, loss of reflexes and ataxia of all limbs, and later, loss of the

ability to stand as well as breathing problems.

Hyperuricosuria (HUU/SLC)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All

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Inheritance Autosomal recessive

Duration 3-5 days

Note Hyperuricosuria is a metabolic disorder that leads to an increased

excretion of uric acid instead of allantoin, which is why the disease is also called "hyperuricosuria and hyperuricaemia". To prevent the formation of calculi, affected dogs should get a low purine diet.

Additionally, adequate hydration is vital.

### Hypomyelination/Shaking Puppy Syndrome (SPS)

Material EB 1 ml, buccal swab

Method Sequencing

Breed English Springer Spaniel, Weimaraner

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note This disease is caused by abnormal formation of the myelin sheath

of the spinal cord. At the age of 12-14 days, affected dogs show generalised tremor, whose severity varies greatly. The dogs are able to walk, but have a hopping gait in the hind legs. Tremor is not present when the dogs are at rest or asleep and decreases considerably from an age of 3-4 months onwards and may even disappear

completely.

#### Hypophosphatasia (HPP)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Karelian Bear Dog
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note HPP has been described in Karelian Bear Dogs and in humans.

Defective variants of the non-tissue-specific alkaline phosphatases are formed. This impairs the release of phosphate from inorganic compounds and leads to insufficient mineralisation of the skeleton. At the age of 2 – 10 weeks, dogs show growth retardation, movement disorders as well as muscle weakness and seizures. In the serum of affected puppies, total protein, albumin and urea levels may be elevated, and more PEA (phosphatase substrate phosphoethanolamine) is excreted in the urine. Affected animals usually die after a

few weeks or are euthanised.

## Ichthyosis in Great Danes > see Congenital Ichthyosis p. 310

#### Ichthyosis (American Bulldog)

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed American Bulldog Inheritance Autosomal recessive

Duration 3 – 5 days

Note Ichthyosis is a congenital disorder of the normal desquamation of

the skin caused by a change in keratinisation. In addition, the skin may also appear differently pigmented. The first signs of the disease

appear after only a few weeks of life.

#### Ichthyosis\* (Golden Retriever)

MaterialEB 1 ml, buccal swabMethodPartner laboratoryBreedGolden RetrieverInheritanceAutosomal recessive

Duration 1 – 2 weeks

Note See Ichthyosis in American Bulldogs.

### **Ichthyosis Type 2**

Material FB 1 ml, buccal swab

Method Sequencing
Breed Golden Retriever
Inheritance Autosomal recessive

Duration 1 – 2 Wochen

Note In addition to the PNPLA1 variant, which has been known since

2012, another variant in the ABHD5 gene was now found in the Golden Retriever, which can also cause the typical signs of ichthyosis (called type 2 here). So far, the type 2 variant has mostly been identi-

fied in American breeding lines.

# Imerslund-Gräsbeck Syndrome (IGS)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay (Beagle, Border Collie)

Sequencing (Komondor)

Breed Beagle, Border Collie, Komondor

Inheritance Autosomal recessive

Duration 3 – 5 days (Beagle, Border Collie)

1 – 2 weeks (Komondor)

Note Malabsorption of vitamin B12 leads to neurological signs and

irreversible damage of the brain and nervous system.

## Inflammatory Myopathy (IM)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Dutch Shepherd
Inheritance Autosomal recessive

Duration 1-2 weeks

Note Inflammatory myopathy (IM) is the result of homozygous inheritance

of a variant in the SLC25A12 gene. The genetic defect causes a decreased activity of the mitochondrial aspartate-glutamate transporter and a resulting proinflammatory milieu as well as oxidative stress in the muscles. Beginning at 3 – 9 months of age, affected dogs show progressive muscle weakness up to the inability to walk. Serum CK is permanently elevated. Affected animals were euthanised at about

2 years of age.

#### Inflammatory Pulmonary Disease (IPD)

Material EB 1 ml, buccal swab
Method TaqMan SNP assay
Breed Collie (rough and smooth)
Inheritance Autosomal recessive

Duration 3 – 5 days

Note Just a few days after birth, IPD causes coughing, shallow breathing,

heavy breathing sounds, foamy vomiting and fever. The dogs respond well to treatment with antibiotics and secretolytics but tend to relapse

without antibiotic treatment.

# Junctional Epidermolysis Bullosa (JEB)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay, if necessary sequencing

Breed German Short-Haired Pointing Dog

Inheritance Autosomal recessive; the genetic test detects a mutation that is

inherited together with the causative mutation.

Duration 3-5 days (1-2 weeks for sequencing)

Note Due to a defect in the cutaneous basement membrane, erosions

and encrustations occur in the area of the foot pads, at pressure points on the limbs, on the inside of the auricles, and in areas of the

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gums, tongue and lips.

#### Juvenile Brain Disease (JBD)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay, if necessary sequencing Breed Jack Russell Terrier, Parson Russell Terrier

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

Note JBD is a disorder with an early onset at the age of 6 – 12 weeks

which leads to epileptic seizures. The disease progresses rapidly

and causes irreversible brain damage leading to death.

# Juvenile Epilepsy (JE)

MaterialEB 1 ml, buccal swabMethodTaqMan SNP assayBreedLagotto RomagnoloInheritanceAutosomal recessive

Duration 3 – 5 days

Note Between the 5<sup>th</sup> and 12<sup>th</sup> week of life, affected dogs suffer from

episodes of slight trembling, unsteady gait or inability to walk and

spastic paralysis.

# Juvenile Laryngeal Paralysis & Polyneuropathy (JLPP)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Black Russian Terrier, Rottweiler

Inheritance Autosomal recessive

Duration 3 – 5 days

Note JLPP is a hereditary disease that leads to breathing difficulties when

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excited or physically stressed already at an age of three months. As the disease progresses, weakness and coordination problems in the hind limbs will develop, which eventually will extend to the front limbs and swallowing difficulties appear. This disease cannot be cured and is fatal within a few months after the onset of the symptoms.

# Juvenile Myoclonic Epilepsy (JME)

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Rhodesian Ridgeback Inheritance Autosomal recessive

Duration 3 – 5 days

Note For Rhodesian Ridgebacks, JME is a typical form of epilepsy with

frequent myoclonic twitches. The dogs suffer from involuntary, sudden muscle jerks which especially occur at rest. First symptoms appear at the age of about 6 months. In more than 85% of the cases, seizures occur daily. In the course of the disease, 40% of the

dogs develop generalised tonic-clonic seizures.

## L-2-hydroxyglutaric Aciduria (L-2-HGA)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Staffordshire Bull Terrier)

Sequencing (Yorkshire Terrier)

Breed Staffordshire Bull Terrier, Yorkshire Terrier

Inheritance Autosomal recessive

Duration 3 – 5 days (Staffordshire Bull Terrier)

1 – 2 weeks (Yorkshire Terrier)

Note L-2-HGA produces a variety of neurological deficits, including

psychomotor retardation, seizures and ataxia. Signs are "wobbly" gait, muscle stiffness as a result of exercise or excitement, and

altered behaviour.

#### **Lafora Disease**

Material EB 1 ml, no buccal swabs!

Method Special FLP

Breed Basset Hound, Beagle, Chihuahua, Dachshund (Dackel), French

Bulldog, Newfoundland, Welsh Corgi Cardigan, Welsh Corgi Pem-

broke

Inheritance Autosomal recessive

Duration 2 – 3 weeks

Note Lafora disease is a glycogen metabolism disorder that causes

progressive myoclonic epilepsy. Soluble glycogen is transformed to insoluble polyglucosan that aggregates to form Lafora bodies and accumulates in the neuronal somatodendritic compartments of the brain as well as in muscles, heart, skin and liver. The signs of this disease are: poor vision/blindness, generalised tonic-clonic seizures, myoclonic jerks, panic attacks, dementia, aggression and, in later stages, faecal and urinary incontinence. The first signs usually

appear from 7 years of age onwards.

# **Lagotto Storage Disease (LSD)**

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Lagotto Romagnolo

Inheritance Autosomal recessive with incomplete penetrance

Duration 3 – 5 days

Note Lagotto storage disease (LSD) is a storage disease with neurode-

generative symptoms, which leads to cerebellar damage in affected animals. These are the cause of movement control and balance disorders. In some affected dogs, nystagmus as well as behavioural changes like aggression and restlessness can be detected. The first

signs appear at an age of four months to four years.

# Laryngeal Paralysis (LP)

Material EB 1 ml, buccal swab

Method FLP

Breed Bull Terrier, Miniature Bull Terrier

Inheritance Autosomal recessive with incomplete penetrance

Duration 1 – 2 weeks

Note In Bull Terrier and Miniature Bull Terrier breeds, a genetic variant was

identified to be a major genetic risk factor for an early form of laryngeal paralysis. Homozygous dogs have a ten to twenty times higher risk of laryngeal paralysis. Because of the high clinical relevance of LP (voice impairment, stridor, limited exercise tolerance, dyspnoea, collapse), mating should be done with at least one of the breeding animals tested as homozygous-clear to avoid homozygous-affected

puppies.

# Laryngeal Paralysis with Polyneuropathy Type 3 (LPPN3)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Labrador Retriever, Leonberger, St. Bernard

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Dogs suffering from LPPN3 often show respiratory problems that

can lead to laryngeal paralysis. Other typical signs of polyneuropathy such as gait disorders may also occur. In addition to this mutation, there are other causative mutations that lead to LPN1 or LPN2,

which are similar diseases in the Leonberger.

#### Late Onset Ataxia (LOA)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Jack Russell Terrier, Parson Russell Terrier

Inheritance Autosomal recessive

Duration 3-5 days

Note The disease leads to progressive restrictions of the musculoskeletal

system and loss of balance. In affected animals, symptoms usually appear between 6 and 12 months of age. SCA can also cause this

clinical picture, but usually has an earlier onset.

#### Leonberger Polyneuropathy (LPN1 and LPN2)

Material EB 1 ml, buccal swab

Method FLP (LPN1)

Seauencina (LPN2)

Breed Leonberger

Inheritance Autosomal recessive (LPN1)

Autosomal dominant with incomplete penetrance (LPN2)

Duration 1 – 2 weeks

Note LPN types 1 and 2 are characterised by increasing exercise intoler-

ance and uncoordinated gait, especially in the hind legs. Eventually, the animals can hardly carry their own weight. Furthermore, there are

distinct breathing sounds, altered barking and dysphagia. The onset of LPN1 is at about 2 – 4 years of age and it shows a severe progression. The LPN1 mutation causes around 11% of all cases of polyneuropathy in Leonbergers. The average onset of LPN2 is around 6 years of age. LPN2 causes around 21% of all cases of polyneuropathy in Leonbergers. In addition to these two mutations,

there are other unknown causative mutations.

# Lethal Acrodermatitis (LAD)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Bull Terrier, Miniature Bull Terrier

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Already in the first weeks of life, LAD is characterised by typical skin

lesions, especially on the paws, growth retardation and immunodeficiency. Initially, the skin lesions resemble a zinc deficiency, later on, severe infections (malassezia, candida) as well as hyperkeratosis and deformation of the nails occur. Additionally, diarrhoea and pneu-

monia occur. LAD usually leads to death in 1 – 2 years.

# Lethal Lung Disease (LAMP3)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Airedale Terrier

Inheritance Autosomal recessive with incomplete penetrance

Duration 1 – 2 weeks

Note In Airedale Terriers, a variant in the LAMP3 gene was found which

encodes for a membrane protein of the lamellar bodies. Since lamellar bodies are involved in surfactant formation in the pulmonary alveoli, synthesis of the surfactant is severely impaired. Homozygous affected puppies are already lethargic at birth, refuse to suckle and develop dyspnoea/tachypnoea and severe oxygen deficiency within

the first days or weeks; they are usually euthanised.

In screenings, the LAMP3 gene was found to be homozygous in a 6-year-old female dog who, according to her owner, never showed any signs of respiratory disease. This suggests that there might be an unknown protective variant causing an incomplete penetrance of

LAMP3.

#### Leukocyte Adhesion Deficiency III (LAD3)

Material EB 1 ml, buccal swab

Method Sequencing
Breed German Shepherd
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note LAD3 is an inherited immunodeficiency disease. It is caused by

a recessive mutation affecting the cell-to-cell contact. Thereby, granulocytes are unable to migrate to the site of the infection. Animals suffering from LAD3 can neither form pus nor develop neutrophilia. Affected dogs develop severe, often life-threatening infections at a very early stage, which cannot be treated even with

high doses of antibiotics.

#### Leukoencephalomyelopathy (LEMP)

Material EB 1 ml, buccal swab

Breed

Method TaqMan SNP assay (Leonberger)

Sequencing (Great Dane, Rottweiler) Great Dane, Leonberger, Rottweiler

Inheritance Autosomal recessive with incomplete penetrance

Duration 3 – 5 days (Leonberger)

1 – 2 weeks (Great Dane, Rottweiler)

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Note LEMP is a neurodegenerative disease of the white matter of the

CNS, in which lesions of the myelin sheath lead to coordination and movement disorders. The first signs appear at the age of 1-3 years; only a few months after the onset of the first signs, the affected dogs will not be able to stand up or walk anymore. Since about 1% of the examined dogs without symptoms are tested as homozygous affected, it is assumed that the penetrance is incomplete and an

influence of modifier genes or factors is expected.

## Leukoencephalopathy (LEP)

Material EB 1 ml, buccal swab

Method Sequencing Breed Schnauzer

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In LEP, there are defects in myelin proteins and/or metabolic defects

of the oligodendrocytes with insufficient formation/maintenance of the myelin sheath. Signs include dysphagia, tetraparesis and ataxia, walking circles, distemper, head tilt, strabismus and tonic-clonic seizures or sudden death. Brains of affected dogs showed lesions of the cerebellar white matter, a reduced distinction between grey and white matter and mild hydrocephalus. Affected puppies are usually

euthanised a few days after birth.

#### **Lundehund Syndrome (LHS)**

Material EB 1 ml, buccal swab

Method Sequencing

Breed Norwegian Lundehund Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note The typical signs of LHS are similar to those of protein-losing entero-

pathy (PLE). They include gastritis, protein loss, chronic inflammation,

lymphangiectasia and malabsorption.

In addition, poor general condition, frequent vomiting and oedema

can be observed in affected animals.

#### Macrothrombocytopenia (MTC)

Material EB 1 ml, buccal swab

Method Sequencing

Breed American Cocker Spaniel, Bichon Frisé, Boxer, Cairn Terrier,

Cavalier King Charles Spaniel, Chihuahua, English Cocker Spaniel, Havanese, Jack Russell Terrier, Labrador Retriever, Maltese, Norfolk

Terrier, Parson Russell Terrier, Poodle, Shih Tzu

Inheritance Autosomal dominant (intermediate) (American Cocker Spaniel,

Bichon Frise, Boxer, Cavalier King Charles Spaniel, Chihuahua, English Cocker Spaniel, Havanese, Jack Russell Terrier, Labrador Retriever, Maltese, Parson Russell Terrier, Poodle, Shih Tzu)

Autosomal recessive (Cairn Terrier, Jack Russell Terrier, Norfolk Terrier,

Parson Russell Terrier)

Duration 1 – 2 weeks

Note MTC is a hereditary disorder affecting platelet production. Two

mutations have been identified in the  $\beta$ 1-tubulin gene, one of which is recessive, while the other one is dominant. Congenital MTC leads to thrombocytopenia, with counts ranging between 100.000 and 50.000 per  $\mu$ I or even below. Moreover, many platelets are larger than normal. Heterozygous carriers of the dominant mutation have counts that lie between those of affected and normal dogs. Affected dogs do not have a bleeding diathesis, but as treatment with antibiotics or steroids is contraindicated for congenital MTC, this genetic test should be considered as an important method for differential diagnosis.

## Macular Corneal Dystrophy (MCD)

Material EB 1 ml, buccal swab

MethodSequencingBreedLabrador RetrieverInheritanceAutosomal recessive

Duration 1 – 2 weeks

Note MCD is a progressive disease which affects the stroma of the

cornea. The disease is caused by a genetic variant in the CHST6 gene, which encodes for an enzyme involved in the synthesis of keratan sulphate. Keratan sulphate is thought to be relevant for corneal hydration. MCD leads to increasing corneal opacity at 4-6 years of age and severe visual impairment over time. In some dogs,

vascularisation of the corneal epithelium may also occur.

# Malignant Hyperthermia (MH)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed A

Inheritance Autosomal dominant

Duration 3 – 5 days

Note This disease is caused by inhalation narcotics and muscle relaxants

and is characterised by increased body temperature, hypercapnia, rhabdomyolysis, cardiac arrhythmias and renal failure. Damage to neural, hepatic and renal tissue and death occur if narcotics and

muscle relaxants are further administered.

#### May-Hegglin Anomaly (MHA)

Material EB 1 ml, buccal swab

Method Sequencing Breed Pug Dog

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Animals with MHA show persistent thrombocytopenia and greatly

enlarged platelets which are variably altered in morphology. In addition, cytoplasmic inclusions can be detected in neutrophil granulocytes. In affected animals, coagulation is delayed.

#### MDR1 Gene Variant\* (Ivermectin Hypersensitivity)

Material EB 1 ml, buccal swab Method Partner laboratory

Breed Australian Shepherd, Border Collie, Collie (rough/smooth), Elo,

German Shepherd, Long-haired Whippet (Silken Windsprite), McNab Shepherd, Miniature American Shepherd, Old English Sheepdog, Shetland Sheepdog (Sheltie), Silken Windhound, Wäller, White Swiss

Shepherd

Inheritance Autosomal recessive; hypersensitivity is also to be expected in

carriers

Duration 1 – 2 weeks

Note Hypersensitivity to the antiparasitic drug ivermectin is caused by a

variant in the multi-drug resistance transporter (MDR1). In addition to ivermectin and loperamide, **numerous other pharmaceutical substances** are known which are expected to easily enter the brain tissue if applied in combination with an altered MDR1 transporter.

# Microphthalmia (RBP4)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Irish Soft Coated Wheaten Terrier

Inheritance Autosomal recessive with maternal influence

Duration 3 – 5 days

Note Microphthalmia can be caused by a hereditary prenatal vitamin A

deficiency. Homozygous affected puppies only show signs if their mother is also homozygous affected and suffers from disturbed vitamin A transport. If the mother is heterozygous for the genetic defect,

the puppies will probably not show any signs.

#### Mucopolysaccharidosis Type IIIa (MPS3a)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Dachshund (Dackel), New Zealand Huntaway

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note MPS3a-affected animals suffer from severe degeneration of the

central nervous system. First neurological signs usually appear from the age of eighteen months onwards, rapidly worsen, culminating in

ataxia, and often lead to the death of the affected dog.

#### Mucopolysaccharidosis Type IIIb (MPS3b)

Material EB 1 ml, buccal swap

Method FLP

Breed Schipperke

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In Schipperkes, MPS3b is a lysosomal storage disease that is also

known as "Sanfilippo syndrome type 3b". An enzyme defect prevents the breakdown of heparan sulphate, which accumulates in the lysosomes. Signs include tremor and imbalance, even with falling to either side. The onset of the signs is between 2-4 years and they become worse, so that these animals are usually euthanised 1-2

years later.

# Mucopolysaccharidosis Type VI (MPS6)

Material EB 1 ml. buccal swab

Method Sequencing

Breed Miniature Pinscher Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note The genetic variant causing MPS6 appears to be relatively common

in the Miniature Pinscher and causes lysosomal storage disease in homozygous cases due to an arylsulphatase B (ARSB) deficiency, so that sulphate cannot be broken down from chondroitin sulphate

Note

Due to extensive fibrosis of the skin and joints, affected dogs suffer from arthrosis and stiffness, have shortened outer toes and a typical flat head shape.

# Mycobacterium avium Complex Sensitivity (MAC)

Material EB 1 ml. buccal swab

Method TaqMan SNP assay, if necessary sequencing

Breed Miniature Schnauzer Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

Note In Miniature Schnauzers, a variant in the CARD9 gene leads to im-

munodeficiency, which is associated with an increased susceptibility to Mycobacterium avium with its subspecies (Mycobacterium avium complex, MAC) and Mycobacterium intracellulare. Starting at the age of 1 – 8 years, this leads to disturbed general condition, nasal discharge, conjunctivitis, diarrhoea and enlarged lymph nodes, liver and spleen. The animals do not respond adequately to treatment and may pose a risk to animal owners with a weakened immune system.

# Myostatin Mutation ("Bully" Gene)

Material EB 1 ml, buccal swab

Method Sequencing Breed Whippet

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In Whippets, a significant correlation between this mutation (in the

heterozygous genotype) and racing performance was found. Dogs with two "bully" alleles (homozygous case) appear extremely muscu-

lar, but their ability to run is limited.

# Myotonia Congenita

Material EB 1 ml, buccal swab

Method TaqMan SNP assay (Miniature Schnauzer)

Sequencing (Australian Cattle Dog, Labrador Retriever)

Breed Australian Cattle Dog, Labrador Retriever, Miniature Schnauzer

Inheritance Autosomal recessive

Duration 3 – 5 days (Miniature Schnauzer)

1 – 2 weeks (Australian Cattle Dog, Labrador Retriever)

Note This disease affects the skeletal muscle ion channels. Clinical signs

are mainly stiff-legged gait, dysphagia and excessive salivation.

All affected Miniature Schnauzers showed abnormal dentition and

overbite, in some cases also abnormal barking.

are detectable in the urine (toluidine blue staining strongly positive). There is no serum ARSB enzyme activity. Severe signs (corneal opacity, disproportionate dwarfism, kyphosis, facial dysmorphia) usually lead to the animals being euthanised as puppies or young adult dogs.

and dermatan sulphate. In case of MPS6, these sulphate compounds

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## Mucopolysaccharidosis Type VII (MPS7)

Material EB 1 ml. buccal swab

Method Sequencing

Breed Brazilian Terrier, German Shepherd

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note This lysosomal storage disease causes corneal clouding as well as

severe skeletal deformities. Dogs cannot walk even at the age of

several weeks to months.

# **Muscular Dystrophy (MD)**

Material EB 1 ml, buccal swab

Method TagMan SNP Assay (Landseer)

Sequencing (Cavalier King Charles Spaniel, Golden Retriever,

Norfolk Terrier)

Breed Cavalier King Charles Spaniel, Golden Retriever, Landseer, Norfolk

Terrier

Inheritance X-linked recessive (Cavalier King Charles Spaniel, Golden Retriever,

Norfolk Terrier)

Autosomal recessive (Landseer)

Duration 3 – 5 days (Landseer)

1 – 2 weeks (Cavalier King Charles Spaniel, Golden Retriever,

Norfolk Terrier)

Note Affected dogs show raised creatine kinase levels in serum, muscle

atrophy with contractures, fibrosis and cardiomyopathy. Generally, first signs appear at the age of three to six months. Affected dogs

usually die between the ages of 4 and 24 months.

Musladin-Lueke Syndrome (MLS)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Beagle

Inheritance Autosomal recessive

Duration 3 – 5 days

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Narcolepsy

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Labrador Retriever)

Sequencing (Dachshund (Dackel), Dobermann)

Breed Dachshund, Dobermann, Labrador Retriever

Inheritance Autosomal recessive

Duration 3 – 5 days (Labrador Retriever)

1 – 2 weeks (Dachshund (Dackel), Dobermann)

Note Narcolepsy is a neurological disease which is characterised by

sleep attacks, cataplexy and sleep paralysis.

**Necrotizing Meningoencephalitis (NME/PDE)** 

Material EB 1 ml. buccal swab

Method Sequencing Breed Pug Dog

Inheritance Autosomal recessive with incomplete penetrance

The test identifies a risk factor which is associated with NME (also

called PDE, pug dog encephalitis).

Duration 1 – 2 weeks

Note Due to the autoimmune inflammation of the central nervous system,

disorientation, confusion and seizures occur. The genetic test deter-

mines the risk for the development of this disease.

**Nemaline Myopathy (NM)** 

Material EB 1 ml, buccal swab

MethodSequencingBreedAmerican BulldogInheritanceAutosomal recessive

Duration 1 – 2 weeks

Note NM in American Bulldogs is characterised by a variety of muscular

disorders, such as muscle weakness, muscular hypotonia, hypo-

ventilation and dysphagia.

Neonatal Cortical Cerebellar Abiotrophy (NCCD)

Material EB 1 ml, buccal swab

Method FLP (Beagle)

Sequencing (Magyar Viszla)

Breed Beagle, Magyar Vizsla Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Affected dogs exhibit signs soon after birth or at a very early age.

These include tremor, ataxia and spastic paralysis.

Neonatal Encephalopathy with Seizures (NEWS)

Material EB 1 ml, buccal swab

Method TagMan SNP assay, if necessary sequencing

Breed Poodle

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

Note NEWS is a malformation of the cerebellum. Already at birth, affected

puppies are relatively small and weak and they often die within the first week of their lives. Those surviving this phase develop ataxia, tremor and seizures. So far, these animals had to be euthanised

before they were 8 weeks old.

Neuroaxonal Dystrophy (NAD)

Material EB 1 ml, buccal swab

Method TagMan SNP assay, if necessary sequencing (Lagotto Romagnolo,

Papillon, Spanish Water Dog) Sequencing (Rottweiler)

Breed Lagotto Romagnolo, Papillon, Rottweiler, Spanish Water Dog

Inheritance Autosomal recessive

Duration 3 – 5 days; 1 – 2 weeks for sequencing (Lagotto Romagnolo,

Papillon, Spanish Water Dog) 1 – 2 weeks (Rottweiler)

Note NAD is generally characterised by a distinct histology and neuro-

degenerative pathology of the central and/or peripheral nervous system. As with most neurological disorders, symptoms may vary greatly. Homozygous puppies typically die shortly after birth due to respiratory failure and, histologically, show swollen, spheroid axons

throughout the nervous system.

Neuronal Ceroid Lipofuscinosis (NCL)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay (American Bulldog, Border Collie, English Setter,

Golden Retriever, Gordon Setter, Tibetan Terrier)

Sequencing (Chihuahua, Chinese Crested Dog, Dachshund (Dackel),

Italian Cane Corso, Saluki)

TagMan SNP assay and sequencing (Australian Cattle Dog, Australian

Shepherd)

Breed American Bulldog, Australian Cattle Dog, Australian Shepherd,

Border Collie, Chihuahua, Chinese Crested Dog, Dachshund (Dackel), English Setter, Golden Retriever, Gordon Setter, Italian

Cane Corso, Saluki, Tibetan Terrier

Inheritance Autosomal recessive

Duration 3 – 5 days (American Bulldog, Border Collie, English Setter, Golden

Retriever, Gordon Setter, Tibetan Terrier)

1 – 2 weeks (Australian Cattle Dog, Australian Shepherd, Chihuahua, Chinese Crested Dog, Dachshund (Dackel), Italian Cane Corso,

Saluki)

Note NCL is a progressive neurodegenerative disease caused by lysoso-

mal storage defects.

Clinical signs include increasing levels of agitation and aggression. The dogs become hyperactive and ataxic and may suffer from epileptic fits and impaired vision. The age of the onset of the disease as

well as its severity may vary greatly.

## Neuronal Ceroid Lipofuscinosis\* (NCL) in the American Staffordshire Terrier

Material EB 1 ml, buccal swab Method Partner laboratory

Breed American Staffordshire Terrier

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note See Neuronal Ceroid Lipofuscinosis (NCL) above.

Obesity

Material EB 1 ml, buccal swab

Method FLP

Breed Flat Coated Retriever, Labrador Retriever

Inheritance As yet unknown
Duration 1 – 2 weeks

Note In Labrador Retrievers and in Flat Coated Retrievers, a POMC (pro-

opiomelanocortin) mutation was found which influences energy homeostasis by disrupting the production of the neuro-affective peptides ß-MSH and ß-endorphin. POMC mutation is associated with greater weight, obesity and increased food motivation. The mutation was particularly often detected in assistance and companion dogs.

## Osteogenesis Imperfecta (Brittle Bone Disease)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay, if necessary sequencing (Dachshund)

Sequencing (Beagle, Golden Retriever)

Breed Beagle, Dachshund (Dackel), Golden Retriever

Inheritance Autosomal recessive (Dachshund)

Autosomal dominant (Beagle, Golden Retriever)

Duration 3-5 days (1-2 weeks for sequencing) (Dachshund)

1 – 2 weeks (Beagle, Golden Retriever)

Note The cause of brittle bone disease (osteogenesis imperfecta) is a

defective formation of type 1 collagen resulting in extremely brittle

bones and teeth already in puppies.

# Paroxysmal Dyskinesia (PxD)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Irish Soft Coated Wheaten Terrier

Inheritance Autosomal recessive

Duration 1-2 weeks

Note Affected dogs suffer from episodes of involuntary, sudden, irregular

and unpredictable movements of the limbs, especially the hind legs, known as hyperkinesia. These attacks last several minutes to hours and occur up to 10 times a day. The first signs typically occur at the

age of about two years and worsen with age.

# Paroxysmal Exercise-Induced Dyskinesia (PED)

Material EB 1 ml, buccal swab

Method Sequencina

Breed Shetland Sheepdog (Sheltie)

Inheritance Probably autosomal dominant (still in research)

Duration 1 – 2 weeks

Note In Shelties, a variant in the PCK2 gene was found which causes PED.

Affected dogs show short to long-lasting episodes of generalised ataxia and hypermetria, increased muscular tension in all four limbs, as well as decreased mental activity and mild tremor. Episodes are triggered by stress or excitement. With good stress management, a specific diet (gluten- and grain-free, seafood-based with high levels of tryptophan) and anti-epileptic treatment, a normal life expectancy

is possible.

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#### Persistent Müllerian Duct Syndrome (PMDS)

Material EB 1 ml, buccal swab Method TagMan SNP assay Breed Miniature Schnauzer Inheritance Autosomal recessive

Duration 3 – 5 davs

Note Persistent Müllerian duct syndrome (PMDS) causes an incomplete

> regression of the Müllerian duct during sex differentiation in male dogs. Normally, the external genitalia are fully developed. In 50% of the affected animals, the testicles do not descend (testicular dystopia),

which can lead to infertility and possibly tumour formation.

# Phosphofructokinase Deficiency (PFKD)

EB 1 ml, buccal swab Material

Method Seauencina

American Cocker Spaniel, English Springer Spaniel, Whippet Breed

Autosomal recessive Inheritance

Duration 1-2 weeks

Note Enzyme deficiency causes the destruction of red blood cells, and

thus leads to a red colouration of the urine, anaemia and icterus as

well as to exercise intolerance and muscle cramps.

# **Pituitary Dwarfism**

Material EB 1 ml. buccal swab

FLP (Czechoslovakian Wolfdog, German Shepherd, Saarloos Wolf-Method

> hond, Tibetan Terrier, White Swiss Shepherd Dog) Sequencing (Karelian Bear Dog, Lapponian Herder)

Breed Czechoslovakian Wolfdog, German Shepherd, Karelian Bear Dog,

Lapponian Herder, Saarloos Wolfhond, Tibetan Terrier, White Swiss

Shepherd Dog

Inheritance Autosomal recessive

Duration 1-2 weeks

Note Dwarfism results from a lack of growth hormone caused by a dys-

> function of the pituitary gland. In Shepherds and Wolfhounds, growth stops at 3 – 8 weeks of age. If left untreated, the animals either keep their puppy fluff or completely lose their coat. Topcoat usually only develops in the head/foot area. Affected Karelian Bear Dogs. Tibetan Terriers and Lapponian Herders gain weight more slowly and keep their puppy coat or suffer from severe hair loss, rather thin skin and

skin inflammation at 2 – 3 years of age.

#### Polycystic Kidney Disease (PKD)

Material EB 1 ml, buccal swab

Method Sequencing Breed **Bull Terrier** 

Inheritance Autosomal dominant

Duration 1 – 2 weeks

Note PKD leads to the formation of cysts in the liver, pancreas and kidneys.

The fluid-filled kidney cysts eventually cause renal failure and lead to

death.

# Postoperative Haemorrhage (P2Y12 mutation)

EB 1 ml. buccal swab Material

Method Sequencing

Great Swiss Mountain Dog Breed Autosomal dominant Inheritance

1 – 2 weeks Duration

In Great Swiss Mountain Dogs, a mutation in the P2Y12 gene leads Note

> to severe coagulation disorders. Affected animals only show severe bleeding, which is often fatal, during major surgery or serious injuries. The genetic test is therefore diagnostically useful as a preventive

measure before surgery.

#### Prekallikrein Deficiency (KLK)

Material EB 1 ml, buccal swab

Method Sequencing Breed Shih Tzu

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Even though KLK leads to deficiency in prekallikrein, a component of

> the coagulation cascade, it is not associated with increased bleeding diathesis. Only in combination with other defects in the coagulation cascade (factor VII, VIII and IX deficiencies), an increased tendency

to bleed has been described in a few cases.

# Primary Ciliary Dyskinesia (PCD)

Material EB 1 ml, buccal swab

TagMan SNP assay, if necessary sequencing (Old English Sheepdog) Method

Sequencing (Alaskan Malamute)

Alaskan Malamute, Old English Sheepdog Breed

Autosomal recessive Inheritance

Duration 3 – 5 days, 1 – 2 weeks for sequencing (Old English Sheepdog)

1 – 2 weeks (Alaskan Malamute)

Note This syndrome is characterised by recurrent infections of the respira-

tory tract as well as reduced male fertility. Approximately 50% of the affected patients develop situs inversus (Kartagener syndrome).

# Primary Hyperoxaluria (PH)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Coton de Tuléar
Inheritance Autosomal recessive

Duration 1-2 weeks

Note PH leads to the accumulation of oxalate and the subsequent

formation of calcium oxalate crystals in the urinary organs. The resulting crystals also accumulate in the kidney tissue and can lead

to decreased renal function.

## **Primary Lens Luxation (PLL)**

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed American Eskimo Dog, American Hairless Terrier, Australian Cattle

Dog, Chinese Crested Dog, Danish-Swedish Farmdog, Fox Terrier, German Hunting Terrier, Jack Russell Terrier, Lakeland Terrier, Lancashire Heeler, Lucas Terrier, Miniature Bull Terrier, Norfolk Terrier, Norwich Terrier, Parson Russell Terrier, Patterdale Terrier, Pug Dog, Rat Terrier, Sealyham Terrier, Teddy Roosevelt Terrier, Tenterfield Terrier, Tibetan Terrier, Toy Fox Terrier, Volpino Italiano, Welsh Terrier,

Westfalen Terrier, Yorkshire Terrier

Inheritance Autosomal recessive: it is estimated that about 2 – 20% of the carri-

ers will develop PLL.

Duration 3 – 5 days

Note Affected dogs may suffer from painful glaucomas and blindness due

to a dislocation of the lens.

# Primary Open Angle Glaucoma (POAG)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Basset Fauve de Bretagne, Basset Hound, Beagle, Norwegian

Elkhound, Petit Basset Griffon Vendeen

Inheritance Autosomal recessive

Duration 1-2 weeks

Note Without any prior eye disease, there is a rise in pressure in the eye-

ball which leads to visual field loss and blindness.

#### Primary Open Angle Glaucoma and Lens Luxation (POAG/PLL)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Shar Pei

Inheritance Autosomal recessive

Duration 3 – 5 days

Note A genetic dysfunction of the connective tissue in the eye leads to

glaucoma (POAG) and often to lens luxation (PLL). POAG can lead

to blindness.

Usually, affected dogs show first signs at the age of 4 - 6 years.

# **Progressive Retinal Atrophy (PRA)**

Progressive retinal atrophy (PRA) is a disease of the retina which continuously progresses and always leads to blindness. Over time, the photoreceptors of the eye will be destroyed. In most forms, rods are initially affected and cones are affected later, so that night blindness occurs first. Clinical signs usually appear in early youth, but the time of onset varies in different dog breeds. The ophthalmologic signs are similar in all forms (bilateral mydriasis, hyperreflective tapetum lucidum, atrophy of the retinal vessels).

**Bas PRA** 

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Basenii

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Further forms of PRA are suspected. The form of PRA in Basenjis,

which can be detected by genetic testing, has an onset at about 5

vears of age.

# CNGA1\_PRA (Shet\_PRA)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay, if necessary sequencing

Breed Shetland Sheepdog (Sheltie)

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

Note There seems to be at least one more mutation.

In Shelties, first signs of PRA are usually seen from the age of two. "Slowly progressing retinopathy" (SPR), which also occurs in Shelties,

is similar to PRA in the early stages and can only be distinguished in

differential diagnosis by means of ERG.

cord1/crd4-PRA

Material EB 1 ml, buccal swab

Method FLP

Breed Beagle, Bolonka Zwetna, Clumber Spaniel, Curly Coated Retriever,

Dachshund (Dackel), English Springer Spaniel

Inheritance Autosomal recessive

Duration 1-2 weeks

Note In cone-rod dysplasia (cord1), the cone cells degenerate first, ap-

proximately from the age of 6 months onwards. In some genetically affected dogs, however, no signs are seen even at a higher age. The connection between this mutation and the occurrence of the disease

is still a subject of scientific debate.

crd-PRA

Material EB 1 ml, buccal swab

Method FLP

Breed Dachshund (Dackel)
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note The early loss of retinal cone cells is characteristic for crd-PRA.

The first clinical signs of crd-PRA can occur at the age of six months. After about 1 to 2 years, the full clinical picture (day blindness) beco-

mes apparent.

crd1-PRA

Material EB 1 ml. buccal swab

Method Sequencing

Breed American Staffordshire Terrier

Inheritance Autosomal recessive

Duration 1-2 weeks Note See crd-PRA.

crd2-PRA

Material EB 1 ml, buccal swab

Method Sequencing

Breed American Pitbull Terrier Inheritance Autosomal recessive

Duration 1 – 2 weeks
Note See crd-PRA.

crd3-PRA

Material EB 1 ml, buccal swab

Method FLP

Breed Irish Glen of Imaal Terrier

Inheritance Still unknown
Duration 1 – 2 weeks

Note A variant in the ADAM9 gene causes crd3. At the age of 12 – 24

months, the cone and later also the rod photoreceptor cells begin to degenerate. It can take several years until complete blindness sets in. At ophthalmological examinations, crd3 can normally only be

detected from 3 – 5 years of age onwards.

**Dominant PRA** 

Material EB 1 ml, buccal swab

Method Sequencing
Breed Bullmastiff, Mastiff
Inheritance Autosomal dominant

Duration 1 – 2 weeks

Early-onset PRA (eo-PRA)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Portuguese Water Dog, Spanish Water Dog

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Early-onset PRA is caused by a variant in the PDE6B gene. Owners

of eo-PRA-affected dogs report initial visual impairment at the age of about 1.5 years and describe the animals as mostly blind by 4.5 years. Early-onset PRA can often only be diagnosed through a clinical ophthalmological examination at a later point in time, after

the owners have already noticed the first changes.

**Generalised PRA** 

Material EB 1 ml, buccal swab

Method Sequencing Breed Schapendoes

Inheritance Autosomal recessive

Duration 1 – 2 weeks

GR PRA1 and GR PRA2

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Golden Retriever

Inheritance Autosomal recessive

Duration 3-5 days

Note The onset of the diseases varies within the breed, but diagnosis is

often not made until about 5 years of age.

IFT122-PRA

Material EB 1 ml, buccal swab

Method Sequencing

Breed Lapponian Herder Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note IFT122-PRA is usually diagnosed between 5 – 12 years of age. It is

caused by a variant of the intraflagellar transport 122 gene (IFT122 gene) and progresses slowly so that some dogs still have some

vision at 13 years of age.

**NECAP1-PRA** 

Material EB 1 ml, buccal swab

Method Sequencing
Breed Giant Schnauzer
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In Giant Schnauzers, a variant in the NECAP1 gene was found. This

gene encodes for a protein that is involved in clathrin-mediated endocytosis (CME) in the synapses. It is assumed that by inhibiting CME, rhodopsin accumulates in the photoreceptors and leads to retinal degeneration. First signs have been described from about 4

vears onwards.

pap\_PRA1

MaterialEB 1 ml, buccal swabMethodTaqMan SNP assayBreedPapillon, PhalèneInheritanceAutosomal recessive

Duration 3 – 5 days

Note There are further forms of PRA in these breeds.

PRA4

Material EB 1 ml, buccal swab

Method FLP

Breed Lhasa Apso

Inheritance Autosomal recessive

Duration 1-2 weeks

Note PRA4 is caused by a variant in the IMPG2 gene. Clinical signs can

appear as early as 2.5 years of age, although the age is very variable. It often takes several years until the owners of affected dogs

notice any visual impairment.

prcd-PRA\*

Material EB 1 ml, buccal swab Method Partner laboratory

Breed American Cocker Spaniel, American Eskimo Dog, Australian Cattle

Dog, Australian Shepherd, Australian Silky Terrier, Australian Stumpy Tail Cattle Dog, Barbet, Bearded Collie, Bolognese, Bolonka Zwetna, Chesapeake Bay Retriever, Chihuahua, Chinese Crested Dog, English Cocker Spaniel, English Shepherd, Entlebucher Mountain Dog, Finnish Lapphund, German Spitz, Giant Schnauzer, Golden Retriever, Jack Russell Terrier, Karelian Bear Dog, Kuvasz, Labrador Retriever, Lagotto Romagnolo, Lapponian Herder, Markiesje, Miniature American Shepherd, Norwegian Elkhound, Nova Scotia Duck Tolling Retriever, Parson Russell Terrier, Poodle, Portuguese Water Dog, Schipperke, Spanish Water Dog, Swedish Lapphund, Wäller,

Yorkshire Terrier

Inheritance Autosomal recessive

Duration 1 – 2 weeks

rcd1-PRA

Material EB 1 ml, buccal swab

Method TaqMan SNP assay, if necessary sequencing Breed Irish Red and White Setter, Irish Red Setter

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

rcd1a-PRA

Material EB 1 ml, buccal swab

Method Sequencing Breed Sloughi

Inheritance Autosomal recessive

Duration 1 – 2 weeks

rcd2-PRA

Material EB 1 ml, buccal swab

Method FLP

Breed Collie (rough/smooth)
Inheritance Autosomal recessive

Duration 1 – 2 weeks

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rcd3-PRA

Material EB 1 ml, buccal swab

Method Sequencing

Breed Chinese Crested Dog, Pomeranian, Welsh Corgi Cardigan

Inheritance Autosomal recessive

Duration 1 – 2 weeks

rcd4-PRA

Material EB 1 ml, buccal swab

Method Sequencing

Breed Australian Cattle Dog, English Setter, Gordon Setter, Irish Red and

White Setter, Irish Red Setter, Old Danish Pointing Dog, Polish Lowland Sheepdog (PON), Poodle, Small Munsterlander, Tatra Shepherd

Dog, Tibetan Terrier

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Type B1-PRA (HIVEP3)

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Miniature Schnauzer Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note New research confirms the correlation between a mutation in the

HIVEP3 gene and this early form of type B PRA in the Miniature Schnauzer. We recommend testing for the HIVEP3 variant as it has a better correlation than the previously offered test for the PPT1 gene.

XL-PRA

Material EB 1 ml, buccal swab

Method Sequencing
Breed Husky, Samoyed
Inheritance X-linked recessive
Duration 1 – 2 weeks

Note XL-PRA is a late form of the disease. The first signs usually occur at

the age of three to five years.

**Protein Losing Nephropathy (PLN)** 

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Irish Soft Coated Wheaten Terrier

Inheritance Heterozygous dogs have a medium risk and homozygous affected

dogs have a high risk of developing signs of the disease.

Duration 3-5 days

Note Hereditary PLN manifests itself as hidden proteinuria from middle

age onwards. The disease can be stable and mild for years. In some cases, however, severe complications such as kidney failure or thrombosis occur. The genetic test provides a risk assessment for

PLN.

Pyruvate Dehydrogenase Phosphatase 1 Deficiency (PDP1)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Clumber Spaniel, Sussex Spaniel

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Even after minimal effort, affected dogs suffer from severe exercise

intolerance which may lead to collapse. There may also be neurolo-

gical symptoms.

Pyruvate Kinase Deficiency (PK)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Basenji, Beagle, Cairn Terrier, Labrador Retriever, Pug Dog, West

Highland White Terrier

Inheritance Autosomal recessive

Duration 1-2 weeks

Note The lack of pyruvate kinase causes severe chronic regenerative

haemolytic anaemia, reticulocytosis, progressive myelofibrosis and

osteosclerosis.

**Raine Syndrome** 

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Border Collie

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Affected dogs show very severe tooth wear and gingivitis, which can

lead to the loss of teeth. The excessive wear of the teeth results from a lack of mineralisation and, thus, reduced hardness of the enamel. The bones of these animals are usually less mineralised as well.

Renal Cystadenocarcinoma and Nodular Dermatofibrosis (RCND)

Material EB 1 ml, buccal swab

Method TaqMan SNP assay, if necessary sequencing

Breed German Shepherd Inheritance Autosomal dominant

Duration 3-5 days (1-2 weeks for sequencing)

Note RCND causes bilateral, multifocal tumours in the kidneys, uterine

leiomyomas in female dogs and nodules in the skin consisting of dense collagen fibres. This form of cancer is inherited in a dominant manner. Animals in which the mutated allele is homozygous most

likely already die in utero.

Renal Dysplasia and Hepatic Fibrosis (RDHN)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Norwich Terrier
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note RDHN in Norwich Terriers is a structural/functional defect of primary

cilia (ciliopathy). Primary cilia are only passively motile, occur on almost all types of cells and are, for example, important for organogenesis. Affected puppies suffer from diffuse cystic, enlarged kidneys, hepatic fibrosis, subcutaneous oedema, pleural effusion and ascites, underdeveloped lungs, cleft palate, diaphragmatic malformation/

hernia and usually die shortly after birth.

Retinal Dysplasia (OSD)

Material EB 1 ml. buccal swab

Method Sequencing (Northern Inuit, Tamaskan)

Partner laboratory\* (Labrador Retriever)

Breed Labrador Retriever, Northern Inuit, Tamaskan

Inheritance Autosomal dominant with incomplete penetrance (Labrador Retriever)

Autosomal recessive (Northern Inuit, Tamaskan)

Duration 1 – 2 weeks

Note Retinal dysplasia (RD), also called retinal folds, is a relatively

common clinical observation in many dog breeds and is per se not a breeding restriction. However, in the Labrador Retriever breed, retinal dysplasia can be associated with a more severe syndrome, the oculoskeletal dysplasia (OSD). Clinical signs of OSD are skeletal malformations, shortened limbs (dwarfism) as well as blindness at

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an early age. OSD in the Northern Inuit and the Tamaskan is caused by a different genetic variant (COL9A3 gene, exon 14) and is very similar to that in Labradors, but vision is not always impaired in these two breeds.

## Sensory Neuropathy (SN)

Material EB 1 ml, buccal swab

Method FLP

Breed Border Collie

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note SN is caused by a degeneration of sensory and (to a lesser extent)

motor neurons. Clinical signs start between 2 and 7 months of age and include progressive proprioceptive ataxia with hyperextension of the limbs and self-mutilation of the limbs. The hind legs are usually more affected. Proprioception and nociception are reduced in all limbs or are no longer present as the disease progresses. Urinary incontinence and vomiting may also occur. Sensory action potentials are reduced or absent, motor nerve conduction velocity is normal or

reduced, the EMG of the innervated muscles is normal.

## Severe Combined Immunodeficiency (SCID)

Material EB 1 ml, buccal swab

Method Sequencina

Breed Frisian Water Dog, Jack Russell Terrier, Parson Russell Terrier

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note SCID is associated with very low immunoglobulin levels and lym-

phocyte counts, which causes a severe weakening of the cellular and humoral immune response. Affected dogs show increased susceptibility to viruses and bacteria and usually die of opportunistic

infections at the age of 8 – 12 weeks.

#### **Shar Pei Autoinflammatory Disease (SPAID)**

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Shar Pei

Inheritance Autosomal dominant with incomplete penetrance (marker test)

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Duration 3 – 5 days

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Note The disease leads to a progressive restriction of the musculoskeletal

system and loss of balance. The onset of the first signs is

usually from the age of 3 months onwards.

# Skeletal Dysplasia 2 (Dwarfism) (SD2)

EB 1 ml, buccal swab Material Method TagMan SNP assay Breed Labrador Retriever Inheritance Autosomal recessive

Duration 3 – 5 days

Note

Note SD2 causes an early halt in growth of long bones. In contrast to

other forms of dwarfism (pituitary dwarfism), the result are "disproportioned" dogs with shortened front limbs and a raising dorsal line,

In addition to the typical fever, SPAID may cause the following sym-

ptoms: arthritis, dermatitis, otitis, systemic amyloidosis, erythema in

the area of the skin folds, skin that is stuck together and thickened,

eye inflammation and recurrent intestinal inflammation. First clinical signs of the disease usually appear at the age of 1 to 6 years.

while torso length and depth are not altered.

# Spinal Dysraphism (NTD)

EB 1 ml, buccal swab Material

Method Sequencing Breed Weimaraner

Inheritance Autosomal recessive

Duration 1-2 weeks

Neural tube defects result from abnormal closure or development Note

> of the neuronal tube during embryogenesis. In Weimaraners, spinal dysraphism is characterised by non-progressive ataxia and causes abnormal hair streams along the back, kinked tails, scoliosis in the lumbar spinal region, bunny-like hopping, crouched stance and

paraparesis.

# Spinocerebellar Ataxia (SCA)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (Fox Terrier, Jack Russell Terrier, Parson Russell

Terrier, Tenterfield Terrier, Toy Fox Terrier)

Sequencing (Alpine Dachsbracke)

Breed Alpine Dachsbracke, Fox Terrier, Jack Russell Terrier, Parson Russell

Terrier, Tenterfield Terrier, Toy Fox Terrier

Autosomal recessive Inheritance

3 – 5 days (Fox Terrier, Jack Russell Terrier, Parson Russell Terrier, Duration

Tenterfield Terrier, Toy Fox Terrier)

1 – 2 weeks (Alpine Dachsbracke)

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## Spondylocostal Dysostosis (Comma Defect)

Material EB 1 ml. buccal swab

TagMan SNP assay, if necessary sequencing Method

Breed Miniature Schnauzer Inheritance Autosomal recessive

Duration 3-5 days (1-2) weeks for sequencing

Note Comma defect is mainly characterised by segmentation disorders of

> the spine and ribs. Already as newborns, affected dogs show disproportionate dwarfism as well as spinal shortening and rib defects. The skull has a prominent forehead and a protruding occiput. In addition, there may be malformations of the toes and abdominal wall defects. Malformed ribs lead to a smaller ribcage and respiratory

insufficiency.

### Spongy Degeneration with Cerebellar Ataxia (SDCA1 and 2)

EB 1 ml. buccal swab Material

Method TagMan SNP assay (SDCA1)

FLP (SDCA2)

Belgian Shepherd, Dutch Shepherd Breed

Inheritance Autosomal recessive 3 – 5 days (SDCA1) Duration 1 – 2 weeks (SDCA2)

Note SDCA is a neurodegenerative disease in Belgian and Dutch Shepherds.

> Puppies with SDCA have an early onset of clinical signs at 5 – 8 weeks of age. They show ataxic gait, which is particularly obvious in the hind limbs. Other clinical signs are stumbling, staggering, intention tremor, muscle spasms, as well as loss of balance and falling. SDCA is a progressive disease, so that affected dogs must

usually be euthanised at the age of 12 weeks.

# Stargardt Disease (Retinal Degeneration) (STGD)

Material EB 1 ml. buccal swab TagMan SNP assay Method Breed Labrador Retriever Autosomal recessive Inheritance

Duration 3 – 5 days

Note In Labrador Retrievers, a variant in the ABCA4 gene was found which

can be associated with STGD and causes clinical signs similar to the human disease. The ABCA4 gene encodes for a membrane transporter protein located in the rods and cones. The gene variant leads to an increased accumulation of lipofuscin in the retinal pigment epithelium and to a degeneration of the cones and, later on, the

rods. Some vision remains throughout lifetime.

#### Startle Disease

Material EB 1 ml, buccal swab Method FLP (Irish Wolfhound)

Sequencing (Galgo Español)

Breed Galgo Español, Irish Wolfhound

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Due to disturbed transport of the neurotransmitter glycine, neurolo-

gical signs appear 5 – 7 days after birth. Affected dogs suffer from extensor rigidity and muscle tremor. The symptoms intensify during movement and cease when dogs are relaxed or sleeping. Affected

puppies usually have to be euthanised.

Laboklin owns the exclusive license to perform this genetic test in

the Irish Wolfhound.

### **Subacute Necrotising Encephalopathy (SNE)**

Material EB 1 ml, buccal swab

Method FLP

Breed Yorkshire Terrier
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note SNE is characterised by ataxia and spasticity as well as central

nervous visual and sensory disorders. The first signs appear in the

first year of life.

# Succinic Semialdehyde Dehydrogenase Deficiency (SSADHD)

Material EB 1 ml, buccal swab

Method Sequencing Breed Saluki

Inheritance Autosomal recessive

Duration 1-2 weeks

Note SSADH is involved in the degradation of the inhibitory neurotrans-

mitter gamma-aminobutyric acid (GABA). In SSADH deficiency, the degradation of GABA is interrupted after the formation of succinic semialdehyde (SSA), which is then reduced to 4-hydroxybutyric acid (GHB), among others. GHB is a major contributor to the clinical picture. The onset of neurological disorders (mild ataxia), seizures and behavioural changes (vocalisation, lethargy) is at 6 – 10 weeks and usually lead to euthanasia. Further abnormalities include absent reflexes (e.g. menace reflex), SSA in urine, GHB in serum and symmetrical spongiform changes in several areas of the brain (histology).

# **Thrombopathia**

MaterialEB 1 ml, buccal swabMethodTaqMan SNP assayBreedBasset Hound, LandseerInheritanceAutosomal recessive

Duration 3 – 5 days

Note Dogs suffering from this hereditary form of thrombopathia have an

unusually high number of veins, haematomas and bruises because their platelets do not respond normally to activation signals.

#### **Trapped Neutrophil Syndrome (TNS)**

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Border Collie

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Dogs with TNS can produce neutrophils but cannot release them

into the bloodstream. Therefore, affected puppies have a weakened immune system. The onset and severity of the disease vary, but

most dogs do not get older than four months.

# **Upper Airway Syndrome (UAS)**

Material EB 1 ml, buccal swab

Method Sequencing
Breed Norwich Terrier

Inheritance Autosomal dominant with incomplete penetrance

Duration 1 – 2 weeks

Note Although Norwich Terriers are considered a mesocephalic breed,

they can suffer from Upper Airway Syndrome (UAS). In this breed, a variant in the ADAMTS3 gene was found which can be associated with UAS. Homozygous affected dogs show an elongated soft

palate, malformed cartilage, everted laryngeal saccules and possibly vocal fold oedema. The resulting constriction of the respiratory tract leads – similar to brachycephalic breeds – to respiratory problems, heat and stress intolerance, cyanosis and the animals can collapse.

#### Van den Ende-Gupta Syndrome (VDEGS)

MaterialEB 1 ml, buccal swabMethodTaqMan SNP assayBreedFox Terrier, Toy Fox TerrierInheritanceAutosomal recessive

Duration 3 – 5 days

Note All affected dogs exhibit a prominent underbite with short maxilla.

Additional symptoms include lack of bone mineralisation, swollen

knee joints as well as luxation of elbow or patella.

#### Ventricular Arrhythmia (IVA)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Rhodesian Ridgeback Inheritance Unclear (see note) Duration 1 – 2 weeks

Note IVA is triggered by a variant in the QIL1 gene. This gene codes for a

protein involved in the formation and distribution of mitochondrial cristae. Affected dogs show ventricular and/or supraventricular tachycardia and other cardiac arrhythmias, usually between 6-18 months of age. In some cases, this leads to sudden cardiac death. This hereditary disease has incomplete penetrance and expression. Only about 60% of dogs carrying the variant have abnormal heart

sounds and in some dogs the signs disappear with age.

#### Vitamin D-dependent Rickets (VDR)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Pomeranian
Inheritance Unknown
Duration 1 – 2 weeks

Note The hereditary form of vitamin D-dependent rickets type II is caused

by a defect in the vitamin D receptor gene. As a consequence, calcium cannot be absorbed intestinally, which results in skeletal malformation and hypomineralisation of bones during growth. Because the VDR gene is also involved in the hair growth cycle,

alopecia may occur as well.

#### Von Willebrand Disease Type 1 (vWD1)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Bernese Mountain Dog, Coton de Tuléar, Dobermann, Drentse

Patrijshond, German Pinscher, Kerry Blue Terrier, Kromfohrländer, Manchester Terrier, Papillon, Poodle, Stabijhoun, Welsh Corgi

Pembroke

Inheritance Autosomal dominant with incomplete penetrance and expressivity

Duration 3-5 days

Note Clinical signs of vWD are prolonged bleeding time and severe bleeding.

#### Von Willebrand Disease Type 2 (vWD2)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed German Short-Haired Pointing Dog, German Wire-Haired Pointing Dog

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Clinical signs of vWD are prolonged bleeding time and severe bleeding.

#### Von Willebrand Disease Type 3 (vWD3)

Material EB 1 ml. buccal swab

Method TagMan SNP assay (Scottish Terrier, Shetland Sheepdog)

Sequencing (Kooikerhondje)

Breed Kooikerhondje, Scottish Terrier, Shetland Sheepdog (Sheltie)

Inheritance Autosomal recessive

Duration 3 – 5 days (Scottish Terrier, Shetland Sheepdog)

1 – 2 weeks (Kooikerhondje)

Note Clinical signs of vWD are prolonged bleeding time and severe blee-

ding.

#### Warburg Micro Syndrome 1 (WARBM1)

Material EB 1 ml. buccal swab

Method Sequencing Breed Husky

Inheritance Autosomal recessive

Duration 1-2 weeks

Note Typical signs include early developmental disorders and polyneuro-

pathy with ocular abnormalties and neuronal vacuolation. In addition, severe progressive ataxia develops, which is why the puppies are

euthanised at 8 to 16 months of age.



#### X-linked Myotubular Myopathy (XL-MTM)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Labrador Retriever, Rottweiler

Inheritance X-linked recessive
Duration 1 – 2 weeks

Note XL-MTM affects all skeletal muscles. Clinical signs for this disease

can already be seen from birth. Symptoms are severe muscle hypotonia, muscle atrophy and progressive weakening of the hind limbs. Impaired breathing can ultimately lead to death by suffocation.

#### X-linked Severe Combined Immunodeficiency (X-SCID)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Basset Hound, Welsh Corgi Cardigan, Welsh Corgi Pembroke

Inheritance X-linked recessive
Duration 1 – 2 weeks

Note The disease is characterised by developmental disorders, increased

susceptibility to pathogens and degeneration of peripheral lymph

nodes. Affected dogs usually die as puppies.

#### 20.2.2 Coat Colour/Coat Structure Dog

The coat colour of dogs is determined by the interaction of several genes that control the formation and distribution of the two main pigments eumelanin (black) and phaeomelanin (red/yellow).

Production is controlled by the gene MC1R (melanocortin-1 receptor), other genes are responsible for colour variants and patterns. The gene for the brown coat colour (TYRP1) modifies the black pigment to brown without the red pigment being involved. Other genes involved in coat colour include Agouti (ASIP), which organises the distribution of black and red pigments, and Dilute (MLPH), which dilutes, among others, black to blue/grey and brown to silver/lilac. There are other genes for the distribution of white patterns and other dilution genes which only play a role in certain breeds. Below, you will find the genetic tests for the inheritance of coat colour in dogs which are carried out at Laboklin.

A-locus: agouti

(Ay: fawn, sable, wheaten; Aw: wolf-coloured; at: black & tan; a: recessive black)

Material EB 1 ml, buccal swab
Method FLP + TagMan SNP assav

Breed All

Duration 1 – 2 weeks

#### B-locus: brown, chocolate, liver(nose) (alleles bd, bc, bs)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All

Duration 3 – 5 days

#### B-locus: b4, be (rare variants)

Material EB 1 ml. buccal swab

Method TagMan SNP assay (Australian Shepherd, Miniature American

Shepherd)

Sequencing (Lancashire Heeler)

Breed Australian Shepherd, Lancashire Heeler, Miniature American Shepherd

Duration 3 – 5 days (Australian Shepherd, Miniature American Shepherd)

1 – 2 weeks (Lancashire Heeler)

#### C-locus: albino (caL and OCA2)

Material EB 1 ml, buccal swab

Method Sequencing

Breed French Bulldog, German Spitz, Lhasa Apso, Pekingese, Pomeranian

Duration 1 – 2 weeks

#### C-locus: albino (OCA4)

Material EB 1 ml, buccal swab

Method Sequencing
Breed Bull Mastiff
Duration 1 – 2 weeks

#### Coat Length I (long or short hair)

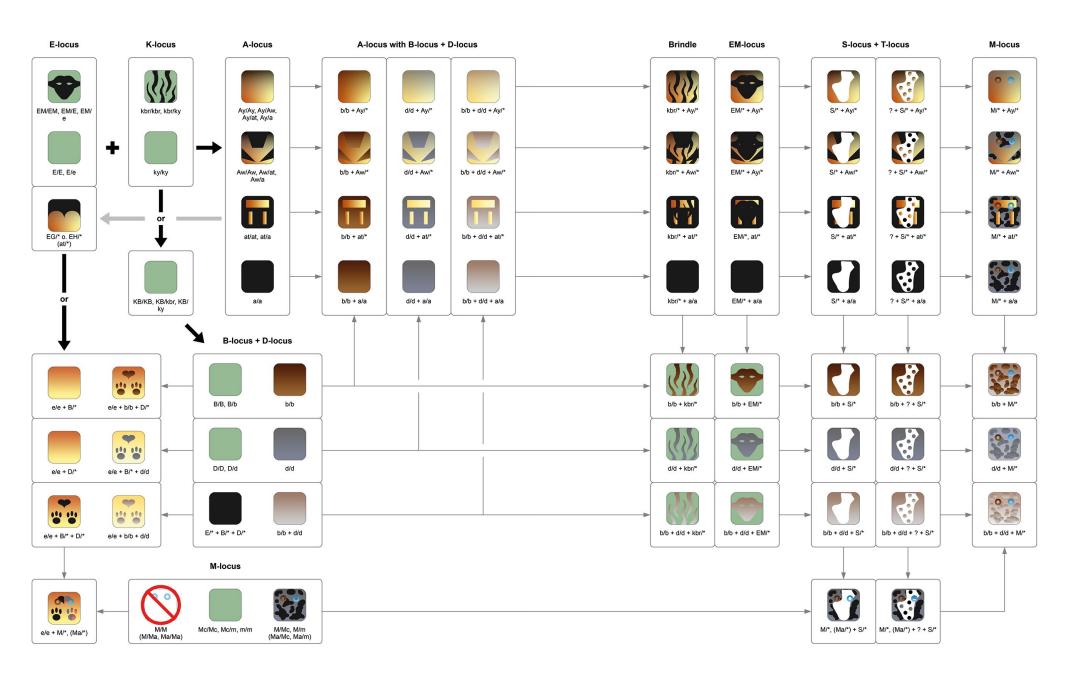
Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed All

Duration 3 – 5 days

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Coat Length II (long or short hair)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Afghan Hound, Akita, Alaskan Malamute, American Akita, Chow

Chow, Eurasian, French Bulldog, Husky, Prague Ratter, Samoyed,

Shar Pei, Shiba Inu

Duration 1 – 2 weeks

Note For these breeds, this test should be carried out in addition to the

test coat length I listed above.

Cocoa: dark brown, dark chocolate

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed French Bulldog Duration 3 – 5 days

Curly: curled hair

Material EB 1 ml, buccal swab Method TagMan SNP assav

Breed All

Duration 3 – 5 days

D-locus: d1 (dilution)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All

Duration 3 – 5 days

D-locus: d2, d3 (rare variants)

Material EB 1 ml, buccal swab

Method Sequencing

Breed d2: Chow Chow, Sloughi, Thai Ridgeback Dog

d3: Chihuahua, Italian Sighthound, Pumi and many more

Duration 1 – 2 weeks

Note For breeds with d2 and d3, testing for d1 + d2 or d1 + d3 is recom-

mended.

E-locus: e1 (yellow, lemon, red, cream, apricot)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Al

Duration 3 – 5 days

Note In Australian Shepherds, Border Collies and other herding dogs, the

colour variant "red" is detected by genetic testing of the B-locus.

E-locus: e2 (rare variants)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Australian Cattle Dog

Duration 1 – 2 weeks

E-locus: EG, EH, eA (special colours)

Material EB 1 ml, buccal swab

Method TagMan SNP assay (eA and EH)

Sequencing (EG)

Breed eA: all ("Husky colouring", similar to domino)

EG: Afghan Hound (domino), Barzoi, Saluki (grizzle)

EH: American Cocker Spaniel, English Cocker Spaniel (sable)

Duration 3 – 5 days (eA, EH)

1 – 2 weeks (EG)

EM-locus (melanistic mask)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All

Duration 3 – 5 days

Furnishing (wire hair)

Material EB 1 ml, buccal swab

Method Sequencing

Breed All

Duration 1 – 2 weeks

H-locus (harlequin)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Great Dane
Duration 3 – 5 days

Note The dominant harlequin allele brightens the merle colour to white

and leads to harlequin colouring with black patches on white background. Dogs with genotype H/H are not viable and already die in

utero.

Hairlessness (powderpuff)

Material EB 1 ml, buccal swab

Method FLP (Chinese Crested Dog, Mexican and Peruvian Hairless Dog)

Sequencing (Deerhound)

Breed Chinese Crested Dog, Deerhound, Mexican Hairless Dog (Xoloitz-

cuintle). Peruvian Hairless Dog

Duration 1-2 weeks

Note Dogs of the breeds Chinese Crested Dog and Mexican or Peruvian

Hairless Dog which carry the heterozygous variant have sparse or no body hair, sometimes abnormal dentition and occasionally malformations of the pinna and the external auditory canal. Embryos with a homozygous genetic variant will already die during gestation. In the Deerhound, another variant (in the SGK3 gene) may be associated with juvenile alopecia (loss of coat in the first weeks of life, permanent

hairlessness).

#### I-locus (phaeomelanin intensity)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All

Duration 3 – 5 days

#### **Improper Coat**

Material EB 1 ml. buccal swab

Method Sequencing

Breed Portuguese Water Dog

Duration 1 – 2 weeks

#### K-locus: only allele KB

Material EB 1 ml, buccal swab

Method Sequencing

Breed All

Duration 1 – 2 weeks

Note The alleles kbr (brindle) and ky are not covered by this test.

#### M-locus\*: merle alleles (Mh, M, Ma+, Ma, Mc+, Mc, m and mosaics)

Material EB 1 ml, buccal swab Method Partner laboratory

Breed All

Duration 1 – 2 weeks

Note Merle (M) is a coat pattern with sections of diluted colour pigments.

It is caused by the gene variants M, Mh (harlequin merle) or Ma (atypical merle). Mc (cryptic merle) does not lead to any colour change. The 4 gene variants are inherited in an incomplete dominant

manner to the normal form (non-merle, m).

The genotype M/M (double-merle) and all combinations of M or Mh with the alleles Mh, M or Ma can lead to severe malformations of the inner ear with hearing loss or deafness as well as to malformations of the eye and are therefore considered as cruel breeding. Such animals often display a very high amount of white or are completely white

The occurrence of merle colouration can be limited to small areas (minimal merle) or may be covered by another colouration (hidden merle). A genetic test is therefore always advisable if merle is present or suspected in a breeding line.

#### **Panda White Spotting**

Material EB 1 ml, buccal swab

Method Sequencing

Breed German Shepherd

Duration 1 – 2 weeks

Note This form of white spotting with areas of unpigmented skin is inherited

as an autosomal dominant trait: the homozygous mutation is lethal.

#### S-locus: white spotting, piebald

Material EB 1 ml, buccal swab

Method FLP Breed All

Duration 1 – 2 weeks

Note A severe form of spotting is often linked to deafness, which mainly

occurs in animals where the white spotting covers the head and

ears.

#### Saddle-tan (A-locus modifier)

Material EB 1 ml, buccal swab

Method Sequencina

Breed Basset Hound, Welsh Corgi Cardigan, Welsh Corgi Pembroke

Duration 1-2 weeks

#### **Shedding**

Material EB 1 ml, buccal swab

Method Sequencing

Breed All

Duration 1 – 2 weeks

Note Shedding in dogs is influenced by the shedding trait in combination

with other coat structural characteristics (furnishing, hair length).

## 20.3 Cat

#### 20.3.1 Hereditary Diseases

#### Acrodermatitis Enteropathica (AE)

Material EB 1 ml, buccal swab

Method Sequencing Breed Turkish Van

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note AE is caused by a variant in the SLC39A4 gene. This gene encodes

for an intestinal zinc transporter; a loss of function of this transporter leads to systemic zinc deficiency. Affected kittens show growth retardation and diarrhoea from 6-8 weeks of age onwards and suffer from severe, rapidly progressing dermatological signs such as scaling, alopecia, moist dermatitis, severe erosions and lesions on abdomen and limbs. Since there is another intestinal zinc transport pathway, zinc deficiency can be treated by high oral doses of zinc.

#### Alpha-Mannosidosis (AMD)

Material EB 1 ml. buccal swab

Method Sequencing Breed Persian

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Alpha-mannosidosis (AMD) is a lysosomal storage disease which

causes clinical signs like malformation in bone structure as well as severe neurological signs such as ataxia, tremor and limited vision. Cats affected by this rare disease usually die after birth or in the first

months of life.

#### **Autoimmune Lymphoproliferative Syndrome (ALPS)**

Material EB 1 ml, buccal swab

Method Sequencing
Breed British Shorthair
Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note In British Shorthair, ALPS has been found in cats in New Zealand

and Australia.

Already from 8 weeks of age, the animals display lymphadenopathy

and splenomegaly.

#### Congenital Myasthenic Syndrome (CMS)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Devon Rex, Sphynx Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note CMS in cats leads to generalised amyosthenia in affected animals,

especially after stress and agitation. Some animals exhibit a typical "squirrel-like" posture. First signs already appear at three weeks of

age. Cats with CMS normally die within two years.

#### Cystinuria

Material EB 1 ml. buccal swab

Method Sequencing

Breed All

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Cystinuria is an inherited metabolic disease with defective absorp-

tion of certain amino acids in the proximal renal tubule. This results in an increased urinary excretion of the amino acid cystine. Because of the excessive accumulation of cystine in the urine and its poor solubility in water, cystine crystallises and calculi are formed. These

uroliths already occur at juvenile age.

#### **Factor XI Deficiency**

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Maine Coon

Inheritance Autosomal recessive

Duration 3-5 days

Note Laboklin has identified a genetic defect to be the cause of factor XI

deficiency in Maine Coon cats. Diagnostically, factor XI deficiency manifests itself in a prolonged partial thromboplastin time while prothrombin time remains normal, and clinically, there is a tendency

for haematoma and minor bleeding after trauma.

#### Gangliosidosis Type GM1, Type GM2

Material EB 1 ml, buccal swab

Method Sequencing

Breed Balinese, Burmese, Javanese, Korat, Oriental Shorthair (OSH),

Peterbald, Seychellois, Siamese, Thai, Tonkinese

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Kittens affected by this lysosomal storage disease have head tremors

at the beginning, later followed by impaired coordination of the limbs which eventually lead to paralysis. In GM2 gangliosidosis, clinical signs usually appear earlier (around the age of 2 months) and worsen more quickly. In GM1 gangliosidosis, the onset of neurological signs

is a little later (3 months) and they progress more slowly.

#### **Genetic Blood Group**

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All, except for European Shorthair

Duration 3 – 5 days

Note The genetic blood typing test looks for the genetic "b" allele which

is necessary for the formation of the serological blood type B. If a female cat has blood type B, the male cat must also have blood type B to avoid neonatal isoerythrolysis in the kittens of the litter.

(See also Chapter 3.3, p. 78)

#### Glycogen Storage Disease Type IV (GSD4)

Material EB 1 ml. buccal swab

Method FLP

Breed Norwegian Forest Cat Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Most affected kittens die at or soon after birth, presumably due to

hyperglycaemia. Survivors of the perinatal period appear clinically normal until the onset of progressive neuromuscular degeneration at

5 months of age which eventually leads to death.

**Head Defect** 

Material EB 1 ml, buccal swab

Method Sequencing Breed Burmese

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Cats with Burmese head defect have a severe craniofacial deformity

and are not viable. One copy of the mutation does not cause any "malformation" but may lead to a shortened facial structure (brachy-

cephalv).

#### Hypertrophic Cardiomyopathy (HCM1, HCM3, HCM4)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Maine Coon (HCM1, A31P mutation), Ragdoll (HCM 3, R820W

mutation), Sphynx (HCM4)

Inheritance Autosomal dominant with incomplete penetrance

Duration 3 – 5 days

Note HCM is caused by two variants in the MYBPC3 gene (HCM1, HCM3)

or one variant in the ALMS1 gene. In HCM1 and HCM3, there is an increased risk of phenotypic expression if the cat is homozygous for the mutation. So far, it is unclear whether the risk of HCM4 is higher in homozygous cats than in heterozygous ones. Furthermore, in the Sphynx, it is assumed that there is at least one other unknown variant that can cause HCM. Generally, not all genetically affected cats

show clinical signs of HCM (incomplete penetrance).

#### Hypokalaemia

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Australian Mist. Burmese, Cornish Rex, Devon Rex, Singapura.

Sphynx, Tonkinese

Inheritance Autosomal recessive

Duration 3-5 days

Note Hypokalaemia, also known as familial episodic hypokalaemic

polymyopathy, is characterised by episodes of skeletal muscle weakness. Affected cats have problems with walking, jumping and

holding their head correctly.

#### Hypotrichosis and Short Life Expectancy

Material EB 1 ml, buccal swab

Method Sequencing

Breed Birman (Sacred Cat of Burma)

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Affected kittens have a thin downy coat that falls out within a week of

birth. Regrowth of a thin coat may occur within the first two months in some animals. Other kittens are born completely bald. In addition, oily and crusty skin in the facial area and anomalies of the claws, tongue and beard are other clinical signs. The disease also leads to stillbirths and early death in kittens in the first thirteen weeks of life

due to impaired immune response.

#### Mucopolysaccharidosis Type VI (MPS6)

Material EB 1 ml, buccal swab

Method Sequencing

Breed Balinese, Birman (Sacred Cat of Burma), European Shorthair,

Javanese, Oriental Shorthair (OSH), Peterbald, Seychellois, Siamese,

Thai, Tonkinese

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note MPS6 is a lysosomal storage disease resulting in a clinically mild

and a severe MPS VI phenotype, characterised by severe disorders of the bone structure and the nervous system as well as dwarfism. First clinical signs of the severe phenotype appear after only a few

weeks of life.

#### Mucopolysaccharidosis Type VII (MPS7)

Material EB 1 ml, buccal swab

Method Sequencing

Breed All

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note MPS7 is a rare lysosomal storage disease that leads to bone and

cartilage malformation, corneal clouding and enlarged abdominal organs due to a dysfunction in the breakdown of mucopolysaccharides. This can already be seen from the age of two months onwards.

#### Myotonia congenita

Material EB 1 ml, buccal swab

Method Sequencing

Breed All

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Myotonia congenita is a disease that affects the skeletal muscles.

Signs of the disease are mainly stiff-legged gait as well as a protruding tongue and a mandible which can hardly be opened. Dysphagia and

excessive salivation are often seen.

#### Osteochondrodysplasia (OCD)

Material EB 1 ml, buccal swab

Method Sequencing Breed Scottish Fold

Inheritance Autosomal dominant

Duration 1-2 weeks

Note A mutation in the TRPV4 gene leads to the characteristic forward-

folded ears in Scottish Fold. Furthermore, this mutation causes osteochondrodysplasia in this breed with malformation in bones and joints of the distal limbs and tail. Homozygous affected cats seem to develop severe malformations, therefore it is not recommended to

mate Scottish fold cats with one another.

#### Polycystic Kidney Disease (PKD)

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed Birman (Sacred Cat of Burma), British Longhair, British Shorthair

(BSH), Chartreux, Colourpoint Shorthair, Exotic Shorthair, Persian, Ragdoll, Russian Blue, Scottish Fold, Selkirk Rex, Turkish Angora

Inheritance Autosomal dominant

Duration 3 – 5 days

Note In PKD, in addition to the formation of cysts in the liver and pancreas,

fluid-filled cysts form in the kidneys which can eventually cause renal

failure leading to the death of an affected cat.

#### **Primary Congenital Glaucoma (PCG)**

Material EB 1 ml, buccal swab

Method Sequencing Breed Siamese

Inheritance Autosomal recessive

Duration 1-2 weeks

Note Cats with primary glaucoma often have congenital malformations

in the eye causing increased intraocular pressure. This results in damage of the retinal ganglion cells and the optic nerve, which leads

to blindness within the first months of life.

#### Progressive Retinal Atrophy (PRA)

b-PRA

Material EB 1 ml, buccal swab

Method TagMan SNP assay, if necessary sequencing

Breed Bengal

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

Note Bengal progressive retinal atrophy causes destruction of the retinal

photoreceptors from about 7 weeks of age onwards and leads to a dilation of the pupils. b-PRA progresses slowly until the cat already has very limited vision at about 2 years of age. Time varies until

complete blindness develops.

pd-PRA

Material EB 1 ml, buccal swab

Method TagMan SNP assay, if necessary sequencing

Breed Birman (Sacred Cat of Burma), British Longhair, British Shorthair

(BSH), Chartreux, Colourpoint Shorthair, Exotic Shorthair, Kartäuser, Persian, Ragdoll, Russian Blue, Scottish Fold, Selkirk Rex, Turkish

Angora

Inheritance Autosomal recessive

Duration 3-5 days (1-2) weeks for sequencing

Note Progressive retinal atrophy in Persians (pd-PRA) causes photo-

receptor degradation in affected animals already at 5 weeks of age, leading to complete blindness by 16 weeks of age. The main signs are uncoordinated eye movements. The ocular fundus shows in-

creased reflectivity.

rdAc-PRA

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed Abyssinian, American Curl, American Wirehair, Balinese, Bengal,

Colourpoint, Cornish Rex, Javanese, Munchkin, Ocicat, Oriental Shorthair (OSH), Peterbald, Seychellois, Siamese, Singapura, Somali,

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Thai, Tonkinese

Inheritance Autosomal recessive

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Duration 3 – 5 days

Note The onset of clinical signs is usually at the age of 1.5 to 2 years (so-

called late onset). At the final stage of the disease, usually at the age of 3-5 years, the photoreceptors are completely destroyed and the

cat becomes totally blind.

rdy-PRA

Material EB 1 ml, buccal swab

Method Sequencing

Breed Abyssinian, Ocicat, Somali Inheritance Autosomal dominant

Duration 1-2 weeks

Note Already at the age of about three weeks, retinal malformations are

visible during examinations (so-called early onset); affected cats usually go almost completely blind at the age of about seven weeks.

Pyruvate Kinase Deficiency (PK)

Material EB 1 ml, buccal swab

Method TagMan SNP assay, if necessary sequencing

Breed Abyssinian, Bengal, Egyptian Mau, European Shorthair, LaPerm,

Maine Coon, Norwegian Forest Cat, Ocicat, Savannah, Siberian, Sin-

gapura, Somali, Turkish Angora

Inheritance Autosomal recessive

Duration 3-5 days (1-2 weeks for sequencing)

Note PK is characterised by chronic regenerative haemolytic anaemia.

Severe haemolytic crises also occur, especially in case of stress or

infection. Occasionally, an enlarged spleen is palpable.

**Spinal Muscular Atrophy (SMA)** 

Material EB 1 ml. buccal swab

Method FLP

Breed Maine Coon

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note SMA is characterised by muscle atrophy and muscle weakness

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which are associated with the degeneration of spinal motoneurons

and already occur at the age of about 12 weeks.

#### 20.3.2 Coat Colour/Coat Structure Cat

#### **Coat Colour Amber**

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed Norwegian Forest Cat

Duration 3 – 5 days

#### Coat Colour Brown (chocolate/cinnamon)

Material EB 1 ml, buccal swab Method TaqMan SNP assay

Breed All

Duration 3-5 days

#### **Coat Colour Russet**

Material EB 1 ml, buccal swab

Method Sequencing Breed Burmese Duration 1 – 2 weeks

#### **Coat Colour Variant Agouti**

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All

Duration 3-5 days

#### **Coat Colour Variant Albino**

Material EB 1 ml, buccal swab

Method Sequencing

Breed All

Duration 1 – 2 weeks

#### **Coat Colour Variant Charcoal**

Material EB 1 ml, buccal swab

Method Sequencing
Breed Bengal
Duration 1 – 2 weeks

#### Coat Colour Variant Colourpoint (Siam/Mink/Burma)

Material EB 1 ml, buccal swab Method TaqMan SNP assay Breed All, except for Bengal

Duration 3 – 5 days

#### **Coat Colour Variant Dilution**

Material EB 1 ml, buccal swab Method TagMan SNP assay

Breed All

Duration 3 – 5 days

#### **Coat Colour Variant Snow**

Material EB 1 ml, buccal swab

Method Sequencing Breed Bengal Duration 1 – 2 weeks

Note Snow is the name for colourpoint colouration in Bengals.

#### Coat Colour Variant White (dominant white/white spotting)

Material EB 1 ml, buccal swab

Method FLP Breed All

Duration 1 – 2 weeks

Note White spotting (ws) and dominant white (W) are caused by insertions

of the endogenous retrovirus FERV1 in the KIT gene; ws is a complete insertion, W is a partial insertion. W is dominant over ws and both are dominant over the wild type (w+). Hearing loss or deafness always occur in the genotype WW, and sometimes occur in Wws and Ww+. The W allele also leads to a typical blue colouration of the iris; in

Wws and Ww+, again, penetrance is incomplete.

#### Coat Length (long or short hair)

Material EB 1 ml, buccal swab

Method Sequencing

Breed All

Duration 1 – 2 weeks

Note This test detects all four known alleles for long hair.

#### **Coat Variant Curly in Selkirk Rex**

EB 1 ml, buccal swab Material

Method Sequencing Breed Selkirk Rex Duration 1-2 weeks

#### Coat Variant Sphynx/Devon Rex

EB 1 ml. buccal swab Material

Method Sequencing Breed Devon Rex. Sphvnx

Duration 1-2 weeks

#### 20.4 Horse

#### 20.4.1 Hereditary Diseases

#### Androgen Insensitivity Syndrome (AIS)

Material EB 1 ml, hair roots Method Seauencina

Quarter Horse and related breeds Breed

X-linked recessive Inheritance Duration 1-2 weeks

Note In AIS, XY (genetically male) horses have a female phenotype (female

external genitalia) and internal testes. These horses often behave

like stallions, but are not capable of reproduction.

#### Cerebellar Abiotrophy (CA)

Material EB 1 ml, hair roots Method TagMan SNP assay

Breed Arabian

Inheritance Autosomal recessive

Duration 3 – 5 days

CA is a neurological disease in which affected foals are born without Note

> symptoms; the first signs usually appear at the age of 6 weeks (up to 4 months): neurological deficits such as head shaking, ataxia and

other deficiencies can occur in varying degrees of severity.

#### **Dwarfism**

Material EB 1 ml, hair roots Method Sequencing

Breed Friesian

Inheritance Autosomal recessive

Duration 1-2 weeks

Note Dwarfism in Friesian horses is characterised by growth retardation

> of the ribs and limbs, while the head and back appear normal. Hyperextension of the fetlock joints is particularly noticeable. The flexor tendon becomes longer. This leads to an abnormal gait with

extreme rotation at the carpus and hocks.

The ribcage is wider than normal with a thickening of the costochondral junction (Th 10-16). The back appears disproportionately long, but the legs are greatly shortened. The abdomen is usually rounded, the muscles of the whole body are only poorly developed.

#### Dwarfism (ACAN, Chondrodysplasia)

Material EB 1 ml. hair roots Method Sequencing

American Miniature Horse, Shetland Pony Breed

Inheritance See note Duration 1-2 weeks

Dwarfism is most common in Shetland Ponies and Miniature Horses. Note

> Phenotypic features include deformed mouths and cleft palates (respiratory problems), limb deformities, a disproportionately large

head, a short neck and abdominal hernia.

considered to be the mildest form.

Four different mutations in the ACAN gene (D1, D2, D3\*, D4), which are inherited in an autosomal recessive manner, can also cause disease in a compound heterozygous form, i.e. two different heterozygous mutations of the same gene are present. Compound heterozygous variants combined with the D1 variant (except for N/D1) often lead to death. A combination with the D2 variant is

#### Equine Malignant Hyperthermia (EMH)

Material EB 1 ml. hair roots TagMan SNP assay Method

Breed ΑII

Inheritance Autosomal dominant

Duration 3 – 5 days

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Note The clinical signs appear after halothane anaesthesia or succinyl-

choline injection and include hyperthermia (> 40 °C) and metabolic acidosis. The animals show generalised spasms of the skeletal muscles, followed by cardiac arrhythmia and renal dysfunction.

#### Foal Immunodeficiency Syndrome (FIS)

EB 1 ml, hair roots Material Method Seauencina

Breed Dales Pony, Fell Pony Autosomal recessive Inheritance

Duration 1-2 weeks

Note Foals with FIS are born apparently healthy but as they lack immunity.

> they develop a number of diseases, especially pneumonia and diarrhoea at a few weeks of age. Foals also suffer from severe progressive anaemia and usually die before the age of three months.

#### Glycogen Branching Enzyme Deficiency (GBED)

Material EB 1 ml. hair roots TagMan SNP assay Method

Appaloosa, Paint Horse, Quarter Horse Breed

Inheritance Autosomal recessive

Duration 3 – 5 days

Note Clinical signs of GBED are abortion, stillbirth or birth of weak foals,

> sudden cardiac death (especially on the pasture) or death caused by seizures, high respiratory frequency due to weakened respiratory

muscles or general weakness (especially when getting up).

#### Hereditary Equine Regional Dermal Asthenia (HERDA)

EB 1 ml. hair roots Material Method TagMan SNP assay

Breed Appaloosa, Paint Horse, Quarter Horse

Autosomal recessive Inheritance

Duration 3 – 5 davs

Note The skin of affected horses is hyperextensible, scarred, and often

shows severe lesions.

#### **Hereditary Myotonia**

Material EB 1 ml, hair roots Method TagMan SNP assay Breed **New Forest Pony** Inheritance Autosomal recessive

Duration 3 – 5 davs

Note The first signs of **congenital myotonia** already appear at a few weeks

> of age. Foals have a stiffed-legged gait, are recumbent and have considerable difficulty getting back on their feet after a long period of lying.

#### **Hoof Wall Separation Disease (HWSD)**

EB 1 ml. hair roots Material Method TagMan SNP assay

American Miniature Horse, Connemara Pony, German Riding Pony Breed

Autosomal recessive Inheritance

Duration 3 – 5 days

Hoof wall separation disease (HWSD) is characterised by a very Note

> unstable hoof wall which can crack and break without any particular strain. Symptoms already appear in the first weeks of life and can be

of varying severity.

#### Hydrocephalus

EB 1 ml. hair roots Material Method Sequencing Breed Friesian

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Hydrocephalus in Friesian horses often results in stillbirth of affected Note

foals and dystocia in dams.

## Hyperkalaemic Periodic Paralysis (HYPP)

Material EB 1 ml. hair roots TagMan SNP assay Method

Breed Appaloosa, Paint Horse, Quarter Horse

Inheritance Autosomal dominant

Duration 3 – 5 days

Note The horses are usually very well-muscled and can be successful

> show/sport horses between episodes of illness with general weakness, muscle spasms and fasciculations. The first episodes of

illness are often observed at the age of 3 to 7 years.



Life-threatening complications are cardiac arrhythmia (secondary to hyperkalaemia) and danger of suffocation by laryngospasm.

#### Idiopathic Hypocalcaemia

Material EB 1 ml, hair roots Method Seauencina Breed Thoroughbred Inheritance Autosomal recessive

1-2 weeks Duration

Note Foals affected by the lethal hypocalcaemic syndrome suffer from

> muscle spasms, stiff gait and increased sweating. They die soon or are euthanised within a few weeks. A genetic variant in the RAPGEF5 gene is inherited homozygously and associated with hypoparathyroidism. The reduced PTH production leads to calcium deficiency. Since the breed is used for improvement breeding, it cannot be ruled out that this hereditary disease is bred into other

breeds.

#### Immune-Mediated Myositis & MYH1 Myopathy (MYHM)

EB 1 ml, hair roots Material Method Sequencing

Breed Appaloosa, Paint Horse, Quarter Horse and related breeds

Inheritance Autosomal dominant with incomplete penetrance

Duration 1-2 weeks

Note A variant of the gene MHY1 inhibits the function of the myosin

> protein and is associated with muscle diseases known as MYH1 myopathy (MYHM). This genetic variant leads to 2 clinical pictures - immune-mediated myositis (IMM) in 8- to 17-year-old horses and

non-exertional rhabdomyolysis in young horses.

**IMM** is a muscular autoimmune disorder with mainly lymphocytic infiltration into muscle fibres and surrounding blood vessels. IMM can lead to weakness, stiffness and severe muscle atrophy with loss of up to 40% of muscle mass in 72 hours. In addition to the genetic disposition, further adverse factors are important triggers. For example, about 39% of IMM-affected horses have suffered from infections like Streptococcus equi subsp. equi or EHV4 for a long time. In young Quarter Horses, non-exertional rhabdomyolysis causes severe, sudden muscle damage which occurs without any physical exertion and is not necessarily associated with muscle atrophy. Inheritance is autosomal dominant with incomplete penetrance. Thus, not all horses that have one or two alleles of the genetic variant will suffer from the disease. Horses with two alleles may be more severely affected.

#### Junctional Epidermolysis Bullosa (JEB)

Material EB 1 ml, hair roots Method Sequencing Breed Belgian Draft

Inheritance Autosomal recessive

Duration 1 – 2 weeks

Note Shortly after birth, foals with JEB (also called hereditary junctional

epidermoylsis bullosa, H-JEB) lose skin parts on the head, neck and

trunk. The hoof horn also separates from the hoof corium.

#### Lavender Foal Syndrome (LFS)

EB 1 ml. hair roots Material Method TagMan SNP assay

Arabian Breed

Autosomal recessive Inheritance

Duration 3 – 5 days

Affected foals show a range of neurological signs, including convul-Note

> sive seizures, opisthotonus or nystagmus. They are normally unable to stand and nurse from their mother and are usually euthanised if

they do not die immediately after birth.

#### **Naked Foal Syndrome (NFS)**

EB 1 ml. hair roots Material Method Sequencing Breed Akhal-Teke

Inheritance Autosomal recessive

Duration 1 – 2 weeks

NFS is a genodermatosis in which foals are born with almost no hair. Note

> They show a mild form of ichthyosis and mostly die in the first weeks after birth. So far, the reason for the early death is unknown; only few

horses reach an age of up to 2.5 years.

#### Ocular Squamous Cell Carcinoma (SCC)

EB 1 ml, hair roots Material Method Seauencina

Belgian Draft (Ardennais, Brabant), Haflinger Breed

Autosomal recessive Inheritance

Duration 1-2 weeks

Note A variant in the DDB2 gene was detected as a genetic risk factor (R)

> for squamous cell carcinoma in the horse's eye in Haflingers and related breeds. Homozygous horses (R/R) develop SCC 5.6 times



(Haflinger) or 4.0 times (Belgian) more often than horses with one copy (R/N) or no copy (N/N). This risk factor does not explain all cases of SCC, but seems to be a significant contributor in Haflingers and Belgian Drafts. In homozygous horses (R/R), routine eye examinations and UV protection are advisable.

#### Overo Lethal White Syndrome (OLWS)

EB 1 ml. hair roots Material Method TagMan SNP assav

Breed Frame overos of all breeds, Paint Horse and related breeds

Inheritance Autosomal recessive

Duration 3 – 5 days

Note OLWS (also called lethal white overo syndrome, LWO) mainly

> occurs when mating frame overo horses. Affected foals are born completely white and die within 24 – 48 hours after birth due to

intestinal aganglionosis and the resulting ileus.

#### Polysaccharid Storage Myopathy Type 1 (PSSM)

EB 1 ml, hair roots Material Method TagMan SNP assay

Breed

Inheritance Autosomal dominant

Duration 3 – 5 days

The clinical signs are similar to those of sporadic exertional rhabdo-Note

> myolysis and include the entire spectrum from reluctance to move to muscle tremor, muscle stiffness, sweating, alternating lameness, stretching of the hind legs up to immobility. Episodes usually begin

after 10 - 20 minutes of light exercise.

Laboklin owns the exclusive license to perform this genetic test.

#### Severe Combined Immunodeficiency (SCID)

EB 1 ml, hair roots Material

Method FI P Breed Arabian

Inheritance Autosomal recessive

Duration 1-2 weeks

Note SCID is a primary, lethal immunodeficiency disease characterised

by the inability to produce B and T lymphocytes. Affected foals are

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extremely susceptible to infections.

#### Warmblood Fragile Foal Syndrome (WFFS)

Material EB 1 ml, hair roots Method TagMan SNP assay

Warmblood Breed

Inheritance Autosomal recessive

Duration 3 - 5 days

Note WFFS is an inherited connective tissue disorder; its signs are

> similar to those of Ehlers-Danlos syndrome in humans. The skin is extremely fragile and tears at even the slightest touch. Not all foals are born after a normal period of gestation; premature births and

miscarriages due to WFFS are also known.

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In Germany, Laboklin owns the exclusive license to perform this

aenetic test.

#### 20.4.2 Coat Colour/Coat Structure Horse

#### Agouti (Bay/Black)

Material EB 1 ml. hair roots

FLP Method ΑII Breed

Duration 1 – 2 weeks

#### Appaloosa Pattern 1 (PATN1)

Material EB 1 ml, hair roots Method Seauencina Breed Appaloosa Duration 1 – 2 weeks

#### **Brindle**

Material EB 1 ml. hair roots Method Sequencing

Breed ΑII

Duration 1 – 2 weeks

#### Camarillo White - W4\*

EB 1 ml, hair roots Material Method Partner laboratory

Breed

3 – 4 weeks Duration

#### Champagne

Material EB 1 ml, hair roots Method TagMan SNP assay

Breed ΑII

Duration 3 – 5 days

#### Chestnut

EB 1 ml, hair roots Material Method TagMan SNP assay

Breed

Duration 3 – 5 days

#### Cream

Material EB 1 ml. hair roots Method TagMan SNP assav

Breed

Duration 3 – 5 days

#### Curly

Material EB 1 ml. hair roots Method Sequencing

Breed ΑII

Inheritance See note Duration 1-2 weeks

Note

Curly Coat leads to a curly coat structure. Curly horses are popular because this coat structure leads to milder or no allergic symptoms in many horse-allergic people. Curly is caused by variants in the genes KRT25 and SP6, which are both separately examined in the test. Horses with variants in both genes or only in KTR25, do not only have curly coat but also develop hypotrichosis. Horses which only have a variant in SP6 just have a curly coat.

Dun

EB 1 ml. hair roots Material

Method FLP Breed ΑII

Duration 1 – 2 weeks

#### **GQ Santana Dominant White W10\***

Material EB 1 ml, hair roots Method Partner laboratory

Breed ΑII

Duration 3 – 4 weeks

#### Greying\*

EB 1 ml. hair roots Material Method Partner laboratory

Breed

Duration 3 - 4 weeks

Note A duplication in the STX17 gene leads to loss of hair pigmentation

> in the first 6 – 8 years. Heterozygous animals often stay dapple grey or flea-bitten grey. Melanoma formation is also associated with the STX17 mutation. 70 – 80% of greys over 15 years of age have one or more melanomas. The risk is higher in homozygous greys than in heterozygous greys and higher in greys that were born black than in

those that were born bay.

#### **Incontinentia Pigmenti**

Material EB 1 ml, hair roots Method Seauencina

Breed ΑII

Duration 1 – 2 weeks

Note IP is an ectodermal dysplasia in which pruritic, exudative skin lesions

> occur soon after birth, sometimes developing into verrucous lesions. There may be areas with alopecia where woolly hair might re-grow. From birth, affected horses show stripes in the coat and can also develop dental, hoof and ocular abnormalities. Due to the X-linked dominant inheritance, IP symptoms can only be seen in mares

(affected male embryos die in utero).

#### **Leopard Complex**

Material EB 1 ml, hair roots TagMan SNP assay Method

Breed ΑII

Duration 3 – 5 days

The leopard gene (LP) is inherited in a dominant manner and is res-Note

> ponsible for the leopard pattern. From birth, homozygous carriers of the gene (LP/LP) are almost always affected by congenital stationary

night blindness (CSNB).

#### Mushroom

Material EB 1 ml, hair roots Method Sequencing Breed Shetland Pony Duration 1 – 2 weeks

#### Pearl

EB 1 ml, hair roots Material Method Sequencing

Breed

1 – 2 weeks Duration

#### Roan Zygosity\*

Material EB 1 ml, hair roots Method Partner laboratory Breed On request Duration 3 - 4 weeks

#### Sabino 1

Material EB 1 ml. hair roots Method Sequencing Breed ΑII

Duration 1-2 weeks

#### Silver Dapple

Material EB 1 ml. hair roots Method Sequencing Breed ΑII

Duration 1-2 weeks

Note

The dominantly inherited silver dapple gene leads to the dilution of black and bay hair, especially on mane and tail. There is a connection between this mutation and ocular malformations. These are more pronounced in homozygous animals than in heterozygous animals,

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where they can also remain undetected.

#### Snowdrop

Material EB 1 ml, hair roots Method Sequencing

Tinker Horse/Gypsy Cob Breed

Duration 1-2 weeks

#### **Splashed White**

Material EB 1 ml, hair roots Method Sequencing and FLP

Breed ΑII

Duration 1 – 2 weeks

Note Splashed White is characterised by an extremely wide blaze, or bald

> face, often with blue eyes and bright white legs. So far, 4 causative mutations have been identified (SW 1 to SW 4), which are inherited in a dominant manner. Some horses with this pattern are deaf, especially if the ears are also white. SW 2 and SW 3 seem to be homozy-

gous lethal.

#### Sunshine

EB 1 ml. hair roots Material Method Sequencing

Breed ΑII

1 – 2 weeks Duration

#### **Tobiano**

Material EB 1 ml. hair roots

Method FLP Breed ΑII

Duration 1 – 2 weeks

#### 20.4.3 Performance Horse

#### **Predictive Height Test**

Material EB 1 ml. hair roots

Method TaqMan SNP assay, if necessary sequencing

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Breed Warmblood

Duration 3 - 5 days (1 - 2 weeks for sequencing)

#### **Speed Gene\* (Myostatin Mutation)**

Material EB 1 ml, hair roots Method Partner laboratory Thoroughbred Breed Duration 1-2 weeks

#### **Tractability**

Material EB 1 ml, hair roots
Method Sequencing
Breed Thoroughbred
Duration 1 – 2 weeks

## 20.5 Cattle

#### Sampling instructions

There should **not** be any **blood samples** sent in for **cattle from multiple births** because of a possible blood chimerism, but if the test allows it, hair roots, sperm or tissue samples can be used. One exception to this is the **free martin test**, for which a **blood sample is mandatory**.

#### 20.5.1 Hereditary Diseases

#### Arachnomelia\* (spider limbs)

Material EB 3 – 5 ml, about 50 hair roots, tissue, sperm

Breed Brown Swiss, Fleckvieh
Duration Approx. 2 weeks

Note Hereditary arachnomelia of Fleckvieh cattle and Brown Swiss is

passed on autosomal recessively. It is characterised by a developmental disorder of the skeletal system leading to the birth of dead or malformed calves and an increased risk of injury to the mother.

#### **Bovine Leukocyte Adhesion Deficiency (BLAD)**

Material FB 1 ml

Breed Holstein-Friesian
Duration Approx. 2 weeks

Note BLAD is a lethal autosomal recessive disease of the immune system

in Holstein cattle. Affected calves suffer from immunodeficiency and die before reaching sexual maturity. Clinical signs manifest themselves in recurrent non-specific infections of the respiratory system and the gastrointestinal tract, delayed wound healing and reduced weight gain as well as leukocytosis with granulocytosis and lympho-

penia as laboratory findings.

#### Bovine Progressive Degenerative Myeloencephalopathy\* (Weaver Syndrome)

Material EB 3 – 5 ml, about 50 hair roots, tissue, sperm

Breed Brown Swiss
Duration Approx. 2 weeks

Note Weaver syndrome is a hereditary CNS disease in Brown Swiss. At

the age of a few months, the first signs appear as weakness of the hind legs, problems getting up and unsteady gait. Disorders are progressive and, after 1 – 3 years, lead to recumbency and death. Weaver syndrome is inherited in an autosomal recessive manner.

#### **Bovine Spinal Dysmyelination\* (SDM)**

Material EB 3 – 5 ml. about 50 hair roots, tissue, sperm

Breed Brown Swiss
Duration Approx. 2 weeks

Note SDM occurs in Brown Swiss, is autosomal recessive and leads to

insufficient myelination.

After birth, calves lie down in a lateral position with their limbs stretched forward. The head is often in a "moon-gazing position"; sometimes hyperreflexia can be observed. The animals are usually

euthanised shortly after birth.

#### Free Martins\*

Material EB 1 ml Breed All

Duration Approx. 2 weeks

Note In case of twins of different sexes, about 90% of infertile, externally

female calves occur during pregnancy, so-called free martins, due to the transfer of male cells to the female embryo. Already in newborn twins of different sexes, the phenotypically female calves can be

examined to see whether they develop as free martins.

#### Spinal Muscular Atrophy\* (SMA)

Material EB 3 – 5 ml, about 50 hair roots, tissue, sperm

Breed Brown Swiss
Duration Approx. 2 weeks

Note This autosomal recessive disease in Brown Swiss calves leads to

the destruction of motor neurons and, at the age of a few weeks, to spinal muscular atrophy. The animals are recumbent, usually without losing their appetite. Spinal reflexes are reduced, pumping respiration is seen, and the animals often develop secondary pneumonia

and die after a few weeks.

#### 20.5.2 Breed Characteristics Cattle

#### Milk Protein

The composition of milk proteins is of considerable importance for further milk processing. In particular, the cheese-making properties of the milk strongly depend on the milk composition. 90% of the proteins in milk consist of the six proteins  $\alpha$ S1-casein,  $\alpha$ S2-casein,  $\beta$ -casein, kappa-casein,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin.

**β-Kasein** 

Material EB 1-2 ml, about 30 hairs with roots, tissue, sperm

Breed All

Duration 2 – 3 days

Note The genetic variants A1 and A2 \( \beta \)-casein can be detected.

Kappa-Kasein\*

Material EB 3 – 5 ml, about 50 hair roots, tissue, sperm

Breed Al

Duration Approx. 2 weeks

Note The kappa-casein gene influences important parameters for milk

processing. Kappa-casein variant B is particularly favourable for

further processing.

Polledness\*

Material EB 3 – 5 ml. about 50 hair roots, tissue, sperm

Breed A

Duration Approx. 2 weeks

Note In polled cattle, the painful procedure of dehorning is no longer

required. Reservations regarding loss of performance in polled lines have largely been eliminated. However, the test cannot evaluate the genetic predisposition for scurs and has not been validated for zebu

cattle.

Red Factor\*

Material EB 3 – 5 ml, about 50 hair roots, tissue, sperm

Breed Black pied Holstein Friesian cattle

Duration Approx. 2 weeks

Note Black pied cattle of the breed Holstein Friesian with a predisposition

for a red coat ("red factor") are popular crossbreeds in red pied breeding. The determination of the genotype of the MSHR gene allows to differentiate between black pied cattle with red factor (Ee)

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and without red factor (EE).

#### 20.6 Small Ruminants and New World Camelids

## 20.6.1 Hereditary Diseases

#### Arachnomelia\* (spider lamb syndrome)

Material 20 – 30 hairs with roots Species Sheep of all breeds

Duration 3 – 4 weeks

Note Hereditary chondrodysplasia leads to underdeveloped muscles in the

lamb and, at about 4 – 6 weeks of age, to skeletal deformities of the head, spine, ribs and abnormally long and bent/twisted limbs (spider lambs). The autosomal recessive disease first occurred in blackheaded sheep of the Suffolk and Hampshire breeds and is based on a mutation in the gene FGFR3 (Fibroblast Growth Factor Receptor 3).

Free Martins\*

Material EB 3 – 6 ml

Species Sheep, goat, llama, alpaca

Duration 3 – 4 weeks

Note In case of twins of different sexes, the transfer of male cells to the

female embryo during pregnancy can result in infertile, externally female animals, so-called free martins. In llamas and alpacas, the risk is 90%, for sheep and goats < 1%, but increases with multiple pregnancies with four or more animals. Already in newborn twins of different sexes, the phenotypically female calves can be examined to

see whether they develop as free martins.

#### **Predisposition for Scrapie**

Material EB 1 – 2 ml Species Sheep Duration 2 weeks

Note Scrapie is a transmissible prion disease in sheep and goats. Prions

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are proteins. If they are pathologically altered, they induce their own proliferation and the vacuolation of nerve cells, especially in the brain stem. Initially, affected animals are lazy, later they show increasing excitability and an unnatural gait and die within six months after the

onset of the disease.

In sheep, there is a genetic predisposition to the classical form of scrapie. Genotype classes are distinguished according to the amino acid pattern. The risk of scrapie varies from an extremely low ("resistant") to a very high risk depending on the genotype class.

#### 20.6.2 Breed Characteristics - Small Ruminants

#### Milk Protein $\alpha$ -S1-Kasein\*

Material 20 – 30 hairs with roots Species Goats of all breeds

Duration 3 – 4 weeks

Note The  $\alpha$ S1-casein gene influences the casein and fat content of goat

milk. A high content of  $\alpha$ S1-casein is positive for cheese production, a low content is beneficial for people with milk intolerance. The gene variants A and B are associated with high  $\alpha$ S1-casein contents,

while only little  $\alpha$ S1-casein is formed in E, F and N.

## 20.7 Pig

#### Malignant Hyperthermia (MH)\*

Material EB 1 ml Species Pig

Duration Approx. 2 weeks

Note MH syndrome or porcine stress syndrome (PSS) is passed on re-

cessively and is mainly found in breeds with increased muscle mass and reduced amount of fat. The disease is caused by a mutation of the ryanodine receptor in the skeletal muscle, which leads to a disturbance of the Ca²-ion exchange and a lowered threshold for muscle cell contraction. It is accompanied by hypermetabolism and increased body temperature caused by inhalation narcotics, muscle relaxants and stress. Damage to nerve, liver and kidney tissue occurs.

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## 21 DNA Profile, Breed, Species

## 21.1 Identity and Parentage

The DNA profile of an animal is also called genetic fingerprint. In contrast to other marking methods, like microchips or tattoos, it cannot be manipulated or destroyed by external factors, such as injuries. It remains unchanged for a lifetime.

On the one hand, a DNA profile provides a lifelong, doubtless identification of the animal. On the other hand, parentage (fatherhood or parenthood) can be proven with certainty by comparing the genetic fingerprints of the family members.

#### Parentage (paternity test)

Material EB 1 ml, buccal swab

Horse and water buffalo: 20 – 30 hairs with roots

Method Microsatellite analysis (STRs)

Species Dog, cat, horse, cattle, sheep, goat, llama, alpaca, water buffalo, pig

Duration 1 – 2 weeks

4 – 5 weeks (alpaca, llama, water buffalo)

Note The proof of parentage (paternity test) makes it possible to check

which parents the offspring has.

The DNA profiles of the parents and offspring are the basis for this. **What is important to know:** Even if only the paternity should be clarified, please send in samples from both parents. In dogs, this applies to the Classic STR DNA profiles (ISAG 2006). The Premium SNP DNA profile (ISAG 2020) in dogs has the potential to solve parentage cases for which only one parent is available (breeds on

request).

## DNA Profile (ISAG 2006)

Dog: Classic STR DNA Profile (ISAG 2006)

Material EB 1 ml. buccal swab

Horse and water buffalo: 20 – 30 hairs with roots

Method Microsatellite analysis (STRs) (according to ISAG 2006)
Species Dog, cat, horse, cattle\*, sheep\*, goat\*, llama\*, alpaca\*,

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water buffalo\*, pig\*

Duration 1 – 2 weeks (dog)

2 – 3 weeks (cat, horse)

3 – 5 weeks (all other species)

Note To create a DNA profile, we test so-called microsatellite markers

(e.g. 22 markers in dogs), recommended in 2006 by the "International Society for Animal Genetics (ISAG)". The DNA profiles we generate are internationally comparable with laboratories working

according to the recommendations of ISAG.

**Dog:** Classic STR DNA Profiles (ISAG 2006) and Premium SNP DNA Profiles (ISAG 2020) are not compatible and cannot be used simul-

taneously within the same parentage analysis.

#### Premium SNP DNA Profile (ISAG 2020)

Material EB 1 ml
Method SNP analysis
Species Dog (all breeds)
Duration 4 – 6 weeks

Note

The Premium SNP DNA Profile follows the recommended 2020 ISAG guidelines (International Society for Animal Genetics) by analysing 230 SNPs (single nucleotide polymorphisms) and allows international comparability between laboratories. Test reliability and power of exclusion for parentage analysis are well above 99.99%.

The Premium SNP DNA profile also has the potential to solve parentage cases for which only one parent is available (breeds on request).

Furthermore, the Premium SNP DNA Profile includes an analysis of the genetic variability (heterozygosity, see figure). Animals with a high heterozygosity are less affected by inbreeding than animals with a low heterozygosity.

Please note: Premium SNP DNA Profiles and Classic STR DNA Profiles are not compatible and cannot be used simultaneously within the same parentage analysis.



#### Heterozygosity

grey shaded area:
genetic variability of the entire
breed population which has
been examined (n > 100);
dashed line:
mean value of the breed;
cross/solid line:
tested dog (value in the example:
43% het.)



Material EB 1 ml, buccal swab

Method Microsatellite analysis (STRs) + database analysis

Species Dog

Duration 2 – 3 weeks

Note If only one parent (e.g. the father) is available for a proof of

parentage, this test allows to calculate a so-called probability value. Values can also be determined to evaluate full or half siblings if only

samples of the potential siblings are available.

What is important to know: The test is limited to the breeds in our database (current information can always be found on our

homepage).

## 21.2 Breed and Species

#### Breed Determination (database analysis)

Material EB 1 ml, buccal swab

Method Microsatellite analysis (STRs) + database analysis

Species List Dog, cat

Duration 2 – 3 weeks (dog)

3 – 4 weeks (cat)

Note The genetic breed determination provides the statistical calculation

of a matching probability to breeds in our database. The test can be used to verify purity of breeding as well as to detect first-generation mongrels. Thus, the test primarily provides a clarification of genetic proportions of so-called "listed dog breeds" or serves to detect non-purebred animals. Among others, the DNA profile of the animal

serves as a basis for the test.

What is important to know: The test is limited to the breeds in our database (current information can always be found on our

homepage).

#### Species Differentiation, Molecular Biological

Material Various (possibly consultation by telephone)

Method Sequencing and database analysis

Duration 2 – 3 weeks

Note This method allows, for example, to assign certain signs to an

animal species, because it is rarely possible to draw conclusions about the origin of samples such as faeces, trails of blood, etc. with the naked eye or with the help of common laboratory methods. In this test, a specific region of mitochondrial DNA is duplicated by PCR, sequenced and its origin analysed. Since this is a comparatively sensitive method, even the smallest sample quantities can be assigned (e.g. blood spatter).

The differentiation of animal species using the latest molecular biological methods can be applied to a large number of questions, e.g. "Does the neighbour's dog/cat do its business in our garden?" We would be pleased to inform you in advance by telephone in

order to clear up any confusion regarding the test.

# 22 Hygiene Examinations

In the veterinary practice, hygiene is one decisive prerequisite for successful treatment. For this purpose, the proper functioning of sterilisers should be checked on a regular basis. The same holds true for testing the disinfection of surfaces or endoscopes. Data from our research indicates that a noteworthy percentage of the devices we tested in the practice no longer showed any level of sterilisation at all.

Please only use printed submission forms to **order** hygiene tests (no online orders). You can request hygiene submission forms from Laboklin or print them out yourself (PDF in My Lab).

The **test materials** for the following tests will be sent to you after we have received your submission form. Samples can be collected up to the expiration date of the test kit, as long as the kit is stored according to the specifications in the accompanying documents. The invoice will be issued after receipt of the order.

## 22.1 Profiles Hygiene

#### Hygiene Monitoring - Steriliser + Surface Disinfection Efficacy Testing

Material Bioindicators + contact plates

Method Culture
Duration 7 days

Note

- Monitoring of a steriliser (heat or steam) + monitoring of 3 surfaces (with contact plates) after disinfection.
- If you participate regularly (2 x per year), you will get a certificate stating the successful annual monitoring of the disinfection performance of your steriliser and the surface disinfection test.
- This test is not available to third countries.

## 22.2 Single Tests

#### **Disinfectant Testing**

Material Disinhibitor broth

Method Culture Duration 3 days

Note For testing the sterility of disinfectants.

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## LABOKI IN

#### **Endoscope Control**

Material Rinse samples + contact plates

Method Culture 3 – 5 days Duration

#### **Heat Steriliser Control**

Bioindicators (contaminated with Bacillus atrophaeus) Material

Method Culture Duration 7 days

Note • If you participate regularly (2 x per year), you will get a certificate

stating the annual monitoring of the disinfection performance of

vour heat steriliser.

This test is not available to third countries.

#### Steam Steriliser Control (autoclave)

Material Bioindicators (contaminated with Bacillus atrophaeus and

Geobacillus stearothermophilus)

Method Culture Duration 7 days

Note • If you participate regularly (2 x per year), you will get a certificate

stating the annual monitoring of the disinfection performance of

your autoclave.

This test is not available to third countries.

#### **Surface Contamination Testing**

Material Contact plates Method Culture Duration 2 days

Note

• This test is also suitable for testing hand contamination.

• Where applicable, the following multidrug-resistant pathogens can

be identified:

MRSA (methicillin-resistant Staphylococcus aureus) and/or MRSE (methicillin-resistant Staphylococcus epidermidis) and/or ESBL (pathogens that produce extended-spectrum β-lactamase).

For this, additional costs will be incurred.

• For testing surface contamination after disinfection, the service

"Surface Disinfection Efficacy Testing" is available.

#### **Surface Disinfection Efficacy Testing**

2 days

Material Contact plates Method Culture Duration

Note

• This test is also suitable for testing the effectiveness of hand

• Where applicable, the following multidrug-resistant pathogens can be identified:

MRSA (methicillin-resistant Staphylococcus aureus) and/or MRSE (methicillin-resistant Staphylococcus epidermidis) and/or

ESBL (pathogens that produce extended-spectrum \(\beta\)-lactamase). For this, additional costs will be incurred.

• If you participate regularly (2 x per year), you will get a certificate stating the annual monitoring of the disinfection performance of vour surface disinfection test.

• For testing the initial contamination, see the service "Surface Contamination Testing".

#### **Testing of Air Settlement Plates**

Air settlement plate Material

Method Culture Duration 2 days

Note For monitoring the microbiological air quality in practice rooms. This

test is not suitable for examinations of barns and stables!

# 23 Reference Ranges

# 23.1 Dog, Cat, Horse

## 23.1.1 Clinical Chemistry

		y	1	1.
	unit	dog	cat	horse
Enzymes 25 °C				
ALT (GPT)	U/I	up to 55	up to 70	-
α-Amylase	U/I	up to 1650	up to 1850	up to 170
AP	U/I	up to 108	up to 140	up to 450
AST (GOT)	U/I	up to 25	up to 30	up to 250
Cholinesterase	U/I	1500 – 3000	1000 - 3000	1500 - 3000
CK	U/I	up to 90	up to 130	up to 130 (190)
GLDH	U/I	up to 6	up to 6	up to 8
γ-GT	U/I	up to 5	up to 5	up to 25
α-HBDH	U/I	up to 50	up to 97	up to 170
LDH	U/I	up to 100	up to 70	up to 400
Lipase (DGGR)	U/I	up to 120	up to 26	up to 250
Substrates				
Albumin	g/l	25 - 44	26 - 56	25 - 54
Albumin/globulin (A/G) ratio		> 0.59	> 0.6	0.7 - 1.1
Bile Acids	μmol/l	up to 20, post-prandial up to 40	up to 20, post-prandial up to 40	up to 12
Bilirubin I (total)	μmol/l	up to 3.4	up to 3.4	8.6 - 59.9
Cholesterol	mmol/l	3.1 - 10.1	1.8 - 3.9	1.81 - 4.66
Creatinine	$\mu$ mol/l	35 - 106	up to 168	71 – 159
Fructosamines	μmol/l	up to 374	up to 340	up to 360
Globulins	g/l	up to 45	up to 55	24 - 51
Glucose	mmol/l	3.05 - 6.1	3.1 - 6.9	3.05 - 4.99
β-HBA	mmol/l	up to 0.6	up to 0.6	up to 0.6
Lactate	mmol/l	0.5 - 3.0	up to 1.0	0.5 - 2.0
NEFA	mmol/l	0.1 - 0.5	0.1 - 0.5	0.1 - 0.5
Protein (total)	g/l	54 - 75	57 - 94	55 – 75
SDMA	μmol/l	up to 0.65	p to 0.75	up to 0.75
Triglycerides	mmol/l	up to 3.9	up to 1.14	up to 0.97
Urea	mmol/l	3.3 - 8.3	5.0 - 11.3	3.3 - 6.7
Electrolytes and Trace Minerals				
Calcium	mmol/l	2.3 - 3.0	2.3 - 3.0	2.5 - 3.4
Chloride	mmol/l	96 – 113	110 – 130	95 – 105
Copper	$\mu$ mol/l	15.7 – 18.9	13.4 - 16.9	7.9 - 21.0
Iron	$\mu$ mol/l	15 – 45	8 – 31	17.9 - 64.5
Magnesium	mmol/l	0.6 - 1.3	0.6 - 1.3	0.5 - 0.9
Manganese	μg/l	up to 20	up to 20	1.11 - 2.96
Phosphate	mmol/l	0.7 - 1.6	0.8 - 1.9	0.7 - 1.5
Potassium	mmol/l	3.5 - 5.1	3.0 - 4.8	2.8 - 4.5
Selenium	μg/l	80 - 250	80 - 250	100 – 200
Sodium	mmol/l	140 – 155	145 – 158	125 – 150
Zinc	μmol/l	7.7 – 19.9	12.2 - 15.3	9.2 - 19.9

Selenium horse: Up to 70  $\mu$ g/l are marginal, more than 300  $\mu$ g/l high/critical. Foals and Iceland horses are sometimes well below these levels.

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## 23.1.2 Haematological Reference Ranges Dog, Cat, Horse

	unit	dog	cat	horse
Erythrocytes	T/I	5.5 - 8.5	5.0 - 10.0	6.0 - 12.0
Haematocrit	1/1	0.44 - 0.52	0.3 - 0.44	0.3 - 0.5
Haemoglobin	g/I	150 – 190	90 – 150	110 – 170
Leukocytes	G/I	6 – 12	6 – 11	5 – 10
Segmented	%	55 - 75	60 - 78	45 - 70
Lymphocytes	%	13 – 30	15 – 38	20 - 45
Monocytes	%	0 - 4	0 - 4	0 - 5
Eosinophils	%	0 - 6	0 - 6	0 - 4
Basophils	%	0	0 - 1	0 - 2
Unsegmented	%	0 - 4	0 - 4	0 - 6
Hypochromasia		neg.	neg.	neg.
Anisocytosis		neg.	neg.	neg.
Platelets	G/I	150 – 500	180 – 550	90 - 300
Differential blood o	ount (absol	ute numbers)		
Segmented	G/I	3 - 9	3 – 11	3 – 7
Lymphocytes	G/I	1 - 3.6	1 – 4	1.5 – 4
Monocytes	G/I	0.04 - 0.5	0.04 - 0.5	0.04 - 0.4
Eosinophils	G/I	0.04 - 0.6	0.04 - 0.6	0.04 - 0.3
Basophils	G/I	up to 0.04	up to 0.04	0 - 0.15
Unsegmented	G/I	up to 0.5	up to 0.6	0 - 0.6
Reticulocytes	/nl	< 110	< 60	-

## 23.1.3 Hormones Dog, Cat, Horse

		don		barras
ACTH	pg/ml	<b>dog</b> 6 – 58	up to 110	horse mid Nov mid July: negative: < 30 borderline: 30 - 50 positive: > 50 mid July - mid Nov.: negative: < 50 borderline: 50 - 100 positive: > 100
Anti- Müllerian Hormone	ng/ml	m-neutered: < 0.1 m-intact: > 2.0 f-neutered: < 0.02 f-intact: > 0.5	f-neutered: < 0.1 f-intact: > 2.0	mare intact: under 4 mare/borderline: 4 - 7 mare with granulosa theca cell tumour: over 7 male neutered: up to 0.1 male intact: over 2
Cortisol	ng/ml	5 - 65	3 - 50 (130)	30 - 67
Insulin	$\mu \text{U/mI}$	8 – 25	10 - 30	up to 20.0***
Oestradiol	pg/ml	prooestrus: 25 - 65 oestrus: up to 25 anoestrus: up to 30 neutered: up to 10 males: up to 15 Sertoli cell tumour: over 30	interoestrus: up to 20 oestrus: 20 - 60 - -	prooestrus: 1.2 - 6.2 oestrus: 7.1 - 13.0 dioestrus: 3.7 - 5.0
Progesterone	ng/ml	prooestrus: up to 1.0 oestrus: up to 30 ovulation*: 4.0 – 8.0 anoestrus: up to 1.0	Preov: up to 1.0 Postov: over 1.0	Levels over 1.0 indicate luteal activity -
Testosterone	ng/ml	m: 1.5 - 8.5 f: up to 0.4 m-neutered: up to 0.5	m: 2.5 - 7.0 - m-neutered: up to 0.5	stallion: 1.0 - 5.0 gelding: up to 0.04** mare: up to 0.04**
TSH	ng/ml	up to 0.6	-	-
TSH	μU/mI	-	over 0.04	-
T3	ng/dl	20 - 206	33 - 167	25 - 180
fT3	pmol/l	3.7 - 9.2	0.8 - 1.4	1.1 - 7.2
T4	μg/dl	1.3 - 4.5	0.9 - 2.9	1.3 – 4.1
fT4	pmol/l	7.7 - 47.6	6.4 - 33.3	9.0 - 44.9

<sup>\*</sup> Time of mating for female dogs: Optimum 24 to 48 hours, maximum 96 hours after ovulation

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# **23.2** Reference Ranges Rabbit, Guinea Pig and Ferret 23.2.1 Clinical Chemistry

		•		
	unit	rabbit	guinea pig	ferret
Enzymes 25 °C				
ALT (GPT)	U/I	up to 61	up to 61	55 – 206
α-Amylase	U/I	up to 459	up to 3159	21 - 59
AP	U/I	9.05 - 94.58	up to 418	18 – 71
AST (GOT)	U/I	3.75 - 32.44	up to 90	43 – 142
СК	U/I	1.63 - 559.53	up to 2143	80 - 453
GLDH	U/I	0.68 - 14.78	up to 17	up to 2
γ-GT	U/I	2.5 - 14.46	up to 13	up to 10
Lipase	U/I	up to 1587	up to 152	86 - 334
Substrates				
Albumin	g/I	36 – 57	-	26 – 42
Cholesterol	mmol/l	0.3 - 1.7	0.3 - 1.7	2.4 - 6.9
Creatinine	$\mu$ mol/l	51.38 - 154.35	up to 77	21 – 69
Fructosamines	μmol/l	248.08 - 501.43	up to 271	128 – 201
Glucose	mmol/l	5.8 - 14.8	5.0 - 16.0	2.7 - 8.6
Protein (total)	g/l	48.66 - 73.64	44 - 66	54 – 78
Triglycerides	mmol/l	0.5 - 3.4	0.3 - 2.4	0.5 - 1.9
Urea	mmol/l	2.63 - 10.28	3.3 – 10.3	5.1 – 16.6
Electrolytes				
Calcium	mmol/l	3.02 - 4.3	2.4 - 3.1	2.0 - 2.6
Iron	μmol/l	20 - 59	26 – 76	16 – 55
Magnesium	mmol/l	0.66 - 1.51	1.0 - 2.6	0.9 - 1.6
Phosphate	mmol/l	0.54 - 2.18	1.0 – 7.0	1.0 - 3.0
Potassium	mmol/l	3.52 - 6.04	4.5 - 8.8	3.8 - 5.5
Sodium	mmol/l	132.61 – 154.0	130 – 150	139 – 166
Hormones				
Androstenedione	ng/ml	-	-	< 428
17-OH- Progesterone	ng/ml	-	-	< 26.1
Testosterone	ng/ml	up to 0.5: neutered 0.5 - 1: questionable over 1: testicular tissue probably present	male/neutered: up to 0.5	male: 1.5 - 8.5 female: up to 0.4 neutered: up to 0.5
T4	μg/dl	3.9 - 5.3	1.1 - 5.2	1.1 – 2.8
f <b>T4</b>	pmol/l	up to 20 (30)	up to 20 (30)	-

<sup>\*\*</sup> Testosterone gelding: No increase in levels to the standard range after HCG stimulation. Testosterone mare: Increased levels indicate a granulosa theca cell tumour.

<sup>\*\*\*</sup> The reference value was test-specifically adapted according to the latest literature.

## 23.2.2 Haematological Reference Ranges Rabbit, Guinea Pig, Ferret

	unit	rabbit	guinea pig	ferret
Erythrocytes	T/I	4.37 - 7.43	4.51 - 6.36	7.4 - 13.0
Haematocrit	1/1	0.28 - 0.48	0.39 - 0.55	0.4 - 0.7
Haemoglobin	g/I	89.63 - 153.82	117 – 169	138 – 209
Leukocytes	G/I	2.71 - 12.23	2.9 - 14.4	3.0 - 16.7
Segmented	%	32 - 64	12 – 62	19 – 79
Lymphocytes	%	13 – 54	28 - 84	16 – 75
Monocytes	%	3 - 14	0 - 9	0 - 7
Eosinophils	%	< 3	0 - 14	0 - 6
Basophils	%	< 9	0 - 2	0 – 2
Unsegmented	%	0	0 - 1	0 – 2
Hypochromasia		neg.	neg.	neg.
Anisocytosis		neg.	neg.	neg.
Platelets	G/I	225.45 - 905.3	273 – 745	297 – 910
Differential blood	l count (absolut	e numbers)		
Segmented	G/I	0.87 - 7.82	0.9 - 5.1	3.2 - 13.1
Lymphocytes	G/I	0.36 - 6.58	1.4 - 10.7	2.6 - 12.5
Monocytes	G/I	0.08 - 1.71	up to 0.7	up to 1.1
Eosinophils	G/I	0.07 - 0.19	up to 1.5	up to 1.0
Basophils	G/I	0.06 - 1.1	up to 0.11	up to 0.3
Unsegmented	G/I	0	up to 0.07	up to 0.3

## 23.3 Reference Ranges Birds

## 23.3.1 Clinical Chemistry

	unit	parakeets	amazon parrots	parrot
Enzymes 25 °C				
ALT (GPT)	U/I	5 – 20	5 – 11	5 – 12
α-Amylase	U/I	187 - 582	100 - 600	200 - 600
AP	U/I	10 - 326	15 – 311	20 - 311
AST (GOT)	U/I	55 - 390	35 – 350	100 - 400
Cholinesterase	U/I	2000 - 4000	2000 - 4000	2500 - 12000
CK	U/I	54 - 300	100 - 500	130 - 400
GLDH	U/I	0 - 9.9	0 - 9.9	0 - 9.9
γ-GT	U/I	1 – 30	1 – 12	1 - 30
LDH	U/I	76 – 450	150 – 400	150 - 400
Substrates				
Albumin	g/l	7 – 18	19 – 35	15 – 33
Bile acids	$\mu$ mol/l	up to 100	20 - 150	19 - 144
Cholesterol	mmol/l	3 - 6	4 - 6	2.6 - 7
Protein (total)	g/l	22 - 50	26 - 50	26 - 53
Triglycerides	mmol/l	1.23 - 3.05	0.66 - 2.25	0.57 - 1.58
Urea	mmol/l	-	0.9 - 9.6	0.7 - 9.5
Uric acid	μmol/l	190 – 833	70 – 595	100 - 654
Electrolytes				
Calcium	mmol/l	1.6 - 3.3	2.0 - 3.5	1.75 – 3.5
Phosphate	mmol/l	0.8 - 2.5	0.3 - 1.8	0.35 - 4.0
Potassium	mmol/l	2.2 - 4.6	2.0 - 4.5	2.0 - 5.0
Sodium	mmol/l	139 – 159	125 – 160	135 – 165

## 23.3.2 Haematological Reference Ranges Birds

	unit	parakeet	amazon parrots	parrot
Haematocrit	1/1	0.48 - 0.58	0.44 - 0.55	0.43 - 0.51
Haemoglobin	g/l	124 – 175	135 – 200	142 – 171
Leukocytes	G/I	3 - 11	1.5 – 15	6 - 15
Heterophils	%	43 - 75	43 - 75	45 - 76
Lymphocytes	%	20 - 58	20 - 55	20 - 51
Monocytes	%	0 - 3	0 – 2	0 – 1
Eosinophils	%	0 - 2	0 - 1	0 - 1
Basophils	%	0 - 1	0 – 2	0 - 1
Unsegmented	%	-	-	-
Hypochromasia		neg.	neg.	neg.
Anisocytosis		neg.	neg.	neg.
Platelets	G/I	34.4 - 38.4	-	46 - 49

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# 23.4 Reference Ranges Farm Animals

## 23.4.1 Clinical Chemistry

	unit	cattle	sheep	goat	pig	alpaca	Ilama
Enzymes 25 °C							
ALT (GPT)	U/I	up to 50	up to 14	up to 14	up to 68	up to 50	up to 26
α-Amylase	U/I	up to 161	up to 120	-	up to 3500	-	-
AP	U/I	up to 300	45 - 235	45 - 235	up to 170	32 - 167	46 - 119
AST (GOT)	U/I	up to 80	up to 60	10 - 50	up to 35	up to 308	up to 450
Cholinesterase	U/I	50 - 160	-	-	-	-	50 - 100
CK	U/I	up to 250	10 - 50	up to 65	up to 500	up to 120	up to 137
GLDH	U/I	up to 8	up to 2	up to 7	up to 4	up to 19	up to 25
γ-GT	U/I	up to 20	up to 32	10 - 20	up to 45	up to 35	up to 28
GPX	U/g Hb	over 130	60 - 180	-	-	-	-
α-HBDH	U/I	up to 700	up to 700	-	up to 300	-	-
LDH	U/I	up to 1500	up to 1500	up to 1500	up to 600	up to 433	up to 695
Lipase	U/I	2 - 8	-	-	-	-	-
Substrates							
Albumin	g/l	30 - 40	24 - 30	30 - 40	18 – 31	29 - 43	29 - 50
Bile acids	$\mu$ mol/l	10 – 25	up to 10	-	-	-	-
Bilirubin I (total)	$\mu$ mol/l	up to 5.0	up to 8.5	up to 8.5	up to 4.3	up to 6.8	up to 8.6
Cholesterol	mmol/l	2.07 - 3.88	1.2 – 1.9	2.07 - 3.88	2.0 - 3.3	0.4 - 2.3	0.3 - 2.3
Creatinine	$\mu$ mol/l	88 - 177	50 - 120	50 - 120	40 - 130	88.4 - 212.2	79.5 – 247.5
Globulins	g/l	27 – 48	27 – 48	27 – 48	51 – 64	21 – 31	up to 32
Glucose	mmol/l	1.94 - 3.05	2.2 - 5.2	2.2 - 5.2	3.9 - 6.4	5.7 - 8.3	5.7 - 7.0
β-НВА	mmol/l	0.2 - 1.0	up to 0.6	up to 0.6	-	-	-
Lactate	mmol/l	0.5 - 3.0	1 – 1.4	1 – 1.4	-	-	-
NEFA	mmol/l	0.4 - 0.8	0.1 – 0.5	0.1 - 0.5	-	-	-
Protein (total)	g/l	60 – 80	50 – 70	60 – 80	55 – 86	57 – 72	47 – 73
Triglycerides	mmol/l	0.17 - 0.51		0.17 - 0.51	•	up to 0.6	up to 0.27
Urea	mmol/l	up to 8	4.5 – 10.7	4.5 – 10.7	3.3 – 8.3	3.6 – 10.1	3.2 – 12.8
Electrolytes and Trace Minerals							
Calcium	mmol/l	2.3 - 2.8	2.1 - 2.7	2.2 - 2.8	2.4 - 3.5	2.1 - 2.5	1.9 - 2.7
Chloride	mmol/l	90 - 110	75 – 114	97 – 110	102 – 106	99 – 122	103 – 122
Cobalt	μg/l	1.0 - 3.5	-	-	-	-	-
Copper	$\mu$ mol/l	8 - 24	7 – 24	16 – 32	16 – 39	2.1 - 12.5	6.1 - 7.9
Iron	$\mu$ mol/l	20 - 40	20 - 30	16 – 35	16.7 - 35.5	18.8 - 37.4	18.6 – 30.8
Magnesium	mmol/l	0.8 - 1.3	0.8 - 1.0	0.8 - 1.0	1.1 – 1.5	0.7 - 1.0	0.8 - 1.1
Phosphate	mmol/l	1.1 - 2.4	1.1 - 2.5	1.61 - 2.26	2.1 - 3.3	1.1 - 2.5	1.5 - 3.6
Potassium	mmol/l	3.5 - 4.5	3.5 - 4.5	4.5 - 6.5	4.0 - 5.0	4.0 - 5.7	3.6 - 6.2
Selenium	μg/l	40 - 85	55 - 170	15 - 40	100 - 200	over 99	over 99
Sodium	mmol/l	135 – 145	145 – 155	135 – 157	140 – 160	146 – 155	148 – 158
Zinc	$\mu$ mol/l	8 - 24	11.0 - 20.5	10.7 - 19.9	10 – 20	3.0 - 14.6	4.1 - 12.4

	unit	cattle	sheep	goat	pig	alpaca	Ilama
Vitamins							
β-carotene	μg/I	over 2500	-	-	-	-	-
Vitamin A	μg/I	130 - 380	-	-	-	-	-
Vitamin B12	pg/ml	over 100	-		-	-	-
Vitamin D3	nmol/l	75 – 125	-	-	-	-	-
Vitamin E	mg/l	over 3	-	-	-	-	-
Hormones							
Insulin	μU/ml	up to 5	-	-	-	-	-
Progesterone	ng/ml	over 1.0**	-	-	-	-	-
T4	μg/dl	3.4 - 8.2	-	-	-	-	-
Other values							-
Haptoglobin	g/l	up to 0.35	-	-	up to 0.68	-	-
lg G	mg/dl	1700 - 2700*	-	-	1700 - 2900	-	-

<sup>\*</sup> cattle (calf: > 800)

## 23.4.2 Haematological Reference Ranges Farm Animals

	unit	cattle	sheep	goat	pig	alpaca	llama
Erythrocytes	T/I	5.0 - 10.0	7.3 - 11.3	8 – 18	5.8 - 8.1	9.4 - 18.1	9.9 - 17.7
Haematocrit	1/1	0.28 - 0.38	0.29 - 0.38	0.24 - 0.48	0.33 - 0.45	0.22 - 0.45	0.25 - 0.46
Haemoglobin	g/l	90 - 140	80 - 120	80 - 120	108 - 148	102 - 193	115 – 195
Leukocytes	G/I	4 - 10	4 - 10	4 - 13	10 – 22	7.1 - 18.6	8.9 - 22.4
Segmented	%	25 - 45	10 - 50	30 - 48	10 - 39	49 - 65	49 - 65
Lymphocytes	%	45 - 65	40 - 80	50 - 70	49 - 85	21 – 25	21 - 25
Monocytes	%	2 - 6	0 - 15	0 - 4	2 - 4	0 - 5	0 - 5
Eosinophils	%	1 – 10	0 - 8	1 – 8	0 - 6	6 - 22	6 - 22
Basophils	%	0 - 2	0 - 4	0 - 1	0 - 5	0 - 1	0 - 1
Unsegmented	%	0 - 3	0 - 4	0 - 2	0 - 7	0 - 1	0 - 1
Platelets	G/I	300 - 800	200 - 800	200 - 800	175 – 580	200 - 600	200 - 600
Reticulocytes	/ml	up to 0.1	up to 0.1	up to 0.1	-	-	-

<sup>\*\*</sup> values that exceed 1.0 indicate luteal activity

# 24 Conversion Table for Laboratory Diagnostic Parameters

On the diagnostic findings compiled by us, you will find the measured values as well as information on the standard ranges in the internationally valid SI units. During followup checks, you may want to compare the measured values of different findings using identical units of measurement. The conversion factors for the parameters for which we have changed the unit of measurement are listed below.

To convert from one unit of measurement to the other, the corresponding measured value must be multiplied with the conversion factor (e.g. bilirubin in mg/dl x 17.104 = bilirubin in  $\mu$ mol/l).

## 24.1 Clinical-chemical Parameters

	Old unit	Conversion factor to SI unit	SI unit	Conversion factor to old unit
Substrates				
Albumin	g/dl	144.9	μmol/l	0.0069
Bilirubin	mg/dl	17.104	$\mu$ mol/l	0.0585
Cholesterol	g/dl	0.0259	mmol/l	38.664
Creatinine	mg/dl	88.402	$\mu$ mol/l	0.0113
Fibrinogen	mg/dl	0.01	g/l	100
Glucose	mg/dl	0.0555	mmol/l	18.016
Lactate	mg/dl	0.111	mmol/l	9.0080
Protein (total)	g/dl	10	g/l	0.1
Triglycerides	mg/dl	0.0114	mmol/l	87.500
Urea	mg/dl	0.1665	mmol/l	6.0060
Uric acid	mg/dl	59.48	$\mu$ mol/l	0.0168
Electrolytes and Trac	e Minerals			
Calcium	mg/dl	0.2495	mmol/l	4.0080
Chloride	mg/dl	0.2821	mmol/l	3.5453
Copper	μg/dl	0.1574	$\mu$ mol/l	6.3532
Iron	μg/dl	0.1791	$\mu$ mol/l	5.5847
Magnesium	mg/dl	0.4113	mmol/l	2.4312
Phosphate	mg/dl	0.3229	mmol/l	3.0974
Potassium	mg/dl	0.2557	mmol/l	3.9102
Sodium	mg/dl	0.4350	mmol/l	2.2989
Zinc	μg/dl	0.1530	μmol/I	6.5370

## 24.2 Blood Parameters

	Old unit	Conversion factor to SI unit	SI unit	Conversion factor to old unit
Erythrocytes	Mio/μl	1	T/I	1
Haematocrit	%	0.01	1/1	100
Haemoglobin	g/dl	10	g/I	0.1
Leukocytes	1/μΙ	0.001	G/I (= 109/I)	1000
Platelets	$1/\mu$ l	0.001	G/I (= 109/I)	1000
Reticulocytes	%	0.001	1	1000

You will find a converter for easily comparing diagnostic findings with parameters in different units – our **SI calculator** – on our website www.laboklin.com.

## 25 Courier Service

LABOKLIN offers courier services in most EU countries. The samples are generally delivered to LABOKLIN within 24/48 hours. For more information, including prices and the possibilities of sample collection in your area, please contact our Service Department or your local LABOKLIN office.

Our contacts: see page 8 and following.

## 26 Invoicing

All prices listed on the submission forms are quoted without the applicable Value Added Tax (VAT). To receive VAT-free invoices, please provide your international tax number (EU only). We issue invoices at the beginning of the next month with detailed information on costs per sample and tests performed in the previous month, together with animal and owner name. If an invoice is to be sent to the owner, we invoice with a factor 1.4 plus 19% German VAT. This is only possible for genetic tests and when the owners' signature and complete data are supplied.

There are discounts available to veterinarians depending on the monthly invoice revenue: For more information, please contact us or your local LABOKLIN office.

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