LABOKLIN aktuell

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Pre-analytics in Canine and Feline Endocrinology

What is Pre-analytics?
And How Can It Affect My Results?

Pre-analytics includes all processes before laboratory analysis, from selecting the appropriate test based on the patient's history and clinical examination to sample collection, transport, storage and processing; in short, the entire journey of the sample up to the point of analysis.

Numerous studies have investigated sources of error in laboratory testing. These studies demonstrate just how important what happens before the analysis is. From selecting the correct test to sample collection and transport conditions, there are many pre-analytical factors that can influence results (Fig. 1). This is particularly relevant in endocrinology, where the often delicate nature of hormones, their stability, and the susceptibility of measurements to sample quality place high demands on sample handling.

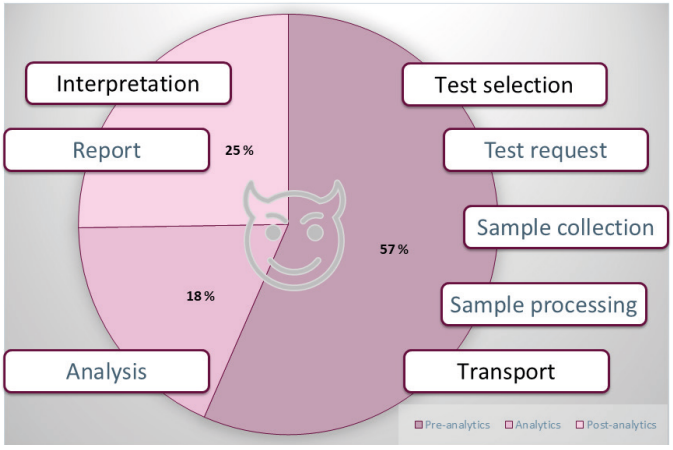

Fig. 1: The greatest source of error in laboratory testing occurs during the pre-analytical phase (adapted from Nordin et al. 2024). *Image source: Laboklin*

The sample requirements described below refer to the analytical methods currently used by Laboklin for the measurement of various analytes. As analytical methods continue to evolve, these requirements may change over time. If in doubt, contact our laboratory before sample collection.

As a general rule, marked haemolysis and lipaemia should be avoided. Common causes of haemolysis include prolonged venous occlusion, difficult venepuncture, clotting in the refrigerator, and failure to separate serum or plasma before shipment. Fasting blood samples are generally recommended for hormone measurements to minimise lipaemia.

Thyroid Gland

T4 (Total T4, TT4; Free T4, fT4) and T3

Indications: Screening for hypothyroidism; screening and diagnosis of hyperthyroidism.

Important considerations before sample collection: T4 concentrations may be reduced by non-thyroidal illness (NTI) and by various medications. Free T4 is somewhat less affected by these factors, but its concentration may also be decreased by NTI and certain drugs. Differentiation from genuine hypothyroidism requires further diagnostic investigation.

Analytical methods: Measurement can be performed using two different methods.

1. CLIA (Chemiluminescent Immunoassay): This assay is based on the immunological binding of the T4 molecule. For initial screening, this more cost-effective method is often sufficient.

2. LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry): This method is increasingly regarded as the gold standard. It is considered particularly precise because it measures only the T4 molecule itself, thereby avoiding interference from metabolites or antibodies. At Laboklin, LC-MS/MS testing is available for T4, T3 and rT3 (see below).

Sample material: Serum (mandatory for LC-MS/MS; preferred for CLIA).

Good to Know: TT4 and fT4 remain stable at room temperature for three to five days. For fT4 it is being discussed that dissociation of T4 from its binding proteins may occur after more than five days and/or when exposed to high ambient temperatures. This may result in elevated fT4 concentrations, potentially leading to misinterpretation of results.

TSH

Indications: Investigation of hypothyroidism; treatment monitoring.

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Important considerations before sample collection: In the presence of a low T4 concentration and compatible clinical signs, an elevated TSH concentration generally confirms hypothyroidism. However, approximately 30% of hypothyroid dogs have low TSH concentrations. In such cases, further diagnostic investigations are required to differentiate between NTI and hypothyroidism.

Analytical method: CLIA

Sample material: Serum

Good to Know: TSH is stable for 24 hours at room temperature and for seven days when refrigerated (2 to 8°C).

Reverse T3 (rT3)

Indications: Aid in differentiating between NTI and hypothyroidism in dogs with reduced T4 concentrations and no increase in TSH; support in cases of suspected hyperthyroidism in cats when T4 concentrations are not elevated as expected.

Important considerations before sample collection: rT3 is a highly promising parameter. However, interpretation should always be performed in the clinical context and in combination with other thyroid parameters.

Analytical method: LC-MS/MS

Sample material: Serum

Good to Know: rT3 remains stable at room temperature for up to 14 days.

Autoantibodies (against Thyroglobulin, T3 and T4)

Indications: Suspected interference with T4 measurement by CLIA; breeding health screening and breeding approval programmes.

Important considerations before sample collection: Immunosuppression (medication) and immune stimulation (for example vaccination) may alter antibody concentrations. This should be taken into account when interpreting results.

Analytical method: ELISA. Results are reported either quantitatively (percentage proportion in the sample) or qualitatively (positive/negative).

Sample material: Serum

Good to Know: Antibodies are relatively stable. However, samples should be transported refrigerated if transport times exceed 48 hours. Sample quality is particularly important for antibody testing. Serum must be cleanly separated, and haemolysis and lipaemia must be avoided.

Parathyroid Gland

Parathyroid Hormone (PTH)

Indications: Investigation of hyperparathyroidism or hypoparathyroidism.

Important considerations before sample collection: As interpretation depends on correlation with the blood calcium concentration at the time of sampling, PTH and calcium should be measured from the same serum sample or from serum collected at the same sampling time.

Sample material: Serum

Analytical method: CLIA

Good to Know: PTH is unstable. Consequently, proper pre-analytical handling is of great importance. The sample should be allowed to clot for no longer than 30 minutes, then centrifuged immediately and the serum transferred into a separate tube. The separated serum must be stored and transported frozen. At a minimum, shipment should be carried out using pre-frozen cooling packs; transport on dry ice is ideal.

Adrenal Gland

Cortisol

Indications: Screening parameter for ruling out hypoadrenocorticism; measurement as part of the ACTH stimulation test and the dexamethasone suppression test.

Important considerations before sample collection: Cortisol secretion increases in response to both emotional and physical stress. This can affect the results of functional tests. In particular, the dexamethasone suppression test should be performed only after concurrent diseases have been resolved and under conditions that are as stress-free as possible.

Analytical methods: CLIA; LC-MS/MS may be used for specific clinical questions. In routine diagnostics, cortisol is generally measured using immunological assay methods (CLIA). In human medicine, however, LC-MS/MS is increasingly becoming established as a highly specific and precise method for cortisol measurement.

Sample material: Measurement can be performed using plasma. However, serum is preferred to ensure standardisation of hormone testing. In particular, the same sample material should always be used for follow-up measurements and functional tests to ensure consistent and comparable results.

Good to Know: Cortisol in serum samples is generally stable at room temperature for 3 to 5 days. Marked lipaemia may affect cortisol measurements performed using immunoassays. Although fasting is not strictly required for functional testing, it may be advisable to feed only a small and/or low-fat meal

before testing in order to minimise lipaemia-related interference.

Urine Cortisol-to-Creatinine Ratio (UCCR)

Indication: Screening for possible Cushing's syndrome.

Important considerations before sample collection: Urine samples for UCCR determination should ideally be collected in the morning and in a stress-free environment, with at least three (better five) days between sample collection and a veterinary visit. Before submission, urinary tract infection should be excluded by performing urinalysis, including sediment examination.

Analytical method: For one of the immunoassays widely used in veterinary medicine for urinary cortisol measurement (CLIA, Immulite 2000®, Siemens, Germany), changes made by the manufacturer to the assay substrate have led to concerns regarding the reliability of results obtained from canine urine samples. The assay used by Laboklin is not affected by this issue.

Endogenous ACTH (eACTH)

Indications: Further differentiation of Cushing's syndrome and hypoadrenocorticism (Addison's disease).

Analytical method: CLIA

Sample material: EDTA plasma

Good to Know: Endogenous ACTH is unstable in biological samples, particularly in dogs. It is rapidly degraded by endogenous enzymes in the blood, which may lead to falsely low results. When stored frozen at -20°C in separated plasma, eACTH remains stable for extended periods. Blood should therefore be collected into pre-cooled EDTA tubes, ideally chilled in a freezer at -20°C before sampling. Centrifuge and separate immediately, transfer plasma to an uncoated tube and ship frozen. Ideally, the sample should arrive at the laboratory still frozen. Glass tubes are not suitable, as eACTH binds to glass surfaces, which can reduce the measured concentration.

Aldosterone

Indication: Investigation of hyperaldosteronism.

Important considerations before sample collection: The patient should be adequately hydrated, as the renin-angiotensin-aldosterone system (RAAS) is influenced by hypovolaemia and dehydration.

In azotaemic patients, measurement of aldosterone alone is not sufficient for the diagnosis of primary hyperaldosteronism, as aldosterone concentrations may also be elevated in renal disease. Feeding time can have an effect on the RAAS; therefore, collection of a fasting blood sample is recommended.

Sample material: Serum

Analytical method: LC-MS/MS. This method is considered highly precise and superior to immunological assay methods. It is important to note that LC-MS/MS typically measures lower aldosterone concentrations than other analytical methods. This has been taken into account in the reference intervals provided by Laboklin.

Good to Know: The sample should be as fresh as possible and no older than 48 hours. Allow to clot for ≤30 minutes, centrifuge immediately and transfer serum to a separate tube. The separated sample should be cooled as quickly as possible and must arrive at the laboratory under refrigerated conditions.

RAAS Profile (Calculation of Renin Activity, ACE Activity and the AA2 Ratio)

Indication: Investigation of hyperaldosteronism in patients with concurrent azotaemia.

Important considerations before sample collection: The patient should be adequately hydrated, as the renin-angiotensin-aldosterone system (RAAS) is influenced by hypovolaemia and dehydration. Feeding time can have an effect on the RAAS; therefore, collection of a fasting blood sample is recommended.

Sample material: Serum

Analytical method: Renin activity is calculated following measurement of aldosterone, angiotensin I and angiotensin II by LC-MS/MS. The method has been validated for both cats and dogs.

Good to Know: Angiotensin I and angiotensin II are unstable and must not be exposed to temperatures of 4 to 8°C. Consequently, the sample must arrive at the laboratory frozen. The sample should be allowed to clot for no longer than 30 minutes, then centrifuged immediately and the serum transferred into a separate tube. The serum needs to be frozen immediately at -20°C. The sample must not be stored in a refrigerator prior to freezing and, once frozen, must not be allowed to thaw before arrival at the laboratory (!). Shipment on dry ice is mandatory.

Catecholamines (Metanephrine, Normetanephrine)

Indications: Investigation of a pheochromocytoma or catecholamine-producing paraganglioma.

Important considerations before sample collection: Catecholamines are released in response to stress. Consequently, stressful situations may lead to increased catecholamine secretion. Urinary measurement is less affected by episodic catecholamine release than blood measurement. Urine samples may be collected either by cystocentesis or as a free-catch sample. Collection in the home environment is not mandatory. However, a urinary tract infection should be excluded before sample submission by performing a urinalysis, including sediment examination.

Medications such as phenoxybenzamine, metoclopramide, β-blockers, calcium channel blockers or sympathomimetics (epinephrine, dopamine, dobutamine, phenylpropanolamine and terbutaline) may cause falsely elevated results. Particular attention should be paid to ensuring that sample collection takes place before initiation of any potential treatment with phenoxybenzamine.

Analytical method: LC-MS/MS

Sample material: EDTA plasma or urine (free-catch or cystocentesis sample)

Good to Know: Catecholamines are unstable in blood samples. Blood should therefore be centrifuged immediately and the plasma separated without delay. After transfer into an uncoated sample tube, the plasma should either be stored refrigerated at 4°C, where it remains stable for up to three days, or frozen at -20°C. Samples should be shipped under refrigerated conditions and must arrive at the laboratory chilled. Shipment with pre-frozen cooling packs is the minimum requirement; transport on dry ice is ideal. In urine, catecholamines remain stable for several days even at room temperature. The acidification of urine samples that was previously recommended is no longer performed. Studies in both human and veterinary medicine have demonstrated that acidification is not necessary.

Pituitary Gland

Insulin-Like Growth Factor 1 (IGF-1)

Indications: Acromegaly (hypersomatotropism) and pituitary dwarfism (hyposomatotropism).

Analytical method: CLIA

Sample material: Serum

Good to Know: The sample should be allowed to clot for no longer than 30 minutes, then centrifuged immediately and the serum transferred into a separate tube. Provided that the blood sample is centrifuged and separated promptly, IGF-1 remains stable for 24 hours at room temperature and for up to seven days at 4°C. Cooling of the sample and shippment under refrigerated conditions is recommended, especially if prolonged transportation times are expected.

Endocrine Pancreas

Insulin

Indication: Insulinoma

Important considerations before sample collection: Blood sampling should be performed during documented hypoglycaemia (blood glucose < 3.3 mmol/L or < 60 mg/dL). Glucose and insulin measurements must be performed on serum obtained from blood collected at the same time. Ideally, collect blood from a large vessel (e.g. the jugular vein) using a large-bore needle to minimise haemolysis and facilitate rapid collection.

Analytical method: CLIA (dog), ELISA (cat)

Sample material: Serum

Good to Know: Insulin is unstable in serum at room temperature and may undergo significant degradation within a few hours. The blood sample should therefore be allowed to clot for no longer than 30 minutes, then centrifuged immediately and the serum transferred into a separate tube to minimise degradation. The sample should be stored refrigerated at 4°C. If analysis cannot be performed within 24 hours, freeze serum at -20°C (-80°C for long-term storage). Refrigerated shipment must be ensured using pre-frozen cooling packs; for longer transport times, shipment on dry ice is recommended. The sample should arrive at the laboratory refrigerated (4-8°C). As haemolysis may result in the release of insulin-degrading enzymes, it should be avoided whenever possible.

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Our Endocrinology Services Include

- Individual hormone assays
- Functional endocrine testing
- Specialised diagnostic profiles

Further reading:

Aus der Humanmedizin: Nordin N, Ab Rahim SN, Wan Omar WFA, Zulkarnain S, Sinha S, Kumar S, Haque M. Preanalytical Errors in Clinical Laboratory Testing at a Glance: Source and Control Measures. Cureus. 2024 Mar 30;16(3):e57243.

Spezielle Literatur wird sehr gerne auf Nachfrage zur Verfügung gestellt.