LABOKLIN
LABORATORY FOR CLINICAL DIAGNOSTICS

DIRECTORY OF TESTS
HORSE

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2023/24

www.laboklin.com

Foreword

Dear colleagues

we present to you the latest edition of our Directory of Tests exclusively for horses and equids. Focused on equine lab services, it provides useful information on basic parameters and profiles as well as some unique parameters that are not so frequently found elsewhere. Consequently, you don't have to bother with tests which are not useful for the species your service is covering. It is, of course, packed with background information: e.g. the material needed as well as critical conditions that may have an impact on the quality of the result, to name only some. That is because we want to lend a helping hand not only for the analytic itself, but for all factors ensuring the quality of the test result. The index at the back will help you to quickly find what you are searching for.

Portfolio
Single parameters and profiles – general profiles as well as those for special needs, e.g. mineral, heavy metals, endocrinopathies – are part of our portfolio. Additionally, we have set up a diagnostic for some poisons often found in equine practice, e.g. colchicine, hypoglycin A. Our PCR-portfolio for the detection of infectious diseases is extremely broad with all tests run every working day and usually reported the day of arrival in the lab. We are aware of the importance of the optimum use of antibiotics, thus our reports are tailor-made for equine practice and naturally based on the international guidelines (CLSI).

Genetics are of growing importance in the veterinary field – not only for breeding purposes, but also for ruling out differentials. Laboklin has a strong history of establishing and running genetic tests. For years we have been actively attending the ISAG for which we were also honoured to organise some round trials lately.

Extras
As colleagues, we know that time can be precious and the office quite often lacking when treating horses. This is why we provide the mobile version of our website to easily place an order, search for test outcomes, pass on results for owners, send additional requests to the lab.

As colleagues, we know how cumbersome it can be to remind animal owners of repeated applications, especially when it comes to long intervals. That is the reason we created the app "4Paws" for your clients to download free of charge and use it as a reminder for deworming, vaccination, allergy therapy, etc.

As colleagues, we know that sometimes a second opinion or a quick report in cytology or microscopy of parasites or urinary sediment is required. This is why we created the image analysis into which you can simply upload up to 4 pictures. Our colleagues will come back with a full report as quickly as possible. You will find the service in "my lab" on our website.

Happy to serve you with all the lab work you might need –

Kind regards
Dr Elisabeth Müller
CEO LABOKLIN GmbH & Co. KG

Test Method Abbreviations

AAS	atom absorption spectrometry
CEDIA	cloned enzyme donor immunoassay
CFT	complement fixation test
CLIA	chemiluminescence assay
EIA	corresponds to ELISA
ELISA	enzyme linked immunosorbent assay
cELISA	competitive ELISA
GCMS	gas chromatography – mass spectrometry
HAH	haemagglutination inhibition test
HPLC	high performance liquid chromatography
ICPMS	inductively coupled plasma mass spectrometry
IFAT	indirect fluorescent antibody test
LCMS	liquid chromatography – mass spectrometry
MAT	microscopic agglutination test
MS	mass spectrometry
PCR	polymerase chain reaction
RIA	radioimmunoassay
SAFC	sodium acetate-acetic acid-formalin concentration
SAT	slow agglutination test
VNT	virus neutralisation test
*	partner laboratory

In our “Directory of Tests – Horse” we have compiled specific equine tests and procedures. Basic information concerning pre-analytics, haematology and clinical chemistry, which are fairly similar for all species, can be found in our detailed Compendium.

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1. Sampling and Shipping – Pre-analytics

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.

Preparation of the patient
Fasted blood samples are not unproblematic in horses and only indicated for special tests, e.g. insulin and glucose for diagnosis of Equine Metabolic Syndrome (EMS).
The owner should be informed about the influence of physical activity or stress on blood test results: particularly muscular enzymes but also glucose and lactate can show elevated serum levels.

Labelling
The names of the animal and the owner should be clearly marked on the submission form and the sample.
Alternatively, LABOKLIN bar code labels can be used. For samples that will be tested bacteriologically, it is important to indicate the sampling site. For function tests, it is necessary to specify the time of collection or number the samples consecutively.

Sample material?
Details on the recommended material can be taken from our test descriptions or the submission forms.

Microbiology
It is important to collect samples as sterile as possible to avoid contamination.

Bacteriology and mycology: swab with transport medium

PCR: swab without transport medium (exception: Taylorella equigenitalis/CEM Profiles – swabs with Amies medium are required), whole blood (EDTA), fresh organ specimen (depending on pathogen)

Urine: sterile tube, swab with medium or Uricult

Hair/skin scrapings: sterile tube

Faeces: standard faeces tube

Punctures: sterile tube, swab with medium (for bacteriological examination)

Blood: blood culture flask (written order required, subject to a charge)

Histology/cytology
When submitting tissue samples for histopathological examinations, the following points must be observed:
- artefact-free extraction of a typical lesion sufficient in size (diameter > 0.5 cm)
- immediate fixation (10% neutral buffered formalin, relation of sample to formalin 1:10 – 1:20, if possible)
- submission of an anamnesis including clinical findings and objective

As a sample, a representative piece of tissue free of preparation artefacts (e.g. disruption, squashing, electrocoagulation) should be taken. If in doubt or in case of generalised alterations, several samples should be collected and submitted (accounting will be per case, not per biopsy).

- Skin punches
As skin samples, punch biopsies of all dermal layers with a diameter ≥ 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.

A preliminary report which contains all relevant data, including any suspected clinical diagnosis, helps to ensure the best possible diagnostic exchange with the histologist.

- Cytology
Samples can primarily be taken as wipe test, scraping or puncture (e.g. synovial fluid) with or without aspiration. Fine-needle aspiration is done using a 22 – 27 gauge needle attached to a syringe. A vacuum is created and, if possible, the tissue should be punctured several times in different directions. To avoid the aspirate receding into the syringe, the vacuum must be released before detaching the needle. 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 to spread the material. If the sample is more liquid, a steeper angle – like in a blood smear – should be applied.

For the cytological examination of aspirates, excretions or secretions, the fluids obtained are sent in EDTA test tubes. Ideally, the fluid obtained can already be centrifuged in the practice (2500 – 3000 rpm for 3 to 5 minutes).
Additionally, the supernatant is decanted and the sediment is carefully spread like a blood smear and shipped air-dried. 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 and unstained. 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.

Molecular Genetic Analysis of hereditary diseases, coat colour, performance and identity
An EDTA blood sample (approx. 0.5 ml) is required to perform these genetic tests. For horses, it is also possible to send in hair samples with roots (approx. 20 plucked mane or tail hairs), but only a limited amount of DNA can be extracted from them.

EB (purple top)
For doing a blood count, EDTA blood is the most suitable material. After collecting the sample, tilt the test tube carefully several times to mix the blood with the anticoagulant.

For the determination of clinical chemical and/or serological parameters it is only second choice, since EDTA as an "ion capturer" interferes with a variety of analyses.

- Haematology
We recommend to send an air-dried blood smear for the differential blood count along with the EB sample. This way, blood cells are fixed and alterations caused by ageing of the blood are avoided. If the smear is done using EDTA blood: please tilt the tube carefully a few times first before taking a drop from the sample to make sure that the composition of cells in the smear represents the composition of the whole blood.

- PCRs from whole blood
EDTA is the most suitable anticoagulant; heparin in contrast may inhibit the PCR.

Serum (red top)
With only a few exceptions, serum is the universal sample material for the determination of clinical chemical, serological and endocrinological parameters. Before separating the serum from the clotted blood, coagulation should be completed (allow to stand for at least 30 – 60 minutes before centrifugation; see above for separation process). If non-centrifuged samples are sent, haemolysis caused by transport and ageing might limit the diagnostic value of some parameters. Especially LDH, CK and K are often elevated because of the delay in sample processing in mobile practices. This needs to be taken into account when interpreting the test results.

Plasma
Plasma samples are drawn into tubes with anticoagulants (EDTA = purple top; heparin = green; citrate = blue; fluoride = grey). Please note: the additives may limit the number of analyses; with the exception of PCRs, heparin samples can be used for virtually all purposes.
The samples can be centrifuged immediately after collection (see above).
In citrate samples for the determination of coagulation parameters, the mix ratio of 1:10 is mandatory (please observe the marking line at the edge of the tube).
For exact glucose and lactate values, the additional submission of sodium fluoride blood is necessary. Both parameters are only stabilised reliably by fluoride (centrifugation as early as possible to separate the whole blood from the plasma).

Factors interfering with analysis

- Haemolysis
Haemolysis is caused by leakage of intracellular components of the erythrocytes such as iron, potassium and especially haemoglobin due to damage of the cell membrane. Haemoglobin causes a red colouration of serum/plasma which primarily interferes with the photometric testing done in clinical chemistry.
In horses, the parameters that are most susceptible to haemolysis are potassium (K), LDH and CK.

	Parameter	
haemolysis	LDH, CK, AST, bilirubin, creatinine, PO4, K, Mg, Fe, fructosamine	⬆
haemolysis	Ca, glucose	⬇

- Lipaemia
Lipaemia in horse blood samples is very rare: hyperlipaemic syndrome especially in ponies or with pronounced Cushing's disease.
Lipaemia refers to the milky/turbid discolouration of serum/plasma caused by triglycerides. Lipaemic samples may complicate the measurement of several clinical parameters.

- Icterus
Icterus is the yellowish discolouration of serum/plasma due to excess amounts of bilirubin in the blood. The most common icterus in horses is starvation icterus which already develops after short periods of fasting/anorexia. It must not be mistaken for the physiological yellow colouration of serum/plasma in horses due to physiologically high bilirubin concentrations! If hyperbilirubinaemia is excessive, the determination of some parameters may be complicated.

- Medication
Numerous drugs may influence clinical chemical or haematological parameters; listing them all would exceed the volume of this manual. When interpreting laboratory results, possible effects of the drugs in pre-treated horses should be taken into account.

Packaging and transport of samples
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, sample tubes should be placed in transport containers that are transparent, break-proof and contain absorbent material for leakage protection 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 (total) 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 below.
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 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

Sample preparation/Shipping supplies

Below, you find the abbreviations for the different sample materials. These abbreviations are used in this manual and on our submission forms.

A. **EB = EDTA blood**

B. **EP = EDTA plasma**
EDTA blood has to be centrifuged and the supernatant needs to be transferred into a neutral test tube (e.g. Eppendorf tube).

C. **HB = heparin blood**

D. **HP = heparin plasma**
Heparin blood has to be centrifuged and the supernatant needs to be transferred into a neutral test tube (e.g. Eppendorf tube).

E. **NaFB = sodium fluoride blood**

F. **NaFP = sodium fluoride plasma**
NaF blood has to be centrifuged and the supernatant needs to be transferred into a neutral test tube (e.g. Eppendorf tube).

G. **S = serum**
The coagulated blood should be centrifuged and the supernatant serum should be transferred into a neutral test tube or another serum tube (remove beads before!).

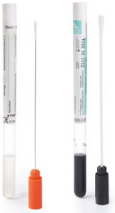
H. **CB = citrate blood**

I. **CP = citrate plasma**
The sample should be centrifuged and the supernatant should be transferred into an uncoated test tube (e.g. Eppendorf tube).

J. **Blood smear**
Blood smears should always be sent in air-dried, unfixed and unstained.
For transportation, the depicted transport covers (shipping containers) are suitable.

K. **Shipping container**
for blood tubes or urine tubes

L. **Swab with transport medium**
(orange: thin swab, amies medium clear; black: thick swab, amies with charcoal)



M. **Swab without transport medium**

N. **Shipping container for swabs**

O. **Urine tube**

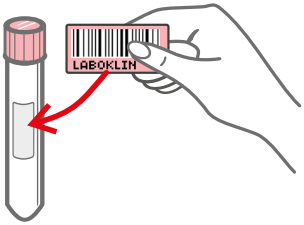
P. **Container for histology**
(formalin tube with shipping container)

Q. **Faeces tube**
with shipping container

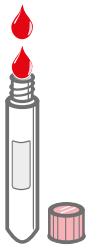
R. **Blood culture flask set**
(aerobic and anaerobic)

S. **Blood culture bottle Peds Plus™**

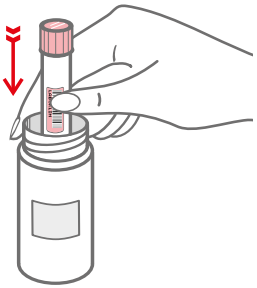
Labelling and shipment of samples



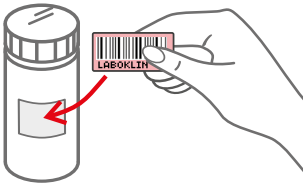
Step 1
Stick the bar code onto the test tube



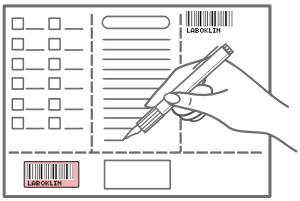
Step 2
Fill the test tube



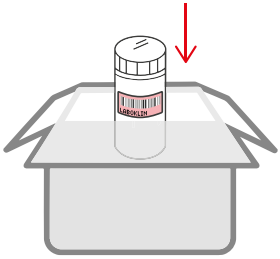
Step 3
Test tube into shipping container



Step 4
Additional bar code onto the shipping container



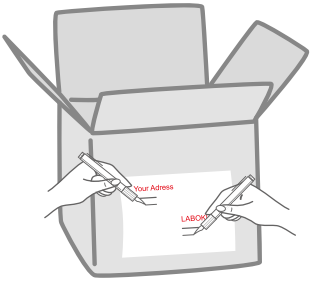
Step 5
Completely fill in submission form and, if necessary, sample list



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

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

Exempt Animal Specimen



Step 8
Pay attention to correct shipping information

2. Equine Profiles (clinical chemistry and haematology)

Our profiles are a combination of several clinical chemical and haematological parameters that allow for a quick overview of the horse's health status. The organ profiles are designed to confirm suspected diagnoses and for further monitoring of the patients.
Many profiles can be requested with an additional blood count, for which a reduced price is invoiced in these combinations.
Our profiles are subject to a certain "flow", as the included parameters are continuously checked to ensure they are up to date: with the ongoing progress in equine medicine, individual parameters are replaced, added or removed, so that LABOKLIN is able to always offer you profiles based on the latest scientific knowledge.
Information on our other horse profiles (PCR profiles, bacteriological/parasitological profiles, allergy profiles) can be found in the respective chapters.

2.1 Screening Large

Monitoring of all important organ functions as well as common minerals and trace elements

Parameter: AP, γ-GT, GLDH, total bilirubin, cholesterol, triglycerides, glucose, AST (GOT), LDH, CK, total protein, albumin, globulin, urea, creatinine, PO4, Ca, Mg, K, Na, Fe, Zn, Cu, Se

Material: S + NaFB

Results: Day of sample receipt (Se possibly the next day)
Method: Photometry, AAS

2.2 Screening Large + SAA

Parameter: like 2.1 + SAA

Material: S + NaFB

Results: Day of sample receipt (Se possibly the next day)
Method: Photometry, AAS, ELISA

2.3 Screening Small

Cost-effective starter profile for liver, kidney, muscles and metabolism

Parameter: GLDH, γ-GT, triglycerides, AST (GOT), LDH, CK, urea, creatinine, total protein

Material: S

Results: Day of sample receipt
Method: Photometry

2.4 Performance Profile

Focus on muscles/muscle metabolism, supplemented by the most important liver, kidney and metabolic parameters plus electrolytes

Parameter: AP, γ-GT, GLDH, total bilirubin, cholesterol, triglycerides, glucose, lactate, AST (GOT), LDH, CK, total protein, albumin, globulin, urea, creatinine, PO4, Ca, Mg, K, Na, Fe

Material: S + NaFB

Results: Day of sample receipt
Method: Photometry

2.5 Senior Profile

This profile includes the most important liver, kidney and metabolic parameters as well as those electrolytes and trace minerals that are essential for older horses. Many parameters that indicate Cushing's disease are included; therefore, it is absolutely necessary to send in NaFB for accurate glucose results! However, only fasting glucose has any reliable informative value.

Parameter: Urea, creatinine, PO4, Ca, total bilirubin, γ-GT, GLDH, lipase (DGGR), total protein, albumin, globulin, glucose, triglycerides, Zn, Se, SDMA

Material: S + NaFB

Results: Day of sample receipt (Se possibly the next day)
Method: Photometry, AAS

2.6 Foal Profile

Includes parameters important in foal medicine.

Parameter: Triglycerides, urea, creatinine, total protein, γ-GT, Na, Ca, Mg, PO4, serum electrophoresis

Material: S

Results: Day of sample receipt
Method: Photometry and capillary electrophoresis

2.7 Liver

- Initial Screening:
A quick overview of the most important liver enzymes

Parameter: AST (GOT), γ-GT, GLDH

Material: S

Results: Day of sample receipt
Method: Photometry

- Further Diagnostics:
Additional parameters provide information on liver function and possible pathogenesis.

Parameter: GLDH, γ-GT, total bilirubin and II, AST (GOT), AP, total protein, albumin, globulin, bile acids

Material: S

Results: Day of sample receipt
Method: Photometry

2.8 Kidney

- Initial Screening:
Quick overview

Parameter: Urea, creatinine

Material: S

Results: Day of sample receipt
Method: Photometry

- Further Diagnostics:
Additional parameters provide information on the extent of impairment.

Parameter: Urea, creatinine, total protein, albumin, Na, K, Ca, PO4

Material: S

Results: Day of sample receipt
Method: Photometry

2.9 Muscular Screening

This profile is useful for diagnosis and follow-up, especially in myopathies. It includes the most important muscle enzymes plus electrolytes and minerals.

Parameter: CK, AST (GOT), LDH, Na, K, Ca, PO4, α-HBDH, Mg, Fe

Material: S

Results: Day of sample receipt
Method: Photometry

2.10 Muscular Screening Extended

Parameter: CK, AST (GOT), LDH, K, Ca, PO4, α-HBDH, Mg, vitamin E, Se

Material: S (cooled)

Results: Clinical chemical parameters on day of sample receipt, vitamin E after approx. 3 days, Se possibly the next day
Method: Photometry, HPLC, AAS

2.11 Equine Cushing Profile/PPID Profile

This profile is designed to provide as much information as possible from a single fasted blood sample. Aetiological parameters are determined as well as indicative parameters. At the same time, insulin resistance is also confirmed or excluded.

Parameter: Insulin, ACTH, glucose, fructosamine, triglycerides, γ-GT, RISQI, I/G ratio, MIRG (for proxies see EMS Profile)

Material: Non-haemolysed EP 2 ml (cooled) + non-haemolysed S 2 ml (cooled) + NaFB 2 ml

Results: Day of sample receipt
Method: Photometry, CLIA

For further information concerning diagnostics of Cushing's see Chapter 6.5.

2.12 EMS Profile (equine metabolic syndrome)

Apart from fasting insulin and glucose, further parameters are determined to assess the metabolic status of the horse.

Parameter: Insulin, glucose, fructosamine, RISQI, I/G ratio, possibly MIRG

Note: Fructosamine reflects the average blood glucose level of the past 2 to 3 weeks. RISQI (= reciprocal inverse square of insulin) is a "proxy", i.e. a mere calculation of insulin and glucose and represents a measure for insulin sensitivity. The proxies "insulin/glucose ratio" and MIRG (= modified insulin to glucose ratio) (only stated in borderline cases) reflect pancreatic β-cell function.

Material: S + NaFB

Results: Day of sample receipt
Method: Photometry, CLIA

For further information concerning EMS diagnostics see Chapter 6.6.

2.13 Complete Blood Count

Includes erythrocytes, haemoglobin, haematocrit, platelets and leukocytes as well as a differential blood count. Depending on the clinical issue, the complete blood count can be ordered together with each profile. In addition to automated analysis, all samples with evident morphological abnormalities are also evaluated microscopically.

Material: EB 1 ml (if possible, send a blood smear along with the EB sample)

Results: Day of sample receipt
Method: Laser light – possibly microscopy

2.14 Blood Donation Profile

Based on the German guidelines for the collection, storage, transport and administration of blood and blood products in the veterinary sector.

Parameter: Complete blood count
Urea, creatinine, Na, K, Ca, PO4, bilirubin, ALT, AP, AST, GLDH, protein, albumin, glucose, fibrinogen, equine infectious anaemia virus (Coggins test), equine arteritis virus (VNT), babesia (cELISA), urinalysis

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

Duration: 5 working days
Method: Laser light – possibly microscopy, photometry, dry chemistry, serology as stated

2.15 Mineral and Trace Elements Profile (Mineral Profile II)

Parameter: Na, K, Ca, Cl, PO4, Mg, Fe, Cu, Zn, Se, Mn

Material: Non-haemolysed S

Results: Day of sample receipt (Se and Mn possibly the next day)
Method: AAS, Photometry

2.16 Heavy Metal Screening

If heavy metal poisoning is suspected.

Parameter: Arsenic, lead, cadmium, chromium, copper, manganese, mercury, thallium, zinc

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

Duration: 7 days
Method: ICPMS

2.17 Neurology Large

Parameter: Serum amyloid A (SAA), antibodies: West Nile virus (IgG and IgM), tick-borne encephalitis virus (TBEV) (IgG and IgM), PCR: bornavirus, EHV 1 & 4

Material: S 1 ml + EB, CSF, swab

Duration: 3 – 4 working days

2.18 Neurology Small

For neurological issues.

Parameter: West Nile virus (IgG and IgM), tick-borne encephalitis virus (TBEV) (IgG and IgM)

Material: S 1 ml

Duration: 2 – 3 working days; IgG: preliminary results

2.19 Tumour Diagnostics

Thymidine kinase, complemented by serum amyloid A and haptoglobin (for detailed parameter description see Chapter 3.3, 3.4, 3.6)

Parameter: Thymidine kinase, SAA, haptoglobin

Material: S 1 ml

Duration: 3 – 4 working days

3. Specific (Single) Parameters

3.1 Foal IgG

Insufficient supply of maternal immunoglobulins through the colostrum is one of the most predisposing factors for infectious diseases in foals. IgG determination in the blood of newborn foals allows for early diagnosis – before clinical symptoms occur – and initiation of therapeutic measures.

Material: S

Result: Day of sample receipt
Method: Electrophoresis

3.2 Lactate

Is the end product of anaerobic glycolysis in muscle tissue. Determination of lactate concentration may be helpful for the diagnosis of myopathies or – within the scope of an exercise stress test – be used to check the individual fitness level of a horse.

Procedure:
- Blood collection before exercise = baseline value
- After some warm-up laps, physical exercise: approx. 10 min at 5 m/s (equivalent to canter)
- 2nd blood collection 3 min after exercise
- 3rd blood collection 15 min after exercise
- 4th blood collection 30 min after exercise

Interpretation:
- baseline value < 2 mmol/l
- 3 min value: not more than twice the baseline value
- 15 min value: 30% lower than 3 min value

It may be useful to submit non-haemolysed serum from the baseline blood sample and the 30-minute value so that other muscle enzymes can be monitored, too.

Material: NaFB or NaFP in each case

Results: Day of sample receipt
Method: Photometry

3.3 Serum Amyloid A (SAA)

SAA is considered a “major APP” (acute-phase protein) in horses and can be used to detect subclinical diseases or for monitoring of treatment/healing processes.

Material: S

Results: Day of sample receipt
Method: ELISA

3.4 DGGR Lipase

DGGR lipase is a specific and sensitive biomarker for the diagnosis of pancreatitis. Pancreatitis can be associated with colic or other gastrointestinal disease. Elevated DGGR lipase values can also be seen in horses with high intensity training.

Material: S, (HP, EP)

Result: Day of sample receipt
Method: Photometry

3.5 Symmetric Dimethylarginine (SDMA)

SDMA is an end product of the protein metabolism and – for the most part – excreted via the kidneys. SDMA serum concentrations reflect glomerular filtration rate and there is a good correlation between SDMA and creatinine. In contrast to creatinine, SDMA is affected little by muscle mass or -wasting. SDMA can be used for early detection of renal disease.

Material: S, HP

Result: Day of sample receipt
Method: Photometry

3.6 Glutathione Peroxidase (GPX)

GPX is an enzymatic peroxidase which acts as an antioxidant. Deficiency of antioxidants results in oxidative stress which accompanies many diseases in horses.

Material: EB

Results: Day of sample receipt (Mo – Fr)
Method: Photometry

3.7 Iodine/Creatinine Ratio

The iodine supply can be assessed very well by the determination of the iodine excretion via the urine. The correlation between alimentary iodine intake and renal iodine excretion is very good.

Material: Urine – cooled

Method: ICPMS, Photometry

3.8 Thymidine Kinase

Thymidine kinase can be used as a biomarker for lymphoma diagnosis in horses. Horses with lymphoma show significantly higher activity of thymidine kinase than control groups.

Material: S 0.5 ml (cooled)

Duration: 3 – 4 days
Method: CLIA

3.9 Alpha Fetoprotein (AFP)

AFP can be used as a tumour marker; especially in case of liver tumours values may be elevated.

Material: S

Results: Day of sample receipt
Method: CLIA

4. Infectious Diseases

4.1 Bacteria
4.1.1 Borreliosis
4.1.2 Lawsonia intracellularis
4.1.3 Leptospirosis
4.1.4 Listeriosis
4.1.5 Rhodococcus hoagii (formerly R. equi)
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4.1.7 Salmonella
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4.2 Viruses
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4.2.2 Bovine Papillomavirus 1 & 2 (equine sarcoid)
4.2.3 Equine Coronavirus
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4.2.6 Equine Herpesvirus 3 (EHV3)
4.2.7 Equine Infectious Anaemia (EIA)
4.2.8 Equine Influenza
4.2.9 Equine Parvovirus
4.2.10 Equine Viral Arteritis (EVA)
4.2.11 Tick-borne Encephalitis Virus (TBEV)
4.2.12 West Nile Virus

4.3 Blood Parasites
4.3.1 Anaplasmosis
4.3.2 Theileria equi/Babesia caballi – Babesiosis – Piroplasmosis

4.4 PCR Profiles
4.4.1 Abortion
4.4.2 Anaemia Small
4.4.3 CEM
4.4.4 Eyes
4.4.5 Diarrhoea Pathogens (foal)
4.4.6 Neurology
4.4.7 Respiratory

There are 2 different ways to approach the diagnosis of infectious diseases:

1) direct detection of the pathogen by culture or PCR or
2) indirect serological examination that reveals previous contact with the pathogen by detection of antibodies
   The clinical relevance of antibody titres often can only be assessed by looking at the titre curve. Epidemiological factors have to be considered as well. In equine medicine, the examination of paired titres, for example, is common in many clinical issues when it is not only a matter of the antibody concentration but also especially of fall or rise in titre.
   It should also be taken into account that significant IgG titres can only be measured from about 3 weeks onwards after initial contact with the pathogen.

4.1 Bacteria

4.1.1 Borreliosis

In Europe, this tick-borne disease is presumably caused by 3 pathogenic Borrelia species of the group Borrelia burgdorferi sensu lato: B. burgdorferi sensu strictu, B. afzelii, B. garinii.
Although the suspected diagnosis of Lyme borreliosis is becoming increasingly common in practice, the diagnosis of the disease is still difficult. In endemic areas, serological prevalence is high even in healthy horses. Numerous clinical findings in horses are associated with Borrelia: performance reduction, chronic intermittent or alternating lameness, alterations of the skin, eyes or heart up to neurological deficits and abortions. Whether or not this spirochete infection causes any clinical symptoms in horses at all is still being controversially discussed.

Detection:
- DNA detection in skin biopsies, CSF, synovia or in ticks by PCR
- quantitative antibody detection: IgM and IgG by IFAT in serum or EP, HP
- qualitative antibody detection: Borrelia blot: Borrelia IgG Line immunoassay in serum (suspicious titres should always be confirmed by Line immunoassay)

4.1.2 Lawsonia intracellularis

Over the past years, this obligate intracellular, gram-negative bacterium has proven to be an important pathogen in the differential diagnosis of diarrhoea in foals. Especially weanlings (< 6 – 7 months of age) are affected, in which L. intracellularis colonises the crypt cells of the ileum causing proliferative enteropathy. This may result in intestinal malabsorption and/or (mostly) chronic diarrhoea. In most cases, individual animals fall ill, though multiple illnesses have been described in some farms. Clear findings in abdominal ultrasound and hypoalbuminaemia are indicative of Lawsonia infection.

Detection: Since only very few pathogens are excreted with the faeces, detection by PCR is the method of choice. However, false negative results may occur due to the very low excretion rate.
Antibody detection: IFAT; this way, particularly in problematic populations, carriers and possible shedders can be determined.

4.1.3 Leptospirosis

Leptospira infections, which are spread through the urine of rodents, are usually clinically inapparent in horses. Thus, the seroprevalence in healthy horses is high. 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.

Special case: equine recurrent uveitis (ERU)
Intraocular persistent leptospira infections seem to contribute to the aetiology of ERU – but are not the only possible aetiology. Resulting 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) in aqueous humour or tissue of the vitreous body indicate ERU.
Cave: serum antibody titres are irrelevant in ERU!

Detection:
- DNA detection by PCR in EB (acute febrile illness) or urine (chronic disease) or aqueous humour or tissue of vitreous body (ERU)
- quantitative antibody detection: MAT for serovars:
  L. interrogans Australis, Autumnalis, Bratislava, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Saxkoebing, Sejroe in serum (systemic disease) or in aqueous humour/tissue of vitreous body (with ERU symptoms)

During acute systemic disease, a significant rise in titres of one or more serovars is to be expected.

4.1.4 Listeriosis

Listeria are ubiquitous pathogens with a wide range of hosts. They can also be found in the intestine of healthy animals. In horses, only L. monocytogenes plays a role. Transmission between horses is possible, but it is more likely that the pathogen is taken up from the environment, e.g. via contaminated feed. Listeria particularly multiply in silage. Clinical manifestation as septicaemia, encephalitis or abortion is rare. Listeriosis is a notifiable disease!

Detection: - quantitative antibody detection by IFAT
- Culture from CSF, abortion material, etc. is possible but requires special culture media. It is therefore essential that suspected listeriosis is noted on the submission form.

Because of the widespread distribution of the pathogen, a significant rise in titre for 2 – 3 weeks in conjunction with acute symptoms would indicate listeriosis.

4.1.5 Rhodococcus hoagii (formerly: R. equi)

Rhodococcus hoagii causes severe pneumonia in foals. Infection occurs during the first days of life by inhalation of pathogens bound to dust particles. The course of the disease is gradual. At the earliest, clinical symptoms occur at the age of 3 – 4 weeks, sometimes even after several months. Purulent bronchopneumonia and abscess formation in the lungs occur. When the pathogens are swallowed, they reach the gastrointestinal tract where they proliferate and may cause granuloma and diarrhoea. They are excreted with the faeces and thus released into the environment – a source of infection for other foals. Older animals also excrete the pathogen but do not fall ill themselves. Moreover, R. equi shows an affinity for bones and joints.

Detection: - bacterial culture: most suitable sample material is TBS (!) or faeces. Examination of nasal swabs is possible, too, with decreased sensitivity, though.
- by PCR: this highly sensitive method can help to identify clinically healthy shedders.

4.1.6 Methicillin-resistant Staphylococci

In horses, too, methicillin-resistant strains of Staphylococcus aureus can be detected regularly, e.g. MRSA in infected wounds. If staphylococci are detected in bacteriological culture, another screening is done by chrome agar to test for methicillin resistance. Methicillin resistance can also be tested by PCR (detection of the MecA gene).

Material: swab with medium

4.1.7 Salmonella

Salmonella spp. belong to the family Enterobacteriaceae and are found in the intestines of animals and humans. Combined with predisposing factors like immunodeficiency or stress, salmonellosis in horses can lead to diarrhoea and fever, septicaemic forms often occur in young animals.
Asymptomatic „carriers“ are described as well, they do not get sick but are a source of infection for other animals and humans.
In Germany, Salmonellosis in horses is a reportable disease.
Detection: - bacteriological culture
- by PCR from faeces

4.1.8 Streptococcus equi

The globally spread equine disease strangles is caused by an infection with **Streptococcus equi subspec. equi.** The main clinical signs are purulent infections of the lymph nodes of the head. Further symptoms are: high fever, nasal discharge, coughing and lethargy. Strangles is a highly contagious disease, transmission is not only possible by direct contact but also indirectly (e.g. objects). Therefore, well-coordinated herd management is mandatory. The problem is: approx. 2 – 10% of the affected animals become carriers, i.e. the pathogen is not completely eliminated but retreats into the guttural pouch and is excreted intermittently. Carriers are often asymptomatic and therefore easily overlooked. However, identification of carriers is absolutely necessary to avoid a permanent manifestation in the herd and an outward spread of the disease. PCR is more sensitive than bacterial culture but as it cannot distinguish between live and dead organisms, it remains unclear whether or not an animal is infectious. Thus, a combination of both methods is recommended.
Clinically, an infection with Streptococcus equi subsp. equi cannot always be distinguished from an infection with **Streptococcus equi subsp. zooepidemicus**, a facultative pathogenic commensal in horses. Infections can cause respiratory disorders and purulent bronchopneumonia.

Detection:
- material: guttural pouch lavage! (gold standard, highest sensitivity), pharyngeal flush, aspirate (lymph node), TBS, BAL, swab (nose)
- PCR: swab without medium; it can be chosen between the single detection of Streptococcus equi equi or the combined detection of Streptococcus equi equi and Streptococcus equi zooepidemicus
- bacteriological examination: swab with medium
- antibody detection by ELISA (serum). 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.

At LABOKLIN, the following titre levels are indicated: 1:200 (weakly positive), 1:800 (positive), 1:3,200 (strongly positive) and 1:12,800 (very strongly positive).
Especially if haemorrhagic purpura or metastatic abscesses are suspected, antibody detection may be very helpful; these animals regularly have high titre levels.

4.1.8 Taylorella equigenitalis

Taylorella equigenitalis is the causative agent of contagious equine metritis (CEM). Information on the diagnosis, which is mainly performed as part of export tests, can be found in Chapter 5.1.

4.1.9 Tetanus – Vaccination Titre

The detection of antibodies against Costridium tetani toxin is particularly useful for determining the immunisation of the horse.

Detection: antibody detection by ELISA in serum
analysis is semi-quantitative: reliable immunisation provided
or: immunisation provided
or: no tetanus antibodies can be detected.

4.2 Viruses

4.2.1 Borna Disease

In horses, Borna disease virus (BDV) causes non-purulent encephalomyelitis; BVD can be found in a variety of species and leads to neurological symptoms as well as behavioural disorders. Horses and sheep seem to be the most susceptible species. Whether BDV is pathogenic to humans is still being discussed. Infected horses are mainly found in East Germany and in Switzerland. However, epidemiological studies show a seroprevalence of approx. 11.5% in all federal states of Germany. In horses from "Borna livestocks", seropositivity increased to 33%. In general, asymptomatic infections and clinical cases must also be expected outside the endemic areas. Shrews were identified as virus reservoir.

Detection:
- quantitative antibody detection by IFAT
- detection of antibodies in CSF would also confirm the diagnosis
- pathogen detection by PCR in CSF, EB (viraemia), intraocular fluid, retina, brain tissue

4.2.2 Bovine Papilloma Virus 1 & 2 (equine sarcoid)

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.

Detection: Pathogen detection by PCR from scurf, hairs (hair roots!) or tissue (tumour) samples. Positive PCR results confirm the suspected clinical diagnosis. Histopathology continues to be the gold standard for the diagnosis of equine sarcoid.

4.2.3 Equine Coronavirus (ECoV)

Equine coronavirus (ECoV), a beta coronavirus, has recently been detected in the USA, Japan and Europe and is associated with fever, colic and diarrhoea. Particularly adult horses are affected in the cold season. Neurological abnormalities have rarely been described: secondarily caused by hyperammonaemia.
Infections can affect several animals of a herd but seem to be self-limiting. However, secondary complications can worsen the course of the disease. Transmission occurs mainly via the faecal-oral route.

Detection: PCR from faeces

4.2.4 Equine Herpesvirus 1 & 4 (EHV1 & EHV4)

Infections with EHV1 as well as with EHV4 primarily cause diseases of the respiratory tract. The severity of the clinical symptoms depends on the age and immune status of the infected animal.
Particularly infections with EHV1 are able to spread beyond the respiratory mucosa and cause severe manifestations of the disease: abortions, perinatal foal death, neurological diseases. Once infected, horses remain carriers of the virus for life. The virus can be reactivated endogenously under adverse conditions (stress, etc.).
Lymph organs, the leukocyte fraction and trigeminal ganglia 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 EHV1 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).
In horses, 2 different variants (DNApol D752 vs. DNApol N752) with varying neuropathogenicity have been described. The D752 variant is associated with most of the neurological disease outbreaks and is therefore referred to as neuropathogenic. However, only some of the infected horses develop neurological symptoms. The N752 variant is mainly found in case of abortions, but also in a smaller number of neurological diseases. Differentiation is particularly interesting from the epidemiological point of view.

Detection:
- PCR from nasal swabs/secretions of the respiratory tract, CSF or abortion material including foetal membranes.
  Recent studies suggest examining a sample of EDTA blood in parallel with the organ material. This is supposed to increase the probability of detection.
  In case of a positive EHV1 result by PCR, the differentiation of the EHV1 variant is performed automatically and free of charge.
  The detection of EHV1 or EHV4 can be ordered as a single test. The detection of EHV1 and EHV4 is included in the different respiratory profiles (see Chapter 4.4.6). Furthermore, the detection of EHV1 and/or EHV4 by

PCR is part of the following profiles: Neurology Large (see Chapter 2.18), Abortion (see Chapter 4.4.1) and Uveitis (see Chapter 4.4.3).
- quantitative antibody detection by ELISA which can sensitively distinguish between EHV1 and EHV4 antibodies.

A clear increase in titre of paired serum samples (interval 10 – 14 days) indicates an acute EHV infection. Vaccination titres cannot be distinguished from infection titres! In acute cases of illness, however, we recommend direct pathogen detection by PCR.

4.2.5 Equine Herpesvirus 2 & 5 (EHV2 & EHV5)

The involvement of EHV2 and/or EHV5 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 EHV2 and 5 are precursors of other viral and bacterial infections of the respiratory tract. Especially in young animals, EHV2 and/or 5 were detected in treatment-resistant, partly catarrhal-purulent, partly necrotising or abscessing bronchopneumonia. EHV5 was recently presented as aetiological agent of "equine multi-nodular pulmonary fibrosis" (EMPF).

Detection:
- PCR from TBS/BAL or pulmonary tissue or from conjunctival swabs (preferably with Cytobrush)
- The detection of EHV2 or EHV5 can be ordered as a single test, the profile Eyes includes the detection of both pathogens.

4.2.6 Equine Herpesvirus 3 (EHV3)

Coital exanthema caused by EHV3, which only sporadically occurs in Germany, is a mildly progressing venereal infection in horses. Clinically, blisters, pustules and erosions appear on the mucous membrane of the vestibule, 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. Coital exanthema is a typical venereal infection. However, transmission is also possible through close contact as well as rectal and vaginal examinations. Infected animals remain carriers of the virus for life.

Detection: PCR from swab without medium (lesions on vestibule, penis, prepuce or surrounding skin) or tissue (lesions)

4.2.7 Equine Infectious Anaemia (EIA)

Equine infectious anaemia (EIA) is a worldwide distributed disease in equids with acute lethal to chronic recurrent forms. EIA is characterised by 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.

Detection: - 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 within 3 – 4 weeks.

Method: - "Coggins test" (= agar gel diffusion test)
To date, the Coggins test is the standard for all official procedures as well as for export tests.
The method-specific test duration is approx. 3 days!
- cELISA
This test offers a considerably higher sensitivity than the "Coggins test"; that is why false positive results may occur in rare cases. However, these would only have implications if they were confirmed by a positive Coggins test. Negative cELISA results, in contrast, can be considered reliable. If the sample arrives in time, results are available on the day of receipt.

Cave: EIA is an epizootic disease that is notifiable upon suspicion in many countries!

4.2.8 Equine Influenza

Equine influenza is caused by the subtypes Influenza A equi 1 (H7N7) and A equi 2 (H3N8), although H7N7 has not been very present anymore over the past 30 years. In susceptible equids, 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.

Detection: - PCR from nasal swabs, TBS/BAL allows a quick and reliable diagnosis
- quantitative antibody detection by HAH: testing of paired serum at an interval of 10 – 14 days

A equi 1 (Prague 56) and A equi 2 (Newmarket 1/93 & Newmarket 2/93) are detected. A fourfold increase in titre would generally indicate an acute infection. Vaccination titres cannot be distinguished from infection titres.

4.2.9 Equine Parvovirus

Equine serum hepatitis, also known as Theiler's disease, is caused by infection with equine parvovirus-hepatitis virus (EqPV-H). EqPV-H is a hepatotropic single-stranded and non-enveloped DNA virus. To date, two different modes of transmission have been assumed: One is the administration of products prepared from equine sera containing equine parvovirus. These include tetanus antitoxin, botulinum antitoxin, stem cell

preparations, and equine plasma products in general. On the other hand, EqPV-H outbreaks also occur in horses that have not received a biological preparation in the past. In this case, transmission from horse to horse or spread by insects is assumed, but is currently still the focus of research. It is presently thought that perhaps 2% of infected horses develop clinical signs.
Clinical signs of EqPV-H infection occur approximately 4 – 10 weeks after administration of a biological product contaminated with the virus. The spectrum of disease progression ranges from asymptomatic to fulminant liver failure. Acute hepatitis may present with neurologic symptoms, such as manic behavior, head pressing, and ataxia, in addition to lethargic behavior with associated anorexia. Colic, recumbancy or death within 72 hours have also been described.

Detection: - DNA detection by PCR from EDTA blood or liver tissue (native)

4.2.10 Equine Viral Arteritis (EVA)

Equine Viral Arteritis (EVA) is a worldwide distributed, contagious viral infection of equids caused by the equine arteritis virus (EAV).
Confirmed outbreaks seem to have increased in recent years. The majority of naturally acquired infections is subclinical, nevertheless, seroconversion still occurs.
When clinical symptoms appear, they vary in type and severity: fever, depression, anorexia and peripheral oedema, conjunctivitis (“pink eye”), urticaria and abortion. In young animals, fulminant 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, virus shedding can also occur by other body secretions: aerosolised secretions of the respiratory tract, urine, abortion material, etc.

Detection:
- detection of RNA by PCR from stallion semen/ejaculate, in systemic affected animals from secretions of the respiratory tract, urine, abortion material, etc.
- During viraemia (fever!), PCR can be attempted from EB.
- quantitative antibody detection by VNT; if titres are low or borderline, a second testing is recommended after 3 to 4 weeks. Vaccination titres cannot be distinguished from infection titres.

Duration: The method-specific test duration is 1 – 3 days for PCR and approx. 5 days for VNT.

Cave: In Germany, EVA is a reportable disease (horses, donkeys, etc.).

4.2.11 Tick-borne Encephalitis Virus (TBEV)

Increasingly, TBE is also detected in neurologically affected horses. Like the West Nile virus, the TBE virus (TBEV) belongs to the genus Flavivirus.

Detection: ELISA: IgG and IgM in serum, IgG in CSF
PCR: pathogen detection in CSF, serum, possibly in tick

4.2.12 West Nile Virus

The virus is transmitted by blood-sucking insects.
Susceptible dead-end hosts are horses and humans; birds can become infected and serve as the virus reservoir, thus possibly transferring the virus over long distances. Infected horses show symptoms of encephalitis, but also ataxia, muscle tremor and paralysis can be found. The mortality rate in infected horses is said to be about 30%. In Germany, the virus was detected for the first time in 2018 in horses, birds and humans.

Detection: ELISA: (IgM and IgG in horses, only IgG in birds), required material: S 0.5 ml
RNA detection by PCR in EB, CSF, tissue (brain, spleen)

Cave: WNV is an epizootic disease that is notifiable upon suspicion.

The very short viraemia phase (approx. 1 – 3 days) makes pathogen detection difficult. Furthermore, this phase is prior to the onset of the first clinical signs.

4.3 Blood Parasites

4.3.1 Anaplasmosis

(formerly: “equine ehrlichiosis”)
Anaplasma phagocytophilum (formerly: Ehrlichia equi), an obligate intracellular gram-negative, coccoid bacterium is the causative agent of equine granulocytic ehrlichiosis. In Europe, tick-borne granulocytic anaplasmosis is predominant. Inoculation and subsequent lymphogenic and haematogenous spreading are followed by colonisation of the target cells: neutrophil and eosinophilic granulocytes. Initial clinical signs include fever, apathy, limb oedemas and reluctance to move. Laboratory findings are thrombocytopenia and possibly mild anaemia. Animals > 4 years show more pronounced clinical symptoms than younger horses. Once the infection is overcome, horses acquire immunity which lasts for approximately 2 years; this is independent of a latent infection or carrier status.

Detection:
- DNA detection by PCR in EB of affected horses approx. 5 days after inoculation, i.e. at the same time as the onset of fever, whereas microscopic detection of blood parasites is not possible until about 5 days after the onset of the febrile illness.
- quantitative antibody detection by IFAT

4.3.2 Theileria equi/Babesia caballi – Babesiosis – Piroplasmosis

Equine babesiosis or piroplasmosis is a tick-borne protozoal infection which is endemic in most tropic and subtropical territories and even reaches the temperate zones. Due to transportation of horses and expansion of the vector's distribution area, clinical cases and seropositive horses can now also be expected in Germany. Causative agents are Babesia caballi and Theileria equi (formerly Babesia equi) which can be found in the erythrocytes of infected animals. Clinical symptoms often are non-specific, the course of the disease is peracute to chronic. Characteristic are: fever – also intermittent –, anorexia, increased respiratory and cardiac rates, depression, anaemia, icterus and haemoglobinuria; in case of a chronic course of the disease: weight loss. Infected animals remain carriers for a long time and are therefore a source of infection for vectors.

Detection:
- PCR (Babesia spp.) in EB. The first step is the detection of Babesia/ Theileria. If the result is positive, differentiation between Theileria equi/ Babesia caballi is performed subsequently by sequencing and free of charge.
- microscopic identification of pathogens in a blood smear
- quantitative antibody detection by
    - CFT (usually required for export-relevant tests)
    - cELISA (particularly for export to the USA, most sensitive test)
    - IFAT (only available for export examinations, if required)

4.4 PCR Profiles

4.4.1 Abortion

PCR detection: Equine herpesvirus 1 and 4 (EHV1, EHV4), equine arteritis virus (EAV), leptospira
Material: Abortion material, swab without medium (genital tract)

4.4.2 Anaemia small

PCR detection: Anaplasma phagocytophilum, babesia/theileria
Material: EB

4.4.3 CEM

Information regarding the CEM profiles (mare and stallion) can be found in Chapter 5.1.

4.4.4 Eyes

- Eye
  PCR detection: Equine herpesvirus 2 and 5 (EHV2, EHV5)
  Material: Swab without medium (conjunctiva)

- Uveitis
  - Antibodies: Leptospira
  - PCR detection: Leptospira, equine herpesvirus 1 (EHV1)
  - Material: Aqueous humour 1 ml

4.4.5 Diarrhoea Pathogens (foal)

PCR detection: Coronavirus, Lawsonia intracellularis, Rhodococcus hoagii (formerly: R. equi)
Material: Faeces

4.4.6 Neurology

Information regarding the profile Neurology Large can be found in Chapter 2.18.

4.4.7 Respiratory

- Respiratory Foal
  - PCR detection: Equine herpesvirus 1 and 4 (EHV1, EHV4), Influenza A virus and Rhodococcus hoagii (formerly: R. equi)
  - Material: Nasal swab (deep) without medium or tracheobronchial secretion (TBS)/bronchoalveolar lavage (BAL)

- Respiratory I
  - PCR detection: Equine herpesvirus 1 and 4 (EHV1, EHV4), Influenza A virus, Streptococcus equi equi/zooepidemicus
  - Material: Nasal swab (deep) without medium or tracheobronchial secretion (TBS)/bronchoalveolar lavage (BAL)

- Respiratory II
  - PCR detection: Equine herpesvirus 1 and 4 (EHV1, EHV4), Influenza A virus, Streptococcus equi equi, equine coronavirus
  - Material: Nasal swab (deep) without medium or tracheobronchial secretion (TBS)/bronchoalveolar lavage (BAL) and faeces

- Respiratory III
  - PCR detection: Equine herpesvirus 1 and 4 (EHV1, EHV4) and Influenza A virus
  - Material: Nasal swab (deep) without medium or tracheobronchial secretion (TBS)/bronchoalveolar lavage (BAL)

- Respiratory IV
  - PCR detection: Equine herpesvirus 1 and 4 (EHV1, EHV4)
  - Material: Nasal swab (deep) without medium or tracheobronchial secretion (TBS)/bronchoalveolar lavage (BAL)

5. Export-relevant Tests

This chapter will briefly discuss the diseases which are required for so-called "export-tests". Requirements vary from country to country. The embassies or consulates of the respective import countries should have information available regarding the current regulations. Mostly, these are also available fully up-to-date on the websites of these countries.

It is essential to take into account the specified test duration, which may be several days, when collecting and sending samples.

5.1 Taylorella equigenitalis (CEM = contagious equine metritis)

CEM is caused by Taylorella equigenitalis. Transmission particularly occurs during mating but also indirectly by contaminated instruments or other items. Stallions may latently carry the pathogen on the mucosa of the penis without becoming clinically ill. In mares, an infection usually leads to endometritis/cervicitis with mucopurulent vaginal discharge and to reduced fertility.
The sampling sites result from the predilection sites of the pathogen:
- In stallions: penile sheath, urethra and fossa glandis
- In mares: fossa clitoridis and med. & lat. sinus clitoridis.

Transportation of these samples in medium with charcoal (e.g. Amies) is mandatory. Current animal health regulations require an examination by bacterial culture for at least 7 days (Canada: 14 days, Norway: 21 days) due to the very slow growth of the microaerophilic bacterium. To get a faster result, a PCR can be performed for Taylorella equigenitalis.

Within the EU, the detection of Taylorella equigenitalis by PCR is also recognised as a suitable test method in addition to the bacteriological examination.

In accordance with Council Directive 92/65/EEC, we offer a CEM PCR profile for 3 samples (stallion) as part of export tests within the EU. This profile includes 3 single tests by PCR for Taylorella equigenitalis from the 3 sites that are specified. There is a corresponding CEM PCR profile available for mares (2 sites).
For CEM export tests, the submission of 2 swabs (mare) or 3 swabs (stallion) with charcoal medium is also required for PCR.

In addition, we also offer 2 expanded profiles for both stallion and mare. These expanded profiles include the examination of sperm for stallions and of a cervix swab for mares in addition to the examination of the above-mentioned swabs of 3 (stallion)/2 (mare) sites.

In Germany, there is an obligation to inform the authorities, if Taylorella equigenitalis is detected.

5.2 Equine Viral Arteritis (EVA)

For further information, see Chapter 4.2.

Material: - antibody detection in serum by VNT
- RNA detection in semen by PCR

5.3 Equine Infectious Anaemia (EIA)

For further information, see Chapter 4.2.

Material: antibody detection in serum by Coggins test (= agar gel immunodiffusion test)

Note: EIA is an epizootic disease that is notifiable upon suspicion in most countries!

5.4 Theileria equi/Babesia caballi – Piroplasmosis – Babesiosis

For further information, see Chapter 4.3.

Materia:l antibody detection in serum by CFT (mandatory for most countries) or cELISA (for export to the USA) or IFAT

5.5 Glanders (Burkholderia mallei)

Glanders is an equine disease caused by Burkholderia mallei, which also has zoonotic potential: Apart from humans, wildcats (zoos!), camelids, bears, wolves and dogs are susceptible. Cattle, sheep and pigs are resistant. The disease is either acute (especially in donkeys and mules) with high fever and respiratory symptoms and death after a few days. In horses, glanders mostly takes a chronic course with nodules and ulcerations on the skin, the mucous membrane and the inner organs. Chronically and subclinically infected animals are 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.

Material: antibody detection in serum by CFT

Note: Glanders is an epizootic disease that is notifiable upon suspicion in many countries!

5.6 Salmonella Abortusequi

Transmission occurs orally, rarely through mating. With regard to miscarriages, this pathogen does not currently play a role in Germany anymore.

Material: antibody detection in serum by MAT

Note: Authorities must be notified if Salmonella Abortusequi is detected.

5.7 Dourine (Trypanosoma equiperdum)

Dourine is a chronic or acute infectious disease in equines, which is transmitted directly between animals during mating. Infected equines 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.

Material: antibody detection in serum by CFT

Note: Dourine is an epizootic disease that is notifiable upon suspicion in many countries!

5.8 African Horse Sickness (AHS)

As the name suggests, AHS is an endemic viral disease in equids particularly in Central Africa; sporadic outbreaks have been observed in the Middle and the Near East as well as in Southern Europe. Generally, the disease is transmitted by Culicoides spp., but also 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 haemorrhages. The mortality rate is about 70 – 95% in horses, approx. 50% in mules and approx. 10% in donkeys.

Material: antibody detection in serum by cELISA

Note: AHS is an epizootic disease that is notifiable upon suspicion in most countries!

6. Endocrinology

6.1 Sex Hormones

6.1.1 Oestradiol

Produced synchronously to the oestrus cycle in the ovarian follicles (“oestrus hormone”); during pregnancy, there is massive oestrogen biosynthesis of the foeto-maternal unit.

Material: S
Method: CLIA

6.1.2 Progesterone

Synthesised by luteal cells of the corpora lutea (c.l.): values ≥ 1 ng/ml indicate luteal function; c.l. during the cycle and especially during pregnancy almost always show much higher values.
During an intact early pregnancy, the concentration of progesterone in the blood should not drop below 4 ng/mg.
Laboratory diagnosis cannot differentiate between cyclic corpora lutea and corpora lutea in pregnancy. If progesterone is used for pregnancy diagnosis on day 18 – 20 post ovulationem and a corpus luteum is detected, it only means that the mare will not return to oestrus at the expected time.

Material: S or HP
Method: CLIA

6.1.3. Testosterone

It is produced in the testicular interstitial cells of Leydig and, to a small extend, also in the adrenal cortex. Sampling should take into account the circadian fluctuation: testosterone levels are low in the morning and highest in the afternoon.
Mares also produce small amounts of testosterone in the ovaries and adrenal cortex.

Material: S or HP
Method: LCMS

Note: Interpreting the analysis of sex hormones can only be done if the results of a clinical examination are considered as well. In some cases, follow-up examinations may become necessary.

6.2 Hormonal Pregnancy Diagnosis

Sometimes, rectal palpation for pregnancy detection is impeded by very pragmatic matters: ponies/miniature horses, aggressive animals, feral and zoo animals, lesions of the rectum, etc. For such situations, we have two pregnancy-specific hormones available in the equine practice:

6.2.1 PMSG/eCG

It is produced by the "endometrial cups" approximately between day 35 and day 120 of pregnancy (in individual cases even longer) with highest values between day 60 and 75. After early embryonic loss, endometrial cups will continue to secrete PMSG for weeks and the test will yield false positive results concerning pregnancy. We therefore recommend re-testing the mares after day 110 of pregnancy by determining their oestrone sulphate levels (see below).

Material: S or HP
Method: ELISA

Note: We recommend sampling between day 45 and 100 post ovulationem.

6.2.2 Oestrone Sulphate

It is produced by the intact foeto-placental unit and thus indicates the presence of a live foetus. The hormone can be detected from approx. day 40 of pregnancy onwards in increasing concentrations; at this early stage, however, there is no reliable differentiation from cyclical hormone secretion possible. We recommend oestrone sulphate testing from day 110 onwards, since mares at this stage of pregnancy have much higher concentrations of oestrone sulphate.

Material: S, possibly HP, EP, U
Method: LCMS

Preferably ≥ day 110 of pregnancy

Note: Not all mares show the typical pattern of secretion. If the test results are borderline or inconclusive, we suggest re-testing after 3 – 4 weeks. A negative test result in mares which have definitely been pregnant for > 120 days can indicate damage to the foetus. In this case, a rectal palpation or an ultrasound examination is mandatory.

6.3 Diagnosis of Ovarian Tumours

Mares with cycle or behavioural abnormalities which also show abnormal rectal/sonographic ovarian findings are often suspected to have an ovarian tumour. In fact, ovarian tumours are among the most frequent neoplasms in horses; the most common diagnosis is granulosa cell tumour (GCT). This kind of tumour is able to secrete oestradiol and testosterone. The determination of these hormones can therefore also be used to make the diagnosis. It must be taken into account, though, that on the one hand, mares with such a tumour may have normal hormone levels and on the other hand, elevated oestradiol and testosterone levels may be found in certain cycle abnormalities or in pregnant mares.

6.3.1 Anti-Müllerian Hormone (AMH)

AMH is a glycoprotein which is crucial for sexual differentiation during embryonic development. In female animals, AMH is secreted by the granulosa cells of preantral and small antral follicles. Since granulosa cell tumours (GCT) are the most frequent tumours of the genital tract in mares, it made sense – as in human medicine – to pursue this diagnostic approach in mares as well. Mares with GCT showed significantly higher levels of AMH than healthy mares. Regarding GCT diagnosis, AMH has a sensitivity of 95%.

Material: S or HP 3 ml, centrifuged and pipetted promptly. Cooling the sample is recommended.
Method: ELISA

Interpretation
“Intact” mares: < 4 ng/ml – individual levels
Ovariectomised mares: < 0.1 ng/ml
Mares with GCT: > 7 ng/ml
Borderline: 4 – 7 ng/ml

Test frequency: daily

6.3.2 Inhibin B

Inhibin is produced by the granulosa cells of the ovary. Around 85% of mares with GCT have clearly elevated serum levels of inhibin B (compared to testosterone: increased in approx. 50% of mares). High inhibin B concentrations are inevitably followed by suppression and non-functionality of the contralateral ovary.

Material: S 3 ml
Method: RIA

Note: The sample is shipped to the USA. Results may take up to 4 weeks. Because of the long test duration, we suggest testing for AMH.

6.4 Diagnosis of Cryptorchidism

Detection of cryptorchid or incompletely neutered male horses. Single determinations of testosterone levels are often of little significance due to pronounced circadian and seasonal fluctuations.

6.4.1 HCG Stimulation Test/"Cox Test"

For a long time, HCG stimulation test was considered gold standard for the diagnosis of cryptorchidism. Principle: HCG has LH-like effects

Material: S
Method: LCMS (determination of testosterone level with 2 samples)

Test procedure:
- In the morning: first blood collection = testosterone baseline value
- Directly followed by i.v. injection of 5000 – 10000 IU HCG/horse
- + 60 min: second blood collection = stimulation value

Interpretation: Completely castrated horses show very low levels and a lack of stimulation. A significant rise in testosterone levels indicates the existence of testosterone-producing tissue. At the same time, the absolute values should also be taken into consideration.

6.4.2 GnRH Stimulation Test

The use of a releasing hormone additionally tests the hypothalamus pituitary axis. This is not really necessary for the mere diagnosis of cryptorchidism.

Test procedure:
- In the morning: first blood collection = testosterone baseline value
- Directly followed by i.v. injection of 0.04 mg GnRH/horse
- + 60 min: second blood collection = stimulation value

Material: S
Method: LCMS (determination of testosterone level with 2 samples)

Interpretation: Depending on the clinical issue

6.4.3 Anti-Müllerian Hormone (AMH)

In stallions, AMH is expressed in the Sertoli cells. Levels stay high until puberty, then decrease parallel to increasing testosterone secretion. Nevertheless, stallions and cryptorchids can clearly be differentiated from castrated animals. AMH in male animals is a useful biomarker for the presence of testicular tissue and can thus be used for the diagnosis of cryptorchidism. Unlike, for example, the determination of oestrone sulphate, this test also works in young castrated animals.

Material: S or HP 3 ml, centrifuged promptly and pipetted off without any cells. Cooling is recommended.
Method: ELISA

Interpretation:
Values < 0.1 ng/ml indicate complete castration
Values 0.1 to 2 ng/ml are to be considered borderline
Values > 2 ng/ml speak for the presence of testicular tissue

Test frequency: daily

6.5 PPID (Cushing's disease)

Equine Cushing's disease is the most frequently diagnosed endocrinopathy in horses. It is a typical disease of old horses and ponies. It is caused by pituitary adenoma – more precisely: pars intermedia hyperplasia (PPID = pituitary pars intermedia dysfunction). In horses, nearly solely pituitary Cushing's is of clinical relevance.
Clinical symptoms are: hirsutism/hypertrichosis, polydipsia/polyuria, weight loss with simultaneous fat redistribution, depression/lethargy, laminitis, insulin resistance, immunosuppression and hyperhidrosis (pathologically increased sweating).
Below, you will find our endocrinological tests for the diagnosis of Cushing's disease. Further information on the **Cushing Profile**, which includes the evaluation of hormones as well as clinical chemical parameters, can be found in Chapter 2.11.

6.5.1 Overnight Dexamethasone Suppression Test (ODST)

This test has long been considered gold standard for the diagnosis of equine Cushing's disease.

Principle:
Exogenous dexamethasone suppresses endogenous corticoid secretion by negative feedback on the pituitary gland – in healthy horses. In PPID horses, there is no feedback as the mutated pars intermedia cells have no corticoid receptors.

Test procedure:
- blood collection between 4 pm and 6 pm = cortisol baseline value
- Directly followed by i.m. injection of 2 mg/50kg bdw of dexamethasone
- (Perhaps additional blood collection after 15 hours. However, long-term suppression after 18 – 20 hours is decisive.)
- blood collection between 10 am and 1 pm the next day = suppression value

Interpretation:
In healthy horses, values are suppressed to well below 10 ng/ml.

Material: S
Method: CLIA (in both samples)

Note: In late summer/autumn, even healthy horses may suppress inadequately!

6.5.2 ACTH Analysis

In horses, the test has a high diagnostic accuracy and is considered the best diagnostic alternative to the dexamethasone suppression test. The determination of endogenous ACTH is especially indicated if the horse is domiciled far away or in laminitic horses.

Test procedure:
Stress-free blood collection: EDTA plasma is required, which is centrifuged promptly and pipetted off without any cells! Cooling is recommended, the temperature of the samples must not rise above room temperature. The sample should arrive at the laboratory the next day.

Interpretation:
Due to seasonal fluctuations, ACTH values should be interpreted as follows:

Mid-November – mid-July:
Negative < 30 pg/ml, borderline 30 – 50 pg/ml, positive > 50 pg/ml

Mid-July – mid-November:
Negative < 50 pg/ml, borderline 50 – 100 pg/ml, positive > 100 pg/ml

Reference values should be seen as guidelines; individual variations can sometimes cause significant deviations. For correct results, it is important to adhere to the pre-analytical recommendations (see Chapter 1).

Material: EP
Method: CLIA

Note: Endogenous ACTH should not be tested during acute laminitis!

6.5.3 TRH Stimulation Test with ACTH Analysis

Test with high specificity and sensitivity for the diagnosis of Cushing's disease.
It is indicated if the results of the ACTH Analysis or the Suppression Test do not correlate with clinical findings or are inconclusive.

Test procedure:
- First blood collection = baseline value
- Directly followed by slow i.v. injection of 1 mg TRH
- Second blood collection after 10 min = stimulation value

Material: EP – see pre-analytics in 6.5.2
Method: CLIA

Interpretation:
Cut off 10 min after stimulation: < 110 pg/ml, borderline: 100 – 200 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.
One note on determining **cortisol levels**:
In most horses suffering from Cushing's disease, cortisol levels are within reference range, as in affected animals, mainly the circadian rhythm of secretion is disturbed. Healthy horses show highest cortisol concentrations in the early morning, lowest in the afternoon/early evening. Further factors influencing endogenous cortisol are stress, exercise, pain, etc.
With regard to Cushing's disease, determination of cortisol is only useful if evaluated in connection with an ODST (see above)!

6.6 Equine Metabolic Syndrome (EMS)

EMS is a severe disturbance of the carbohydrate and lipid metabolism resulting in insulin resistance (IR)/insulin dysregulation (ID): Increased insulin secretion (partly) compensates for decreased insulin efficiency.
EMS comprises adipositas, insulin resistance/dysregulation and laminitis (either anamnestic or present).

Clinical presentation:
- Mainly middle-aged or old horses (approx. 5 – 15 years) are affected, especially easy-keeper breeds such as Ponies, Arabian Horses, Fjord Horses, Mustangs, "Baroque Horses", etc.
- Laminitis: subtle to acute
- Adipositas (approx. 10% of the affected horses have a "lean" appearance, though)

- Polydipsia/polyuria
- Reduced fertility
- Recurrent colic

The diagnosis is based on the detection of insulin resistance (IR)/insulin dysregulation (ID); in older horses, PPID may need to be excluded or clarified.
Information on the **EMS Profile**, which includes the evaluation of insulin as well as other clinical chemical parameters, can be found in Chapter 2.12.

6.6.1 Analysis of Fasting Insulin and Fasting Glucose

Material: S (insulin) and NaFB (glucose)
Both samples should be collected from the fasted animal (hay/straw only ration)!
Method: CLIA, Photometry

Interpretation:
The reference range for insulin is < 20 µU/ml.
Negative: < 20 µU/ml, borderline: 20 – 50 µU/ml, dysregulation of insulin: > 50 µU/ml
IR/ID-horses show significantly higher insulin levels whilst glucose levels are within reference range (= compensated) or elevated (= not compensated).

Note:
To determine the insulin level, non-haemolysed serum is required (centrifuged promptly after blood collection and pipetted off without any cells), as otherwise leukocytic proteases will lead to decreased values. The sample should arrive at the laboratory the day after sample collection. Cooling is recommended, the temperature of the samples must not rise above room temperature.
Sampling should be delayed if the horse is affected by an acute, intensely painful attack of laminitis.

6.6.2 Oral Glucose Tolerance Test with Insulin Determination

Test procedure:
- The horse must fast overnight (hay/straw only ration).
- In the morning, it gets:
  a) 1g/kg bdw of glucose
  or
  b) 0.5 g/kg bdw of glucose per os (can be done by the animal owner)

Blood collection to determine insulin levels after 2 hours

Material: See Chapter 6.6.1

Method: CLIA

Interpretation:
Healthy horses stay below these cut offs:
a) < 85 µU/l insulin
b) < 68 µU/l insulin
EMS horses show higher values.

6.6.3 Oral Sugar Test („karo light syrup® “) with Insulin Determination

More sensitive but also more time-consuming than 6.6.1.

Test procedure:
- Hay/straw only ration
- Administer 0.15 or 0.45 ml/kg bdw of Karo Light Corn Syrup® via dose syringe
- Blood collection after 60 and/or 90 minutes, measurement of insulin and glucose

Interpretation:
0.15 ml/kg dose: > 45 µU/ml insulin indicates ID
0.45 ml/kg dose: > 63 µU/ml insulin indicates ID

6.6.4 Insulin Tolerance Test with Glucose Determination

Test procedure:
- Fed (pasture or hay) state! Do not fast!
- Blood collection at time 0; measurement of glucose and administration of 0.10 IU/kg bdw of regular (soluble) insulin
- Blood collection after 30 minutes, measurement of glucose
- Feed immediately after last sample

Interpretation:
Glucose should be decreased > 50% compared with baseline in non ID. horses.

6.7 Hypoadrenocorticism

In horses, hypoadrenocorticism is a rare endocrine disorder. If it occurs spontaneously in horses, it is mostly primary chronic hypoadrenocorticism (Addison’s disease). However, by far the most common form in veterinary medicine is secondary, iatrogenic hypoadrenocorticism caused by long-term application of exogenous glucocorticoids.

6.7.1 ACTH Stimulation Test

Evaluation of adrenal function

Test procedure:
- First blood collection in the morning = cortisol baseline value
- Directly followed by i.v. injection of 100 IU ACTH
- Second blood collection after 2 hours = stimulation value

Material: S
Method: CLIA

Interpretation:
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.

6.8 Thyroid gland

In horses, the thyroid gland only plays a secondary role. If at all, hypothyroidism occurs. However, hypothyroidism is often secondary, e.g. in horses suffering from Cushing's disease, EMS or other severe diseases. Foals are extremely sensitive to fluctuations in thyroid hormones – already intrauterine.

6.8.1 Thyroid Hormones

Reference values are available for:

T4	1.3 – 4.1	µg/dl*
fT4	9 – 44.9	pmol/l*
T3	30 – 180	ng/ml*
serum iodine	18 – 30	µg/l*

*Depending on age, foals show significantly higher values.

6.8.2 TRH Stimulation Test with Determination of T4

The definite diagnosis “hypothyroidism” should only be made after performing a TRH stimulation test.

Test procedure
- First blood collection = baseline value of T4
- Slow i.v. injection of 1 mg of TRH/horse or 0.5 mg of TRH/pony
- Second sample after 4 hours = stimulation value

Parameter: T4
Material: S
Method: CLIA

Interpretation: Euthyroid horses have a 2- to 3-fold increase in T4 after 4 hours.

7. Allergy

Allergies are an increasing problem in the equine practice, especially at the beginning of the warm summer months. Horses are not only sensitised to pollen and insects – as often suspected – but also to perennial allergens like dust mites, storage mites or moulds which can be found ubiquitously in horse stables, horse feed or in the surroundings of the barn. Allergy is a threshold phenomenon, that is why clinical signs mainly occur in the warmer time of year: It takes a certain concentration of allergen(s) to induce clinical symptoms in patients. If seasonal and perennial allergens appear at the same time, they add up, thus exceeding the individual threshold level.
Horses suffering from allergy show the following clinical signs: severe pruritus, skin lesions, urticaria or respiratory diseases (equine asthma, former RAO complex “recurrent airway obstruction”, formerly known as COPD = “chronic obstructive pulmonary disease”).

General information on allergy testing
The diagnosis of an allergy is a clinical diagnosis which consists of a thorough anamnesis and clinical examination. Subsequent allergy tests are only used to identify the underlying allergens in order to be able to avoid them or to perform an allergen-specific immunotherapy (hyposensitisation).
The administration of glucocorticoids or the wrong time of blood collection can considerably affect the results of the allergy test or falsify the test results. Withdrawal times of up to 3 months are recommended for injectable cortisone preparations and 6 to 8 weeks for prednisolone tablets. But also the use of locally applied cortisone ointments, creams, sprays, etc. should be stopped 2 to 4 weeks before the allergy test.
The right time to perform an allergy test is when the clinical signs have been observed for approximately 4 – 6 weeks. After this time, it can be assumed that the allergen-specific IgE antibodies are already detectable.
Furthermore, the test should be performed within or at the end of the season, as the test may yield false negative test results outside the season.

7.1 Allergy Profiles

Allergy Profile Respiratory
Determination of seasonal and perennial allergens (see 7.3).

Allergy Profile Skin
Determination of seasonal and perennial allergens, insects (see 7.3), Food Panel see 7.4).

Material (both profiles): S
Method: ELISA

7.2 Allergy Screening Test

Detects sensitisation against the 4 main allergen groups:
pollen (grasses, herbs, trees)
house dust and storage mites
fungal spores
insects

Material: S
Method: ELISA

Those groups that are positive in the screening test can then be differentiated into single allergens in the main test. All samples sent in are stored by us for 14 days. Hence, within this time frame, all necessary further tests can be ordered from a sample sent in for a screening test.

7.3 Main Tests Allergy

Pollen/Seasonal Panel: Grasses and Herbs

- 6-grass mix (orchard grass, perennial ryegrass, Timothy grass, meadow fescue, Kentucky bluegrass, meadow soft grass)
- rye (Secale cereale), mugwort (Artemisia vulgaris), lamb's quarters (Chenopodium album), English plantain (Plantago lanceolata), nettle (Urtica dioica), sorrel (Rumex rumex spp.), dandelion (Taraxacum officinalis), rape (Brassica napus), ragweed (Ambrosia elatior)

Trees and Bushes
- hazel (Corylus avellana), alder (Alnus), poplar (Populus), birch (Betula spp.), beech (Fagus sylvatica), willow (Salix spp.)

Mites & Moulds/ Perennial Panel:

Moulds
- Alternaria alternata, Aspergillus fumigatus, Aspergillus niger, Cladosporium sp., Epicoccus nigrum, Helminthosporium sativum, Penicillium notatum, Fusarium, Ustilago, Rhizopus

Dust Mites
- Dermatophagoides farinae, Dermatoph. pteronyssinus

Storage Mites
- Acarus siro, Tyrophagus putrescentiae, Glycophagus domesticus, Lepidoglyphus destructor

Insects:
- Simulium (blackfly)
- Culex tarsalis (mosquito)
- Tabanus (horse fly)
- Musca domestica (housefly)
- Culicoides (gnat)

Material: S
Method: ELISA

7.4 Further Main Tests Allergy

Feathers/Hairs/Epithelia:
Detection of IgE antibodies against epithelia of cat, dog, rabbit, guinea pig, parrot, feather mix

Food Allergy Test:
Determination of antibody titres (IgE & IgG) against 8 individual food allergens: soya, molasses, oats, maize, barley, wheat, barn and lucerne.
The definite diagnosis of a food allergy can only be made after allergen avoidance or feeding an elimination diet (only food components which have yielded a negative result for both antibodies = reaction class 0), followed by allergen provocation.

Material (both tests): S
Method: ELISA

7.5 Allergen-specific Immunotherapy (ASIT, hyposensitisation)

ASIT is considered the most promising therapeutic approach for allergic patients. The pathomechanism consists, among others, in the modulation of the Th2 to a Th1 cell response. Thus, it intervenes causally in the pathophysiological mechanisms of the disease.
The allergen extracts are composed individually for each patient according to the allergy test results. Our LABOKLIN allergy team will be happy to help you select the causative allergens. The allergen extracts are administered subcutaneously once a week with an initial weekly increase in dosage until the maintenance level is reached. Afterwards, the treatment intervals can be individually adjusted (up to monthly injections).
Whether ASIT has been successful should be evaluated after completing the initial and the follow-up treatment. If there is a good response, therapy should be continued for life. In case of seasonal allergies, we recommend starting treatment at the end of the season. Individual adaptations of the dosage plan may be necessary. We will gladly advise you in such cases.

Finally, some basic comments on ASIT:
The outcome of this therapy is better the earlier it is started within the course of the disease (within 1 – 2 years after the occurrence of the first clinical symptoms). Middle aged horses with a short duration of clinical signs (both insect bite hypersensitivity and equine asthma) showed best therapy success; but also elder horses (> 20 years of age) still showed satisfying responses to ASIT. Very young horses (< 1 year of age) should not be treated with ASIT yet.

ASIT treatment

Starter set:
The starter set contains 2 vials of allergen extract in 2 different concentrations (green and red cap) and will last for approx. 6 months of treatment.

Refill set:
The refill set contains only one vial with the higher concentration (red cap) and will last – depending on the individual injection interval – for about 10 months.

Cave:
It is not possible to perform hyposensitisation to food allergens!

Please enclose a veterinary prescription when ordering ASIT!
Delivery to the veterinary pharmacy will take 2 – 3 weeks.
Sending an invoice to the owner is not possible when ordering ASIT.

8. Drug Analysis/Testing for Doping/Intoxication

National and international anti-doping regulations in equestrian as well as in human competitive sports judge every qualitative detection of a substance (with few exceptions) as a positive result (so-called “zero option”). In equestrian sports, these regulations also apply to substances administered for therapeutic purposes. Yet, the subject of doping is in a continuous state of flux as the pharmaceutical industry on the one hand provides more and more agents, and on the other hand analytical procedures are permanently adapted and testing techniques are refined.
Which drug screening to choose for an individual horse depends on anamnesis, suspicious facts, exclusion attempts, etc. From our comprehensive Screening for Doping-relevant Substances up to a targeted analysis of a certain drug group, everything is possible.
Doping samples you send to LABOKLIN will be forwarded to an accredited partner laboratory for analysis. We will take care of the processing for you.

For the most frequent questions regarding drug level testing, we provide the following screenings:

8.1 Screening for Doping-relevant Substances

Testing for drugs and medicines in sport horses, also suitable as a pre-purchase examination.

Material: S 20 ml
Method: LCMS/MS + GCMS + CEDIA

8.2 Antiphlogistics Screening

Analysis of glucocorticoids + NSAIDs + endogenous cortisol.

Material: S 20 ml
Method: LCMS/MS + GCMS

8.3 Glucocorticoid Screening

Including endogenous cortisol

Material: S 10 ml
Method: LCMS/MS

8.4 NSAID Screening

Material S 10 ml
Method GCMS

8.5 Sedatives/Tranquiliser

Material: S 20 ml
Method: LCMS/MS

8.6 Stimulants

Material: S 10 ml
Method: GCMS + CEDIA

8.7 Tricyclic Antidepressants

Material: S 10 ml
Method: LCMS

If administration of a certain medicine is suspected, please note this on the submission form.

Further analyses and detections on request.

If you have any specific requests, please contact us by phone.

8.8 Colchicine

Colchicine is the main poison of Colchicum autumnale. Horses and other grazing animals can ingest the plant directly on the pasture or via hay or silage (colchicine is stable and remains toxic in hay and silage). Often, whole herds are affected. Possible symptoms of intoxication: colic, mucous-watery to bloody diarrhoea with secondary dehydration and electrolyte imbalances, central nervous manifestations such as apathy and unsteady gait, polyuria, haematuria, convulsions, respiratory depression, dyspnoea, cardiovascular failure, myopathies. Consequently, changes in the following laboratory parameters can occur: shift of electrolytes, initially peripheral leukocytosis, with progressing course leukopenia and pancytopenia, increase of liver and kidney values. The detection of critical amounts of poison can help to clarify the cause of the observed clinical symptoms.

Material: Urine (frozen) preferred,
(EB (cooled))

Duration: 1 – 2 working days
Method: LCMS

Note: Colchicine belongs to the category of doping-relevant substances in horses (FEI guidelines); traces may be detectable for up to several weeks after uptake.

8.9 Hypoglycin A (equine atypical myopathy, sycamore poisoning)

Hypoglycine A (HGA) is a non-essential amino acid and can be found in various maple species, such as ash maple, sycamore maple, Japanese maple and others. It has not yet been detected in field and norway maple. The toxin is located especially in seeds and seedlings. This means that pasturing horses are particularly at risk of taking up toxic plant parts in spring (seedlings) and in autumn (seeds). Possible symptoms of HGA poisoning are apathy, ataxia, colic, lateral position, tremor, muscle weakness and pain, stiffness, discoloured urine, hyperthermia, dyspnoea up to respiratory arrest. Severe courses lead to rhabdomyolysis with myoglobinuria, colic and lying down. Finally the animals die from cardiac or respiratory arrest or from the secondary impact of rhabdomyolysis. Laboratory parameters that can give hints: severely elevated levels of CK, LDH, and AST.

Material: S (frozen) preferred,
(urine, frozen)

Duration: 1 – 2 working days
Method: LCMS

Note: The detection of critical amounts of poison can help to clarify the cause of the observed clinical symptoms.

9. Urinalysis

Urinalysis in addition to blood/serum analysis is used for laboratory diagnostic workup of urinary tract diseases. But urinalysis is also useful regarding other issues: e.g. the determination of glucose in the urine of hyperglycaemic horses or the fractional electrolyte excretion, which also can be interpreted with regard to the electrolyte supply of the horse in case of muscular problems.
Please note: Informationen concerning SDMA, a helpful parameter for the early detection of renal dysfunction, can be found on page 27.

9.1 Urinalysis including Urinary Sediment

Specific gravity, total protein, haemoglobin/myoglobin, pH, bilirubin, urobilinogen, glucose, nitrite, ketone bodies as well as erythrocytes, leukocytes, bacteria, yeasts, cylinders, epithelial cells, crystals

Material: Urine 5 ml
Method: Dry chemistry, photometry, refractometry

9.2 Protein/Creatinine Ratio

For the diagnosis of nephropathies and loss of protein

Material: Urine 5 ml
Method: Photometry

9.3 γ-GT/Creatinine Ratio

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

Material: Urine 5 ml
Method: Photometry

9.4 Fractional Electrolyte Excretion (FE)

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 1) the glomerular filtration rate and 2) the tubular reabsorption. If the electrolyte excretion is put into relation with the creatinine excretion (here GFR = excretion), it indicates the FE of the electrolyte.
If tubular reabsorption fails, the FE of one or more electrolytes usually increases and its FE values will be above the normal range.

Interpretation: Persistent or repeated FE increases of one or more electrolytes (especially Na and P) indicate tubular malfunction.

Material: Non-haemolysed S + urine – samples collected at the same time
Parameter: FE of Na, K, P and Cl
Method: Photometry

9.5 Bacteriological Culture – Urine

Microbial count and pathogen differentiation if an infection is suspected.
In case of clinical suspicion of a urinary tract infection or if urinalysis and sediment are suspicious, a bacteriological examination is indicated. The urinary culture test includes a microbial count as well as pathogen differentiation. If the results are clinically relevant, an antibiogram will automatically be performed.

Material: Clean-catch midstream urine, catheter urine

10. Coagulation

Coagulation disorders are rare in horses. The following tests are at your disposal for diagnosis:

Prothrombin time (PT, Quick), PTT, thrombin time and fibrinogen

The most frequently used parameters for monitoring of thrombolysis treatment are prothrombin time and determination of fibrinogen. Fibrinogen can also be interpreted as an acute phase protein.

Material: CP 1 ml for each parameter
Method: Chronometric

Cave:
A correct mix ratio of citrate and blood is extremely important – please observe the indicated fill level!

11. Microbiology and Parasitology

11.1 Pre-breeding Examinations

11.1.1 Breeding Soundness

In addition to gynaecological/andrological examinations of all animals that are intended for breeding, the microbiological examination of swabs is used to detect those animals whose genitalia are colonised by pathogenic and facultative pathogenic agents.
As these infections usually progress subclinically, only bacteriological/mycological examination can provide insight into the microorganisms involved, allowing for a specific antibiotic treatment to make the horses fit for breeding.
Please note: If there are any clinical symptoms please choose the bacteriological examination. Otherwise we will only perform an antibiogram if the following bacteria are detected (as these are considered pathogenic, regardless of symptoms):

- Actinobacillus equuli
- Bordetella bronchiseptica
- E. coli var. haemolytica
- Klebsiella
- Pseudomonas aeruginosa

- Raoultella
- Rhodococcus hoagii (formerly: R. equi)
- Serratia marcescens
- Staphylococcus aureus
- ß-haemolytic Streptococci

Clinically healthy mares in which a high level of
- E. coli in pure culture
- yeasts
- moulds

is detected should also be treated – for hygienic reasons.

A "certificate of good health" can only be issued by the veterinarian who examined the animal taking into account the clinical as well as the bacteriological findings.
We offer the test for breeding soundness as a single examination, in combination with CEM testing (detection of Taylorella equigenitalis by culture) or combined with a mycological examination or with a mycological examination and an endometrial biopsy (see Chapter 12.1.1).

Material: Mare: Swab with medium (cervix or uterus)
Stallion: Swab with medium (penile sheath, urethra, glans penis)

If CEM is to be tested, too, a swab with charcoal transport medium is required (e.g. Amies). If CEM should be detected by PCR as well as by bacteriological culture, a separate swab is required for each examination method.

11.1.2 Taylorella equigenitalis/CEM
See Chapter 5.

11.2 Strangles
See Chapter 4.

11.3 Skin Diseases

11.3.1 Parasitological Examination
If mites are suspected to be the causative agent, the depth of the scraping has to be adapted to the living habits of the mites:
Chorioptes bovis (leg and tail mange): superficial skin scraping
Psoroptes equi (head mange): combed out hairs, ear detritus
Demodex caballi (eye lids, mouth)/Demodex equi (whole body): deep skin scraping

11.3.2 Bacteriological Examination

For bacteriological examinations, an additional swab with transport medium is recommended. Especially in cases of abscesses or wound infections, testing for anaerobes should also be done. In purulent conditions, it is important to collect the sample from the area of transition between healthy and infected tissue.

11.3.3 Mycological Examination

For mycological examinations, sampling of hairs from the area of transition between intact and altered skin is recommended in addition to skin scrapings. It is best to send in skin scrapings and hairs in sterile tubes or small paper bags.
In addition to the fungal culture on a special growth medium, a slide for microscopic examination is prepared from each scraping. An antimycogram can be performed on special request.
For the diagnosis of dermatophytosis, a routine **dermatophyte PCR** test is available in addition to fungal culture. This molecular method helps to significantly reduce the time it takes to make a diagnosis (2 – 4 working days), allowing for an early antimycotic therapy. The dermatophyte PCR is approximately as sensitive as fungal culture and it is not susceptible to moulds, but negative results cannot completely rule out an infection – neither in culture nor in PCR.

11.3.4 Dermatophilus congolensis

Dermatophilus congolensis is the causative agent of “mud fever”. The disease develops when pre-injured skin (micro traumata!) is permanently exposed to wet conditions. Symptoms are scurfs, thick scabs and alopecia. Detection is done by microscopic examination of a stained preparation.
Material: scabs

11.4 Faecal Analysis

11.4.1 Microbiological Faecal Analysis

The bacteriological faecal analysis detects facultative pathogenic organisms as well as salmonella and indicates possible dysbacteriosis. It is included in some of our profiles: For horses, the small and the large Faecal Profile are available, and for foals, the Foal Faecal Profile and the PCR profile Diarrhoea Pathogens (foal). These profiles include the detection of coronaviruses, Lawsonia intracellularis and Rhodococcus hoagii (formerly: R. equi).

Material for the profiles: Faeces

11.4.2 Maldigestion/Malabsorption

To find out whether or not food components are sufficiently broken down in the gastrointestinal tract, the particle size can be determined.

11.4.3 Parasitological Faecal Analysis

The parasitological examination of faecal samples from horses includes flotation and enrichment by SAFC (sodium acetate-acetic acid-formalin concentration). The number of detected parasite stages is indicated as “low, medium and high-grade”. In order to counteract the increasing development of resistance of endoparasites against anthelmintics, alternative deworming strategies have been devised. If the concept of selective deworming is used in a horse population, the faecal sample should be examined by using the modified McMaster method. Here, the exact egg count/amount of oocysts per gram of faeces is determined (epg/opg) by counting in a counting chamber. Deworming is only carried out if there are > 200 strongyle eggs per gram of faeces in a horse.
However, in case of tapeworm infestation (Anoplocephala perfoliata) or if nematodes (Parascaris equorum) are detected, deworming should generally be performed, regardless of the degree of infestation.

New: Equine Endoparasite Profile, including flotation, SAFC and modified McMaster. As worm eggs are shed intermittently, it is recommended to send in a 3-day pooled faecal sample in order to increase the probability of detection. If oxyurids are suspected, it may be helpful for diagnosis to send in a tape test of the anal region.

Note:
It is not possible to differentiate between large and small strongyles based on the eggs. Instead, a larval culture would be required.
Tapeworm eggs are only shed intermittently and in very small numbers; the probability of detection can be increased by repeated examinations of several animals of a population.

New: Serum EIA is another possibility to diagnose Anoplocephala. Compared to faecal examination, sensitivity and specificity are better; but please note: antibodies can persist for months.

11.5 Autovaccines

In the context of bacteriologic culture and depending on the clinical issue, there is basically the possibility to produce an autovaccine (so-called farm-specific vaccine according to the German Epizootic Diseases Act). For this, an individually adjusted bacterial lysate is produced which can be applied orally or subcutaneously or by inhalation – depending on the affected organ system and the clinical signs. The immune system is stimulated both specifically and non-specifically.
In horses with "faecal water syndrome", for example, an orally applied autovaccine can induce the production of secretory IgA, thus stabilising the mucosal barrier.
Autovaccines can be ordered within one week after the arrival of the samples, as pathogens from the bacteriological culture are stored for 7 days.
A veterinary **prescription** is required to order an autovaccine; billing the animal owner is therefore not possible. The delivery time to the veterinary pharmacy is approximately 3 weeks.

12. Pathology

The normal processing time is 1 day for cytological exams (= results available on the day of sample receipt (Mo – Fr)) and 2 – 4 days for histological specimens.

Informing about the anamnesis or the presumptive diagnosis is extremely important. This way, our pathologists' comments can be more case-specific and more beneficial for the colleague who ordered the examination.

12.1 Histopathology

12.1.1 Endometrial Biopsies

Endometrial biopsies can be taken from the mare at any stage of the oestrus cycle and sent fixed in formalin.
Endometrial biopsies are used to assess the probability of the mare getting in foal. Success of treatment (endometritis) can be monitored and irreversible degenerative changes and disturbances of differentiation in the endometrium can be identified.
The results of the histological examination (endometritis, endometrosis, angiosclerosis and state of endometrial differentiation) are categorised according to Kenney and Doig (1986). Diagnoses are further interpreted according to Schoon et al. (1992).
An endometrial biopsy can also be requested as a combined service together with breeding soundness examination and mycological examination.

12.1.2 Other Tissue Samples

Tumour samples, organ samples and skin biopsies should be sent fixed in formalin and, preferably, have a diameter of at least 5 mm. From this material, it is possible to do a histological and, where applicable, an immunohistological examination. For additional tests (e.g. microbiology), fresh material is required.

12.2 Cytology

12.2.1 Tracheobronchial Secretion (TBS) & Bronchoalveolar Lavage (BAL)

These tests are performed to assess the current state of pulmonary diseases. This is an important factor regarding prognosis and treatment options because differentiation between acute (especially bacterial) infection, allergic inflammation and chronic disease of the respiratory tract is possible. For evaluation purposes, it is important to know whether the sample is a lavage sample or directly obtained secretion and what kind of pre-treatment has taken place.
For cytological examinations, it is very important to prepare the smears directly after the sample collection. Otherwise, the sample will rapidly cease to be analysable because of autolysis and bacterial overgrowth. In addition to the smear, the remaining fluid and, if desired, a swab for bacteriological examination should be submitted.

12.2.2 Other Cytological Examinations

In horses, mainly synovia, abdominal/thoracic fluids and aspirates of cutaneous neoplasms are used for cytological examination. It is recommended to prepare smears directly and submit them together with the remaining material (in an EDTA tube). Because of the low cell count, aqueous samples should be centrifuged (3 – 5 min, 2500 – 3000 rpm) before preparing a smear, and the smear should be prepared from the sediment. Please note on the submission form whether it is a sediment smear or a fresh. If an additional bacteriological examination is required, it is recommended to send in a swab.

13. Molecular Genetic Analysis

Hereditary Diseases/Coat Colours/Performance/Identity and Parentage
(For molecular genetic pathogen detection by PCR see Chapters 4 and 5.)

13.1 Hereditary Diseases

13.1.1 Androgen Insensitivity Syndrome (AIS)
13.1.2 Cerebellar Abiotrophy (CA)
13.1.3 Congenital Myotonia
13.1.4 Congenital Stationary Night Blindness (CSNB)
13.1.5 Dwarfism
13.1.6 Dwarfism (chondrodysplasia)
13.1.7 Equine Malignant Hyperthermia (EMH)
13.1.8 Foal Immunodeficiency Syndrome (FIS)
13.1.9 Glycogen Branching Enzyme Deficiency (GBED)
13.1.10 Graying*
13.1.11 Hereditary Equine Regional Dermal Asthenia (HERDA)
13.1.12 Hoof Wall Separation Disease (HWSD)
13.1.13 Hydrocephalus
13.1.14 Hyperkalaemic Periodic Paralysis (HYPP)
13.1.15 Idiopathic Hypocalcaemia
13.1.16 Immune-Mediated Myositis & MYH1 Myopathy (MYHM)
13.1.17 Incontinentia Pigmenti (Hyperpigmentation)
13.1.18 Junctional Epidermolysis Bullosa (JEB)
13.1.19 Lavender Foal Syndrome (LFS)
13.1.20 Naked Foal Syndrome (NFS)
13.1.21 Ocular Squamous Cell Carcinoma (SCC)
13.1.22 Overo Lethal White Syndrome (OLWS)
13.1.23 Polysaccharid Storage Myopathy Type 1 (PSSM)
13.1.24 Severe Combined Immunodeficiency (SCID)
13.1.25 Warmblood Fragile Foal Syndrome (WFFS)

13.2 Coat Colours and Coat Structure

13.2.1 Agouti (black/bay)
13.2.2 Appaloosa Pattern 1
13.2.3 Brindle 1
13.2.4 Camarillo White – W4*
13.2.5 Champagne
13.2.6 Chestnut
13.2.7 Cream
13.2.8 Curly
13.2.9 Dun

* = Partner laboratory

13.1 Hereditary Diseases

Variances or anomalies which have a genetic basis can already occur at birth or manifest themselves later in life.
Most congenital defects are actually not hereditary.
As research into the equine genome progresses, molecular biological studies could detect the genetic fixation of some of these diseases, though.

Below, we will describe breed dependencies, clinical picture, inheritance and diagnosis of the defects which are proven to be genetically fixed.

To perform a genetic test, an **EDTA whole blood sample** (approx. 0.5 ml) is required. In horses, it is also possible to send in **hairs with roots** (approx. 20 hairs, plucked from mane or tail), however, the amount of DNA which can be extracted from hairs is limited. The **results** of genetic tests are available approx. 3 – 5 working days after the arrival of the sample at the laboratory (exception: tests that are performed by one of our partner laboratories).

DNA extracted for genetic tests is **stored** at LABOKLIN for 10 years. This DNA can then be used for future genetic tests or paternity tests. In most cases, it is therefore not necessary to send in a new sample.

13.1.1 Androgen Insensitivity Syndrome (AIS)

In mammals, gender is determined by the X and Y chromosomes. They are responsible for numerous factors that determine the proper development of the sexual phenotype. The role of androgens is crucial for normal male sex differentiation. The intracellular androgenic effect is mediated by the androgen receptor (AR). If its function is impaired, the cells in the body are not able to respond to the androgen. This leads to a variety of syndromes with severe clinical consequences, most notably androgen insensitivity syndrome (AIS): XY (genetically male) horses have a female phenotype (female external genitalia) and internal testis. These horses often behave like stallions, but are not capable of reproduction.
Affected breeds are Quarter Horses and related breeds.

13.1.2 Cerebellar Abiotrophy (CA)

CA is a neurological disease inherited in an autosomal recessive mode which almost exclusively affects Arabian Horses and their crossbreeds. Affected foals are usually born with no symptoms; already in the first weeks of life, the disease causes the death of neurons in the cerebellum. This leads to neurological deficits such as head shaking, ataxia and other deficiencies. The first signs usually appear at the age of 6 weeks (up to 4 months). The neurological problems may not be apparent to owners and are frequently thought to be a consequence of a fall/an accident rather than CA.
The severity of the symptoms of CA is variable. Some foals show very severe signs, including, most notably, exaggerated gait and lack of balance. Others only show mild clinical signs, but in almost all cases it is impossible to ride these horses later.

13.1.3 Congenital Myotonia

Congenital myotonia is a disease of the skeletal muscles in New Forest Ponies. It is caused by a mutation in the CLCN1 gene, which is responsible for the function of chloride channels in the muscles.
The first signs of the disease already appear at a few weeks of age. Foals have a stiff-legged gait, are recumbent and have considerable difficulty getting back on their feet after a long period of lying. In most cases, it is impossible to lift one single foot, the foals lose their balance very quickly. The eyeballs may also be retracted deeply into the eye sockets due to myotonia.

13.1.4 Congenital Stationary Night Blindness (CSNB)

Homozygous carriers of the leopard gene LP/LP are almost always affected by congenital stationary night blindness (CSNB), heterozygous carriers LP/lp do not fall ill, but already show the dominantly inherited coat colour caused by the leopard gene (from various white spots and patterns up to full leopard). Generally, CSNB may affect all breeds where there are horses with corresponding coat colour. In CSNB, night vision is impaired from the time of birth on. For further information, see Leopard Complex (Chapter 13.2.13).

13.1.5 Dwarfism

Dwarfism in Friesian horses is characterised by growth retardation of the ribs and limbs, while the head and back appear normal. A striking feature of the condition is hyperextension of the fetlock joints. The flexor tendon does not contract as usual when the foals grow older, but tends to stretch further. As a consequence, these dwarf Friesians develop an abnormal gait in which the limbs undergo extreme rotation at the carpus and hocks. The head in adult dwarf Friesians is the same size as in healthy animals, the ribcage is wider than normal with a thickening of the costochondral junction (Th10-16). The back appears disproportionally long, but the legs are greatly shortened. The abdomen is usually rounded, the muscles of the whole body are only poorly developed. Dwarfism in Friesian horses is caused by a mutation in the B4GALT7 gene, which has an autosomal recessive mode of inheritance.

13.1.6 Dwarfism (Chondrodysplasia)

Dwarfism is most common in Shetland ponies and miniature horses. Phenotypical features of this hereditary disease are breathing problems due to a cleft palate, deformed mouths, shortened limbs and bowed forelegs, disproportionately large cranium and shortened neck, protruding eyes, abdominal hernia and a shortened rib cage. As a result, affected animals are often not viable or have to be euthanized due to the poor quality of life. A mutation in the ACAN gene is responsible for this form of dwarfism. Four different mutations, which cause the autosomal-recessive disease, are known so far. These are named D1, D2, D3*, and D4 and are also pathogenic as compound heterozygous genes. Compound heterozygous variants together with D1 (except N/D1) are more deleterious and often lead to death. A combination with the D2 variant is considered as the mildest form of dwarfism.

13.1.7 Equine Malignant Hyperthermia (EMH)

Equine malignant hyperthermia (EMH) is an inherited disorder of skeletal muscle characterised by rhabdomyolysis, generalised spasms of the skeletal muscles, cardiac arrhythmia and renal dysfunction. The clinical signs appear after exposure to muscle relaxants, halothane anaesthesia or stress. EMH appears to increase the symptoms of PSSM. It is inherited in an autosomal dominant manner and especially affects Quarter Horses and related breeds.

13.1.8 Foal Immunodeficiency Syndrome (FIS)

Foal immunodeficiency syndrome (FIS) is a hereditary disease that, so far, has only been detected in Fell Ponies and Dales Ponies. Foals with FIS appear to be normal at birth but as they lack immunity, they develop a number of diseases, especially pneumonia and diarrhoea at a few weeks of age. The foals also suffer from severe progressive anaemia and usually die before 3 months of age.

13.1.9 Glycogen Branching Enzyme Deficiency (GBED)

Until recently, GBED had not been recognised as a disease, mainly because the clinical signs are very similar to other diseases that typically affect foals. Furthermore, routine staining of muscle tissue post mortem was not suitable to detect GBED.
Since molecular biology was able to detect a genetic defect and provide a genetic test, epidemiological studies have revealed that about 10% of the horses belonging to Quarter Horse, Paints and related bloodlines carry the mutation. It is assumed that GBED is responsible for at least 3% of the abortions in Quarter Horses.
GBED is inherited in an autosomal recessive manner. Affected foals lack the enzyme GBE that is necessary for synthesis and storage of glycogen. Predominantly affected tissues are skeletal muscles, brain and heart.
Clinical signs associated with GBED are:

- abortion, stillbirth or birth of weak foals
- sudden cardiac death – especially on the pasture – or death caused by seizures
- tachypnoea due to weakened respiratory muscles
- generalised weakness, especially when getting up

All cases known so far were euthanised or died at the latest by the age of 18 weeks.

13.1.10 Graying*

Horses with this mutation are born coloured but gradually lose their hair pigmentation (not skin pigmentation) until they are completely grey or white at an age of about 6 – 8 years.

A duplication in the gene STX17 causes the greying phenotype, the mode of inheritance is autosomal dominant. This means, even horses carrying only one copy of the mutation (heterozygous horses G/g) will turn grey/white. In most cases, these horses never become completely white, but often stay dapple grey or flea-bitten grey throughout their lives. Horses that are homozygous for the mutation (G/G) will turn completely white at an early age.
Horses with the greying mutation show a high incidence of dermal melanomas (70 – 80% of greys over 15 years have one or more melanomas). The incidence is statistically higher for horses that are homozygous carriers of the duplication compared to heterozygous horses. It also depends on the base colour of the horse, which is determined at the agouti locus. The risk of developing melanoma is significantly higher in greys that were born black than in white horses that were born bay.

Note on ordering: If this genetic test does not appear in your online order system, please use the printed submission form Genetics Horse which you can either request from LABOKLIN or download as PDF document from the LABOKLIN website.

13.1.11 Hereditary Equine Regional Dermal Asthenia (HERDA)

Hereditary equine regional dermal asthenia (HERDA) is a degenerative skin disease affecting predominantly American Quarter Horses. Within the population, the number of carriers of this autosomal recessively inherited disease is about 1.8 – 6.5%.
Normally, foals are born without any signs of the disease. Skin areas that later develop lesions are focal and irregularly distributed all over the body with a predilection for the area on the back. Consequently, the disease is often only discovered when the horses are saddle broken – at about 2 years of age.
The skin of affected horses is hyper-extensible, scarred, and often shows severe lesions.
Histological examinations of skin biopsies are not suitable for diagnosis of HERDA and can only provide hints.

13.1.12 Hoof Wall Separation Disease (HWSD)

Hoof wall separation disease (HWSD) in the Connemara Pony is characterised by a very unstable hoof wall which can crack and break without any particular strain. The coronary band appears normal. Symptoms already appear in the first weeks of life and can be of varying severity. However, these horses are usually not suited for riding later.

13.1.13 Hydrocephalus

Hydrocephalus in Friesian horses is a developmental disorder that can lead to a progressive enlargement of the head. It has been associated with dystocia in dams and stillbirths of the affected foals. It can eventually lead to fatal complications for the dam at parturition. Affected foals that are still alive may be euthanised at birth to facilitate parturition.
Hydrocephalus in Friesian horses is caused by a mutation in the B3GALNT2 gene, which is inherited in an autosomal recessive manner.

13.1.14 Hyperkalaemic Periodic Paralysis (HYPP)

Hyperkalaemic periodic paralysis (HYPP) has been reported in Quarter Horses (QH), Paints, Appaloosas and other bloodlines descending from the QH stallion “Impressive”. Most of the affected horses are very well-muscled and may be very successful sport or show horses between episodes of illness.
The main clinical symptom is general weakness; muscle spasms and fasciculations may occur. The manifestation of the clinical symptoms ranges from subclinical to severe. Life-threatening complications are cardiac arrhythmia and risk of suffocation caused by laryngospasm. When clinical symptoms occur, hyperkalaemia can be found by laboratory diagnostics; muscle values are usually within the reference range or slightly above.
The first episodes of clinical illness are usually observed at the age of 3 – 7 years. HYPP is caused by a mutation of a base in the gene that encodes for the sodium channels of skeletal muscles. It is inherited as an autosomal dominant trait. Homozygous carriers of the defect gene become more severely ill than heterozygous animals.
In contrast to sporadic exertional rhabdomyolysis, which is always associated with movement in horses, HYPP usually does not occur in connection with exercise, but during periods of rest, at feeding time or in stressful situations (transport, change of food, fasting); periods of standing and a diet rich in K can also trigger clinical signs.

13.1.15 Idiopathic Hypocalcaemia

A lethal idiopathic hypocalcaemia was described in 1997 for thoroughbred foals. The affected foals suffer from muscle spasms and seizures within the first weeks of life due to calcium deficiency in the blood. Other manifestations can be a stiff gait and increased sweating. The foals died within a few weeks or were euthanized due to the poor prognosis.
In addition to calcium deficiency, the blood count also shows a magnesium deficiency and an increased phosphate level. The parathormone (PTH), which is produced by the parathyroid, increases normally with calcium deficiency. However, no increased PTH concentration was found in the affected foals.

In 2020, the genetic cause underlying calcium deficiency could be described. A gene variant in the RAPGEF5 gene is associated with a subfunction of the parathyroid gland (hypoparathyroidism). This subfunction in turn causes a reduced PTH production, which causes the calcium deficiency.
This is a recessive hereditary disease, i.e. only animals carrying two copies of the disease-causing gene variant show symptoms.
The gene variant has so far only been described in English thoroughbreds. However, since thoroughbreds are used in the breeding of other breeds, further distribution cannot be excluded.

13.1.16 Immune-Mediated Myositis & MYH1 Myopathy (IMM/MYHM)

Appaloosas, Paint Horses and Quarter Horses are susceptible to an autoimmune muscle disease called immune-mediated myositis (IMM) which can cause severe atrophy, resulting in the loss of up to 40% of muscle mass within 72 hours. IMM is characterised by the infiltration of inflammatory cells, especially lymphocytes, into muscle fibres und surrounding blood vessels, which manifests itself in stiffness, weakness and non-specific malaise. Affected horses are usually 8 years and younger or 17 years and older. Environmental factors combined with genetic susceptibility are important triggers for the development of muscle atrophy or severe rhabdomyolysis. About 39% of the horses with IMM have already been suffering from a triggering factor for some time such as an infection with Streptococcus equi subsp. equi or EHV4.
A variant in the MYH1 gene which inhibits the function of the myosin protein in muscle cells, is associated with increased susceptibility to develop IMM. Another clinical presentation of the MYH1 variant in young Quarter Horses is severe, sudden muscle damage which occurs without any physical exercise and is not necessarily associated with muscle atrophy (non-exertional rhabdomyolysis). IMM and non-exertional rhabdomyolysis belong to the group of muscle diseases known as MYH1 myopathy (MYHM).
The mode of inheritance for MYHM is autosomal dominant with incomplete penetrance, which means that not all horses that have one (genotype N/My) or two alleles (genotype My/My) of the genetic variant will develop IMM or non-exertional rhabdomyolysis. Horses with two copies (My/My) may be more severely affected.

13.1.17 Incontinentia Pigmenti (Hyperpigmentation)

Incontinentia pigmenti (IP) is an ectodermal dysplasia in Quarter Horses and related breeds. It is characterised by skin lesions evolving over time, as well as dental, nail and ocular abnormalities.
Soon after birth, pruritic, exudative lesions occur in affected horses, sometimes developing into verrucous lesions. There may be areas of alopecia with occasional

woolly hair re-growth. Affected horses also show streaks of darker and lighter coat colour from birth, similar to brindle coat colour. Due to the X-linked dominant inheritance, IP symptoms can only be seen in mares while affected male embroys die in utero.

13.1.18 Junctional Epidermolysis Bullosa (JEB)

Junctional epidermolysis bullosa (JEB) is an inherited disease of the skin which mainly affects Belgian Draft Horses but can also be found in American Saddlebred Horses. The mode of inheritance is autosomal recessive and causes blistering and detaching of the skin. Affected foals are already born with lesions or develop them after only 2 days at the latest. Primary skin lesions are vesicles that rupture easily and become sharply contoured ulcers with exudation and encrustation. Predilection sites are the coronary band, where changes up to sloughing may occur, mucocutaneous junctions of the lips, anus, vulva, eyelids and nostrils; in addition, all skin areas covering prominent bones are affected (fetlock, carpus, hips, etc.). Another sign is dental dysplasia. The foals eventually have to be euthanised due to infections.
So far, LABOKLIN can only test Belgian Draft Horses; in American Saddlebred Horses, the mutation is found at another gene locus.

13.1.19 Lavender Foal Syndrome (LFS)

Lavender foal syndrome (LFS) is an autosomal recessive defect which occurs in a subgroup of the Arabian thoroughbred, the Egyptian Arabian. Affected foals show a range of neurological signs, including convulsive 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.
The name “Lavender Foal Syndrome” originates from the fact that the causative gene for LFS is linked to another gene that is responsible for the dilution factor “lavender”. That is why affected foals often show the characteristic “lavender” coat colour.

Note: Not every foal born with a “lavender”-coloured coat is necessarily a (homozygous) carrier of the mutation.

13.1.20 Naked Foal Syndrome (NFS)

NFS is a genodermatosis in the Akhal-Teke horse breed. Affected horses are born with almost no hair. They show a mild form of ichthyosis and mostly die within days to weeks after birth. So far, the reason for the early death is not known; only few hairless foals have survived up to 2.5 years. The first records of hairless Akhal-Teke foals date back to 1938, and since then, the number of such foals has been steadily increasing. Many horses with NFS were probably registered as stillborn or not registered at all.

13.1.21 Ocular Squamous Cell Carcinoma (SCC)

Squamous cell carcinoma (SCC) is the second most common type of tumour in the horse and the most frequent tumour in the horse‘s eye. Factors thought to increase risk of SCC include UV exposure, pigmentation and genetic factors. As a risk factor for the development of SCC, a variant in the DDB2 gene in Haflingers, Ardennes Horses and Belgian Drafts was identified.
When originating at the limbus, SCC can spread into the cornea and quickly lead to visual impairment and destruction of the eye.
Horses homozygous (R/R) for the risk factor are 5.6 times (Haflinger) or 4.0 times (Belgians) more likely to develop ocular SCC than those with one copy (R/N) or no copies (N/N) of the risk factor. This risk factor does not explain all cases of ocular SCC but it appears to be a major contributor in Haflingers and Belgians.
Homozygous horses (R/R) are advised to have routine eye exams for early detection and better prognosis, and to wear a UV protection fly mask when out during the daylight hours.

13.1.22 Overo Lethal White Syndrome (OLWS)

OLWS is a lethal autosomal recessive defect which mainly occurs when mating frame overo Paints. However, also miniature horses, Arabian crossbreeds, thoroughbreds, Quarter Horses, Mustangs as well as Tobiano Paint Horses without the typical coat pattern can be carriers of the mutation of the endothelin-B receptor gene.
Affected foals are born completely white and show intestinal aganglionosis. The foals develop severe colic due to the resulting functional ileus and normally die within 24 to 48 hours.
Note: Not every foal of the respective breeds which is born white is necessarily a (homozygous) carrier of the mutation.

13.1.23 Polysaccharid Storage Myopathy (PSSM)

Polysaccharide storage myopathy (PSSM) is a glycogen storage disease which weakens the affected horses and may even be life-threatening. Affected breeds are mainly Quarter Horses, American Paints, Appaloosas but also draught horses, warmbloods, ponies and crossbreeds of all breeds mentioned above.
PSSM is characterised by the accumulation of abnormal polysaccharides and the excessive accumulation of the normal form of sugar in muscle tissue.
The clinical signs are similar to those of sporadic exertional rhabdomyolysis 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. The muscles of the mainly affected hind legs are often hard or even painful.

Many horses with PSSM have a history of numerous episodes of muscle problems. If the clinical picture is pronounced, myoglobinuria and, possibly, resulting renal impairment may occur. Generally, PSSM is held responsible for the majority of neuromuscular diseases in the affected breeds.
PSSM is inherited in an autosomal dominant mode, which means that already one copy of the affected allele can cause the disease. Horses that are homozygous for the mutation, i.e. that have 2 affected alleles, are often more severely affected.
Laboklin owns the exclusive license to perform this genetic test.

13.1.24 Severe Combined Immunodeficiency (SCID)

Severe combined immunodeficiency (SCID) is the oldest known hereditary disease in horses. Affected breeds are Arabian Horses and their crossbreeds. It is a primary, lethal immunodeficiency characterised by the inability of the horse to produce B and T lymphocytes. Gamma interferon and IgM are also deficient. The inheritance is autosomal recessive, the incidence is 2 – 3% with a carrier frequency of about 25%. Affected foals are extremely susceptible to infections. Depending on the supply of maternal antibodies, sooner or later (at the latest at 2 months of age), the foals will suffer from opportunistic pathogens, typically also from adenovirus infections. The respiratory and the gastrointestinal tract are particularly affected. In addition, high-grade persistent lymphopenia occurs. Most of the affected foals die before they are 5 months old.

13.1.25 Warmblood Fragile Foal Syndrome (WFFS)

Warmblood fragile foal syndrome (WFFS) is an inherited connective tissue disorder that becomes apparent immediately after the warmblood foal is born. Symptoms are comparable to Ehlers-Danlos-Syndrome in humans. The skin is extremely fragile and tears at even the slightest touch. In addition to numerous injuries all over the body and enlargements of the joints (joint effusion), gingiva and mucous membranes can also be affected. Limb joints are hyperextensible, most clearly visible at the fetlock joints. That is why affected foals are mostly unable to stand properly. As the prognosis is very poor, foals with WFFS are euthanised shortly after birth.
Not all foals are born after a normal period of gestation, premature births and abortions due to WFFS are also known. The disease is inherited in an autosomal recessive manner.
Laboklin owns the exclusive license to perform this genetic test.

13.2 Coat Colour and Coat Structure

Horses have 2 main types of skin pigments that are responsible for the coat colour. One base colour is dark (black or brown/bay) and the other one is red. The many colours and shades in between are the result of genes that regulate the amount, strength and

distribution of the 2 main pigment types.
For information on sample material, test duration and DNA storage see Chapter 13.1.

13.2.1 Agouti (black/bay)

The distribution of the black pigment is controlled by the Agouti (A) locus. In chestnuts, this gene does not play a role in determining the coat colour. A black or brown/bay coat colour can only develop if a horse is either a non-carrier (E/E) or only a carrier (E/e) of the chestnut coat colour. The Agouti gene determines whether the horse develops a black or a brown/bay coat. If the recessive allele is homozygous (a/a), the horse will be black. Otherwise (carrier: A/a or non-carrier (A/A), the coat is brown/bay and black appears only in certain areas like mane or tail.
For breeding purposes, the Agouti gene is particularly important as a “hidden” feature in chestnut horses.

Allele Combination	Agouti A/A	Agouti A/a	Agouti a/a
Extension E/E	brown/bay	brown/bay	black
Extension E/e	brown/bay	brown/bay	black
Extension e/e	chestnut	chestnut	chestnut

13.2.2 Appaloosa Pattern 1

While the gene for the Leopard complex (LP) is responsible for various white patterns, the PATN1 gene determines the extent and distribution of white parts. A mutation in the PATN1 gene is associated with an increased amount of white in in LP horses. In horses that are heterozygous for LP (LP/lp), the presence of the PATN1 mutation usually produces a leopard pattern. In horses that are homozygous for LP (LP/LP), the PATN1 mutation most often results in a few spot pattern. The PATN1 mutation is often present in breeds with LP, including Appaloosa, British Spotted Pony, American Miniature Horse and Knabstrupper. It has been found in other breeds, too, but has no effect on the phenotype without the LP gene.

13.2.3 Brindle 1

The phenotype “brindle” shows irregular vertical stripes in the coat along the neck, back and hind legs. This specific form of brindle is called “brindle 1 (BR1)”. In some BR1 horses, the stripes are differently pigmented. Most horses show a sparse mane and tail. BR1 has an X-chromosomal semi-dominant mode of inheritance. The typical BR1 phenotype with a brindle coat pattern can only be seen in heterozygous females (genotype X(N)/X(BR1)), whereas homozygous females (genotype X(BR1)/X(BR1)) and hemizygous males (genotype X(BR1)/Y) show a sparse mane and tail but no stripes.

13.2.4 Camarillo White W4*

The W4 mutation in the KIT gene is a mutation which is traced back to the stallion "Sultan". He was born in 1912 and Adolfo Camarillo bred him to Morgan Horse mares. These very successful white horses were bred by the Camarillo family until 1987 when they were sold at an auction all over the world.
The genetic test enables breeders who would like to breed this colour to find out whether or not a horse that is a descendant of Sultan and only has few white markings itself, can pass on the mutation.
The genotype W4/W4 has not yet been observed. It is therefore likely that homozygous horses die in utero.
Note on ordering: If this genetic test does not appear in your online order system, please use the printed submission form Genetics Horse which you can either request from LABOKLIN or download as PDF document from the LABOKLIN website.

13.2.5 Champagne

Similar to the cream gene, the champagne gene causes a dilution of the base colour. It is inherited in a dominant manner and the difference in phenotype between homozygous (CH/CH) and heterozygous (CH/ch) carriers can hardly be distinguished. The base colour chestnut is diluted to gold champagne, bay becomes amber champagne and black becomes classic champagne.
Champagne horses are born with pink skin that develops freckles during the first days of life. In most cases, the eyes are blue but darken as the horse gets older.

13.2.6 Chestnut

The Extension (E) locus determines the red factor (chestnut). The dominant allele (E) leads to the formation of eumelanin and thus to the colour bay or black. The disposition for chestnut coat colour (e) is inherited in a recessive manner, i.e. only if the mutation in the McR1 gene is homozygous (e/e), phaeomelanin will be produced and the horse will be chestnut. A horse that is homozygous (E/E) or heterozygous at the E-locus (E/e) will be either black or bay, depending on the Agouti locus.

13.2.7 Cream

Another gene, MATP, determines the different variations of the coat colour cream. Depending on the base colour, which is determined by the genes of the E- and the A-locus, this gene can create the following colour shades:
Non-carriers of the gene (cr/cr) have the base colour chestnut, bay or black.
Chestnut carriers (e/e; CR/cr) are diluted to palomino (isabelline), chestnut homozygous carriers (e/e; CR/CR) become cremello.

Bay carriers (E/e or E/E; A/A or A/a; CR/cr) become buckskin, bay homozygous carriers (E/e or E/E; A/A or A/a; CR/CR) are diluted to perlino.
Black carriers (E/e or E/E; a/a; CR/cr) become smoky black, black homozygous carriers (E/e or E/E; a/a; CR/CR) become smoky cream.
Our test specifically detects a mutation in the MATP gene, which has been shown to be responsible for the described cream colours. Other genes or mutations causing similar coat colours are not identified by this test.

13.2.8 Curly

Curly coat is a special trait in horses and leads to a curly coat structure. It occurs mainly in the American Bashkir Curly Horse, but it can be found in various other horse breeds, too. Nowadays, Curly horses are very popular because their coat structure causes milder or no symptoms at all in many horse-allergic people.
The curly coat structure sometimes appears in combination with hypotrichiosis. Genetic variants in two different genes, KRT25 and SP6, are responsible for the development of the curly coat and hypotrichiosis. Horses that are heterozygous or homozygous for only the KRT25 variant, show curly coat and hypotrichiosis, while horses with only the SP6 variant have curly hair without hypotrichiosis. Horses with mutated alleles in both variants develop curly hair and hypotrichiosis. All horses with the KRT25 variant are hypotrichiotic because of the epistatic effect that KRT25 has on SP6. In our genetic test, both variants are examined separately.

13.2.9 Dun

Dun is a dominant colour dilution gene, which on the one hand changes the base colour of the coat and on the other hand causes so-called “primitive markings”. These include the dorsal stripe, zebra stripes on the legs (leg barring) or head (cobwebbing) and shoulder stripes. The dorsal stripe can be seen on all dun horses for life while the other primitive markings may appear additionally.
The effect of the dun gene on the base colours chestnut, bay and black produces a number of colour shades that range from golden to dark grey to olive. Dun is inherited independently of other coat colour genes and can occur in combination with other genes that affect the base colour.
There are 3 alleles that influence the appearance of the dun dilution and the primitive markings: D (dun dilution and primitive markings), nd1 (no dilution, primitive markings may occur in various forms, e.g. the pseudo-dorsal stripe) and nd2 (no dilution, no primitive markings). D is dominant over nd1 and nd2; nd1 is dominant over nd2.

13.2.10 GQ Santana Dominant White W10*

All horses with this colour can be traced back to the stallion “GQ Santana”, who was born in the USA in 2000. He was a famous stallion in both AQHA and APHA. W10 is inherited as a dominant trait, i.e. all horses that carry this mutation will display white markings to some degree. Horses with the W10 mutation in the KIT gene have pink skin in those areas where the hair is white. The eyes have normal pigmentation and can rarely be blue.

The genetic test enables breeders who would like to breed this colour to find out whether or not a horse that is a descendant of GQ Santana and only has few white markings itself, can pass on the mutation. So far, the genotype W10/W10 has only been detected in aborted foetuses if two carrier animals (N/W10) were mated. It is therefore likely that homozygous horses are non-viable.

Note on ordering: If this genetic test does not appear in your online order system, please use the printed submission form Genetics Horse which you can either request from LABOKLIN or download as PDF document from the LABOKLIN website.

13.2.11 Graying*

Grey horses are carriers of the greying mutation. As the formation of melanoma is directly linked to the greying mutation, it is described together with hereditary diseases (see Chapter 13.1.9).

13.2.12 Incontinentia Pigmenti (Hyperpigmentation)

Incontinentia pigmenti leads to dermal lesions, it is therefore described together with hereditary diseases as well (see Chapter 13.1.15).

13.2.13 Leopard Complex

A single gene inherited in a dominant manner, called leopard complex (LP), is responsible for the development of various white spots and patterns up to “leopard”, e.g. in Appaloosa Horses. Depending on the breeding association, the patterns are approved as “few spot leopard”, “leopard”, “snowcap blanket”, “blanket with spots”, “varnish roan (marble)”, “snowflake”, “frosted”, “speckled” or “mottled”.
Homozygous carriers of the leopard gene (LP/LP) are almost always affected by congenital stationary night blindness (CSNB), whereas heterozygous carriers (LP/lp) are not affected. CSNB is characterised by impaired vision in the dark and is present from birth.
Horses with both the tobiano and the leopard gene are called pintaloosas.

13.2.14 Mushroom

Mushroom is a dilute coat colour in Shetland Ponies that is characterised by a distinctive sepia-toned body hair colour, often accompanied by a flaxen mane and tail. The genetic variant is inherited in an autosomal recessive mode.
Mushroom dilutes red pigment (phaeomelanin), so it is mostly seen in chestnut coat colour. The mushroom phenotype shows a wide variation of shades and has similarities to cream and silver dilutions, although it is genetically distinct from both.
If the basic colour is bay the mushroom variant leads to a sepia coloured body hair. It has no influence on the black mane and tail, however, because the colour here is caused by eumelanin.
The phenotype of black horses is not modified by the mushroom variant, even if the horse is homozygous.

13.2.15 Pearl

In addition to the four most frequent, dominant dilution genes cream, champagne, silver and dun, there is another gene which causes a dilution of the base colour. In Quarter Horses and Paints, this gene was originally called “Barlink factor”, but in Spanish horse breeds, such as Andalusians and Lusitanos, it was called ”pearl”. It is exactly the same mutation, though, and because of the Spanish ancestors of Quarter and Paint Horses, the gene was eventually called “pearl”.
In contrast to the other dilution genes, pearl is inherited in an autosomal recessive manner, i.e. only if the gene is homozygous, the base colour of the horse as well as the mane and tail are diluted evenly. A chestnut becomes apricot, a black horse becomes evenly light grey.
Heterozygous presence of the gene does not change the base colour of the horse. Combined with the heterozygous presence of the cream gene (CR/cr and N/Prl), a phenotype is caused which corresponds to homozygous cream (CR/CR). From their appearance, these horses cannot be distinguished from true cremello, perlino or smoky cream.

13.2.16 Roan Zygosity*

The roan gene causes a mixture of coloured and white hairs on the body which leads to a “dilution” of the original coat colour. However, there are no “diluted” hairs, but the interspersed white hairs in the coat cause this phenotype. The head, legs, mane and tail remain unaffected by the roan gene and always show the base colour.
Foals are already born with the pattern, though it may not be visible in the foal’s coat and only becomes apparent when the coat is changed for the first time.

The white hairs are evenly distributed over the body if the true roan gene is present, not to be confused with the different patterns of white hair called “roaning”. Roaning patterns have an uneven distribution of white hairs and the genes responsible for their inheritance have not yet been identified.
Roan is inherited as a dominant gene and can be found in many different horse breeds, e.g. Quarter Horses, Paints, Paso Finos, Paso Peruanos, Welsh Ponies and Belgian Horses, but not in Arab Horses and thoroughbreds.
Although it has been assumed that roan is homozygous lethal, there are reports about Quarter Horses who have passed on 100% of the gene to their offspring. In these horses, genetic tests have confirmed that the genomic region which contains the roan gene is homozygous.
DNA testing is used to detect mutations which are associated with the roan pattern in Quarter Horses and Paint Horses. The causative mutation has not yet been identified, so the existing test is a marker test.

Note on ordering: If this genetic test does not appear in your online order system, please use the printed submission form Genetics Horse which you can either request from LABOKLIN or download as PDF document from the LABOKLIN website.

13.2.17 Sabino 1

Horses with Sabino 1 show a spotting pattern characterised by white patches of varying sizes and irregular forms and borders. The spots mainly occur on the face, legs and belly. Sometimes, roaning on the belly or all over the body can occur. The spotting pattern can be seen more or less clearly in horses that are heterozygous for the sabino trait, homozygous horses are usually almost completely white from birth. So far, one causative gene could be identified (Sabino 1) but it is likely that there are other genes that are responsible for similar markings.

13.2.18 Silver Dapple

The silver gene/silver dapple gene is another gene causing a dilution of the base colour. Unlike cream and champagne, it has no influence on phaeomelanin, only the black pigmented areas are diluted. The effect is mainly visible on the mane and tail which are often interspersed with white and grey hairs when the silver gene is present. The gene is inherited in an autosomal dominant manner, i.e. one copy of the gene is sufficient to cause this phenotype.

13.2.19 Snowdrop

Different gene variants in the SLC45A2 gene are responsible for various coat colour dilutions such as Cream, Pearl or Sunshine. In 2020, a gene variant that caused the brightening of the coat colour could be detected in a Tinker with brightened cota that did not bear any of the known causal gene variants for Cream, Pearl or Sunshine. This fur colour dilution is called snowdrop and dilutes both red and black pigment, consequently the basic colours chestnut, bay and black are brightened by this gene variant.
The manner of inheritance is autosomal recessive, i.e. only if both alleles of the animal have the variant for snowdrop, the fur colour dilution will occur. To date, the presence of the gene variant in other breeds has not been scientifically described, but cannot be excluded.

13.2.20 Splashed White

Splashed white is an irregular spotting pattern primarily characterised by an extremely wide blaze, or bald face, often with blue eyes and bright white legs. Some, but not all, splashed white horses are deaf. So far, four mutations have been identified – SW-1, SW-2, SW-3 and SW-4 – that cause splashed white markings. SW-1 has already been detected in several breeds, e.g. Quarter Horse, Paint Horse, Trakhener, Miniature Horse, Shetland Pony and Iceland Horse. Horses homozygous for SW-1 have been identified, i.e. this mutation is not homozygous lethal. SW-2 and the (very rare) SW-3 mutation occur only in certain lines of Quarter Horses and Paints. Both mutations seem to be homozygous lethal, thus, mating of two horses that carry SW-2 or SW-3 should be avoided. The SW-4 mutation, which is also very rare, has been detected in Appaloosa Horses and causes splashed white or a wide blaze.
Horses that carry two or more of the splashed white, tobiano or lethal white overo mutations usually have very extensive white patterns or are even completely white.

13.2.21 Sunshine

In addition to the already known colour dilution genes such as Cream and Pearl, a further variant for dilution of the coat colour has been discovered. Sunshine is inherited in an autosomal-recessive manner. This means that only if the gene is homozygous (Sun/Sun) the basic colour of the horse is diluted. It is assumed that this dilution is similar to Pearl. As well as Pearl, Sunshine can interact with Cream to produce pseudo-double Cream dilute phenotypes.

13.2.22 Tobiano

Tobiano is one of the most common pinto patterns in domestic horses. The patches of these horses have smooth edges and distinct borders, the eye colour is usually dark. Tobianos usually have white feet or even more pronounced leg markings, the head markings are comparable to horses without tobiano. The white patches typically cross the dorsal midline in at least one area, but the size of the patch can vary considerably The tobiano pattern is inherited as a dominant trait. Therefore, horses with the heterozygous genotype N/Tob as well as the homozygous genotype Tob/Tob develop the characteristic colour pattern.

13.3 Performance

13.3.1 Predictive Height Test

The base exchange of a specific gene locus regulates the expression of the LCORL gene, which apparently influences the growth of the horse. This gene variation affects the withers of warmblood horses. Other factors such as feeding, keeping and rearing of the horse, especially the offspring, but also the mother mare, are essential for the development, though, and therefore influence the size of the horse as well. Horses with the „small“ genotype (T/T) that we tested had an average height of 164 cm. Horses with the genotypes C/T and C/C were 4 – 8 cm taller on average. The test does not guarantee an exact stick measure of the horse, but if the genotype is known, the size can be narrowed down. Breeders are thus able to increase the chance of the desired phenotype, if the genotype of both parents is known.

13.3.2 Genetic Test for the Detection of various Myostatin Variants (Speed Gene)*

Myostatin and its associated gene MSTN have been detected in humans as well as in different animal species (e.g. cattle, dog, mouse and horse).

Different variants of the myostatin gene are responsible for the development of varying muscle types: “Sprinters”, for example, have a very high proportion of muscle mass in relation to their total body weight and are therefore perfectly suited for fast, short races. In contrast, horses which are better suited for long distances are usually lighter, the ratio of muscle mass to body weight is smaller (“long-distance runners”).
Horses which are heterozygous for both myostatin variants tend to be most successful over middle distances.
The genetic test for the detection of the different myostatin variants provides information about which racing distance is ideal for the tested horse. However, the test provides no guidance on the actual suitability of the horse as a race horse.

Myostatin – Mutation and Inheritance

The mutation which is responsible for the 2 myostatin variants can be detected by a DNA test.

There are three genotypes:
Genotype C/C (homozygous): The tested horse is homozygous for the C allele of the myostatin gene and could be best suited for short-distance races. Mostly, these horses are very precocious.
Genotype C/T (heterozygous): The tested horse is heterozygous C/T in the myostatin gene. The horse might therefore be best suited for middle distances.
Genotype T/T (homozygous): The tested horse is homozygous for the T allele of the myostatin gene and could be best suited for long distances. These horses tend to be late developers.

Note on ordering: If this genetic test does not appear in your online order system, please use the printed submission form Genetics Horse which you can either request from LABOKLIN or download as PDF document from the LABOKLIN website.

13.3.3 Tractability

The test for tractability is designed to show a horse‘s willingness to learn or perform. Since the phenotype is only based on statements of the horse owners or people who have contact with the horse, differentiation is difficult. The test does not provide any information concerning the cleverness of the horse. It should also be noted that factors such as anatomical conditions or diseases can strongly influence the tractability, so genotype and phenotype may not be consistent.

13.4 DNA Profiles for Identity and Parentage Testing

Unlike other identification methods, such as microchips or tattoos, DNA profiles cannot be manipulated or destroyed by external factors, e.g. injuries. They remain unchanged for life.
On the one hand, DNA profiles provide a lifelong, doubtless identification of the animal. On the other hand, parentage can be proven with certainty by comparing the genetic fingerprints of the family members.

13.4.1 DNA Profile

A DNA profile is based on the testing of highly variable DNA markers (microsatellites), which differ between individuals by their length (length polymorphism). The overall combination of microsatellites results in a DNA profile which is unique for every individual.
In order to create a DNA profile, initially, DNA is isolated from nucleated cells. Then the segments of the DNA to be analysed are multiplied million-fold by PCR.
The length of the microsatellites can be determined by a computer-aided analysis in the "Genetic Analyser". This data is then used to create an individual and reproducible code for each animal.
The DNA profile is unique, with an accuracy of more than 99.9%. The only exception are monozygotic multiples. For the identification of an animal, its DNA profile is generated and archived in a DNA database. To create a DNA profile, we test the microsatellites markers which are recommended by the "International Society for Animal Genetics (ISAG)". The generated DNA profiles are internationally comparable with laboratories working according to the recommendations of ISAG.

13.4.2 Parentage

The purpose of parentage testing is to clarify whether supposed parents of a certain animal are the true biological parents.
Each offspring receives 50% of its genetic material from the mother and 50% from the father. Provided that the maternity is confirmed, all non-maternal markers in the DNA profile of the offspring must have been inherited from the father. If at least two markers in the DNA profile do not match, paternity can almost certainly be excluded. The same applies to the mother.

Similar to DNA profiles, the informational value of parentage testing considerably depends on the number of examined microsatellites. The more highly variable DNA segments are examined in parentage testing, the more precisely false parentage can be detected.

If a pedigree certificate is required, not only sample material (see Chapter 13.1) from the offspring to be tested must be submitted, but also samples from father and mother. If paternity should be excluded, blood or hairs of all potential fathers should be submitted in addition to the maternal samples. Results are available approx. 2 – 3 weeks after the samples have arrived at the laboratory.

Note on ordering: If the DNA Profile and Parentage do not appear in your online order system, please use the printed submission form Genetics Horse which you can either request from LABOKLIN or download as PDF document from the LABOKLIN website.

14. Appendix

14.1 Reference Ranges Horse

Clinical Chemistry

Enzymes 37 °C		
α-HBDH	< 221	U/l
AP	< 352	U/l
AST (GOT)	< 568	U/l
CK	< 452	U/l
γ-GT	< 44	U/l
GLDH	< 13	U/l
GPX	50 – 150	U/gHb
LDH	< 455	U/l
DGGR Lipase	< 20	U/l

Substrates		
Albumin	25 – 54	g/l
Bilirubin, total	8.6 – 59.9	µmol/l
Cholesterol	1.81 – 4.66	mmol/l
Fructosamine	< 360	µmol/l – preliminary
Bile acids	< 12	µmol/l
Total protein	55 – 75	g/l
Globulins	24 – 51	g/l
Glucose	3.05 – 4.99	mmol/l
Urea	3.3 – 6.7	mmol/l
Creatinine	71 – 159	µmol/l
Lactate	0.5 – 2.0	mmol/l
A/G ratio	0.7 –1.1	
SAA	< 7	µg/ml
SDMA	< 0.75	µmol/l
Triglycerides	< 0.97	mmol/l

Electrolytes and Trace Minerals		
Calcium	2.5 – 3.4	mmol/l
Chloride	95 – 105	mmol/l
Iron	17.9 – 64.5	µmol/l
Potassium	2.8 – 4.5	mmol/l
Copper	7.9 – 21.0	µmol/l
Magnesium	0.5 – 0.9	mmol/l
Sodium	125 – 150	mmol/l
Phosphate	0.7 – 1.5	mmol/l
Selenium	100 – 200	µg/l
Zinc	9.2 – 19.9	µmol/l
Vitamin E	1 – 2 2 – 3	mg/l (stable) mg/l (pasture)

Haematology

Erythrocytes	6 – 12	T/l
Thoroughbred	8 – 12	T/l
Warm blood	6.5 – 9	T/l
Cold blood	6 – 9	T/l
Pony	5.5 – 8.5	T/l

Haematocrit	0.3 – 0.5	l/l
Thoroughbred	0.35 – 0.50	l/l
Warm blood	0.33 – 0.45	l/l
Cold blood	0.32 – 0.42	l/l
Pony	0.30 – 0.40	g/l

Haemoglobin	110 – 170	g/l
Leukocytes	5 – 10	G/l
Platelets	90 – 300	G/l

Differential blood count	%	absolute: G/l
Segmented	45 – 70	3 – 7
Lymphocytes	20 – 45	1.5 – 4
Monocytes	0 – 5	0.04 – 0.4
Eosinophils	0 – 4	0.04 – 0.3
Basophils	0 – 2	0 – 0.15
Unsegmented	0 – 6	0 – 0.6

Adrenal gland		negative	borderline	positive	
ACTH	Mid-November – mid-July	< 30	30 – 50	> 50	pg/ml
	Mid-July – mid-November	< 50	50 – 100	> 100	pg/ml
Cortisol	Ref. range	30 – 70			ng/ml

A consensus statement of the Equine Endocrinology Group from 2021 further differentiates ACTH reference ranges according to season. The deviations are marginal and final diagnosis should always consider clinical findings.

Endocrinology

Pancreas		
Insulin	< 20	µU/ml

Reproduction & Pregnancy				
Oestradiol	reference values mare	pro-oestrus	1.2 – 6.2	pg/ml
		oestrus	7.1 – 13.0	pg/ml
		di-oestrus	3.7 – 5.0	pg/ml
Progesterone			>/= 1,0 *	ng/ml
Testosterone	reference values	stallion	1 – 5	ng/ml
		gelding	< 0.04	ng/ml
		mare	< 0.04	ng/ml
Anti-Müllerian Hormone		see page 51, 53		
PMSG & Oestrone sulfate		depending on stage of pregnancy		

* reflects corpus luteum function

Thyroid gland		
T4	1.3 – 4.1	µg/dl
fT4	9.0 – 44.9	pmol/l
T3	25 – 180	ng/ml

Serum Protein Electrophoresis

Reference range	%	absolute (g/l)
Albumin	45 – 60	33 – 38
α-Globulins	10 – 20	5 – 8
β-Globulins	10 – 25	8 – 14
γ-Globulins	8 – 22	9 – 14

Urine

Protein/Creatinine ratio	< 1	
γ-GT/Creatinine ratio	< 5.0	U/mmol
Fractional Electrolyte Excretion		
Sodium	0.04 – 0.52	%
Potassium	35 – 80	%
Phosphate	0 – 0.2	%
Chloride	0.7 – 2.1	%

Coagulation

Thrombin time	18 – 55	sec.
PTT	30 – 65	sec.
Prothrombin time	8 – 14	sec.
Fibrinogen	100 – 350	mg/dl

14.2 Reference Ranges Foal

Clinical Chemistry

Age:	1 day	1 week	1 month	6 months	
Enzymes					
AP	< 2671	< 1169	< 866	< 650	U/l
AST (GOT)	< 340	< 620	< 440	< 620	U/l
CK	< 909	< 143	< 585	< 396	U/l
γ-GT	< 43	< 164	< 99	< 26	U/l
Substrates					
Albumin	25 – 36	27 – 34	27 – 34	30 – 35	g/l
Cholesterol	2.85 – 14.56	3.60 – 11.53	2.15 – 6.03	2.15 – 4.48	mmol/l
Total protein	43 – 81	44 – 68	50 – 67	60 – 69	g/l
Globulins	15 – 46	16 – 39	18 – 37	28 – 37	g/l
A/G ratio	0.6 – 1.9	0.7 – 1.8	0.8 – 1.5	0.8 – 1.4	
Glucose	6.72 – 12.93	6.72 – 10.66	7.22 – 11.99	6.10 – 11.65	mmol/l
Urea	1.50 – 6.66	0.67 – 3.33	1.00 – 3.50	2.5 – 4.99	mmol/l
Creatinine	91.5 – 328	76.3 – 129.6	83.9 – 137.3	91.5 – 160.1	µmol/l
Triglycerides	0.34 – 2.20	0.34 – 2.72	0.51 – 1.78	0.4 – 0.87	mmol/l
Electrolytes and Trace Minerals					
Calcium	2.92 ± 0.5	3.12 ± 0.3	3.04 ± 0.3	2.94 ± 0.4	mmol/l
Chloride	102 ± 12	102 ± 8	103 ± 6	105 ± 7	mmol/l
Potassium	4.6 ± 1.0	4.8 ± 1.0	4.6 ± 0.8	4.2 ± 1.4	mmol/l
Magnesium	0.92 ± 0.74	0.83 ± 0.25	0.83 ± 0.41	0.99 ± 0.29	mmol/l
Sodium	141 ± 15	142 ± 12	145 ± 9	143 ± 10	mmol/l

Haematology

Age:	1 day	1 week	1 month	6 months	
Erythrocytes	8.2 – 11.0	7.4 – 10.6	7.9 – 11.1	7.9 – 11.6	T/l
Haematocrit	0.32 – 0.46	0.28 – 0.43	0.29 – 0.41	0.29 – 0.41	l/l
Haemoglobin	120 – 166	107 – 158	109 – 153	108 – 154	g/l
Leukocytes	4.9 – 11.7	6.3 – 13.6	5.3 – 12.2	7.8 – 11.6	G/l
Platelets	129 – 409	111 – 387	136 – 468	128 – 368	G/l

Differential Blood Count – Absolute Numbers

Age:	1 day	1 week	1 month	6 months	
Segmented	3.4 – 9.6	4.4 – 10.6	2.8 – 9.3	2.9 – 5.6	G/l
Lymphocytes	0.7 – 2.1	1.4 – 2.3	1.7 – 4.9	3.2 – 6.0	G/l
Monocytes	0.1 – 0.4	0 – 0.5	0.1 – 0.6	0 – 0.5	G/l
Eosinophils	few individual cells	up to 0.1	up to 0.1	up to 0.6	G/l
Basophils	few individual cells	up to 0.2	up to 0.1	up to 0.1	G/l
Unsegmented	– no reference range, pathological –				

Passive Immune Transfer in Neonatal Foals – Serum IgG

< 2 g/l	= absolute deficiency
2 – 4 g/l	= partial deficiency
4 – 8 g/l	= subnormal
> 8 g/l	= adequate supply

14.3 Reference Ranges Donkey

Clinical Chemistry

Enzymes 37 °C		
AP	< 252	U/l
CK	< 525	U/l
AST (GOT)	< 536	U/l
γ-GT	< 70	U/l
GLDH	< 8	U/l
LDH	< 538	U/l

Substrates		
Albumin	22 – 32	g/l
Bilirubin, total	0.1 – 3.7	µmol/l
Cholesterol	1.4 – 2.9	mmo/l
Bile acids	2.6 – 18.6	µmol/l
Total protein	58 – 76	g/l
Globulins	< 37	g/l
Glucose	3.9 – 4.7	mmol/l
Urea	1.5 – 5.2	mmol/l
Creatinine	53 – 118	µmol/l
Triglycerides	0.6 – 2.8	mmol/l

Electrolytes and Trace Minerals		
Calcium	2.2 – 3.4	mmol/l
Chloride	96 – 106	mmol/l
Iron	6.4 – 25.5	µmol/l
Potassium	3.2 – 5.1	mmol/l
Copper	9.4 – 18.4	µmol/l
Magnesium	0.68 – 1.1	mmol/l
Sodium	128 – 138	mmol/l

Phosphate	0.87 – 1.97	mmol/l
Selenium	50.6 – 179.2	µg/l
Zinc	3.3 – 14.1	µmol/l

Haematology

Erythrocytes	4.4 – 7.1	T/l
Haematocrit	0.27 – 0.42	l/l
Haemoglobin	89 – 147	g/l
Leukocytes	6.2 – 15	G/l
Platelets	95 – 384	G/l

Differential Blood Count

	%	G/l
Segmented	23 – 59	2.4 – 6.3
Lymphocytes	34 – 69	2.2 – 9.6
Monocytes	0.5 – 7.5	0 – 0.75
Eosinophils	0.9 – 9.1	0.1 – 0.9
Basophils	0 – 0.5	0 – 0.07
Unsegmented	0	0

15. Finally, a few Words on the Process

15.1 Courier Service

LABOKLIN offers courier services in most 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 10 and following.

15.2 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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LABOKLIN

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