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Hormone analyses at the IZW

The endocrine laboratory of the *Leibniz Institute for Zoo and Wildlife Research* (IZW) offers measurement of steroid hormones in blood plasma, urine, faecal and hair samples (non-invasive monitoring of hormones) on a service basis.

We are able to analyse progesterone, estradiol, testosterone, cortisol, corticosterone, prostaglandin F2α-metabolite (PGFM) in blood, and their metabolites in urine and faeces reflecting male and female reproductive and adrenocortical activity (stress). Additionally, we offer the option for hair glucocorticoid metabolites (hGCM) measurement.

1. Requirements regarding sample collection and samples

For all samples it is crucial to apply a consistent labeling mentioning at least the ID of the animal and the date of sampling. For some samples, the time of sampling might be relevant as well.

1.1 Blood samples

After sampling, blood should be centrifuged, with isolation of the cell-free fraction, and the resulting plasma/serum should be immediately transferred to the lab under cooled conditions or stored at -20°C until analysis. Our assays are generally validated for plasma rather than serum.
Minimum volumes per sample depend on the specific case study and can be determined after inquiry.

1.2 Urine and faecal samples

For non-invasive and potentially contaminated samples such as urine and faecal samples, it is important to freeze them immediately after collection (-20°C). If not, changes in metabolite concentrations may occur due to microbial or enzymatic activities, leading to artefacts.

1.3 Hair samples

After sampling, hair can be stored at room temperature in dry conditions.

1.4 Prerequisites

Please keep in mind that the secretion of cortisol/corticosterone follows diurnal and pulsatile patterns. Therefore, it is impossible to measure adrenal activity (stress) in an animal based on a single sample. Therefore, samples should be collected over a longer time period.
The same applies for reproductive monitoring. A single elevated progesterone concentration in samples of plasma, urine or faeces does not necessarily indicate pregnancy, it may just as well indicate the luteal phase of a cycling female. Thus a sample collection period exceeding

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the length of an ovarian cycle is necessary, including baseline samples. Pregnancy can only be confirmed based on persisting high progesterone concentrations in samples collected over a certain period.

The sample frequency for diagnostic monitoring will thus be case- dependent. In case of any doubts on how frequent sampling should be, please contact the endocrine laboratory timely, and make sure to provide base-line samples (e.g. from prior to mating or any stress event).

2. Limitations

Steroids are metabolized in a species-specific way, and so-called *biological validation* of a non-invasive hormone measurement is recommended prior to start monitoring reproductive activity in a valuable animal. Biological validation tests the ability of a certain
methodology to detect known differences or changes in hormone levels.

For this reason, we can offer non-invasive hormone monitoring with guaranteed results only for species for which we have prior experience or ready-to-use analytical methods.

Alternatively, we can pursue method optimization and validation for any new species on the condition that the related cost will be covered and adequate samples will be provided.

Please note that if the results are to be published in a scientific journal, referees will most likely require analytical and biological validation.

Our methods are analytically validated for use (sensitivity, specificity, reproducibility (precision), recovery (accuracy), dilution integrity/parallelism,…). However, additional optimization and validation might be needed for a specific application that is not fitting in the current scope (new species, new matrix, …).

3. Estimated costs per sample

Prices will be calculated adapted to the specific case and will depend on a number of factors, including the duration and complexity of the extraction procedure and the batch sample size (reduced prices per sample for larger batches). A case-specific offer will be provided within one week after inquiry (unless otherwise communicated). In what follows, the maximum price per sample for a batch of 100 samples is illustrated (all prices are excl. 19% tax):

- Plasma sample: 13.35 €
- Urine samples: 13.35 €
- Faecal samples: 17.35 €
- Hair samples: 19.85 €

4. Customer risks

If a customer explicitly requests non-invasive hormone measurement for a species (or matrix) for which we have no analytical expertise, we cannot guarantee reliable results unless proper biological and full-spectrum analytical optimization and validation. This implies an additional cost (to be discussed case-by-case). Additionally,
reference samples specifically assigned to the biological and analytical validation should be provided by the customer.

The related costs will have to be paid for all procedures conducted, regardless of a successful outcome.

5. Warranties

Delivery and handling time of samples will be estimated upon consultation. For non-urgent batch-analysis, we will pursue reporting results within 4 weeks.
A more prompt scheduling is possible in case of urgent or real-time reproductive monitoring. *It is best to prenotice our lab about upcoming urgent analysis, in order to guarantee timely analysis.*

A special case is parturition monitoring in elephants where analyses have to be carried out 3 times per week. When hormone concentrations drop, we switch to daily measurements also including weekends.

The final report will include the data and its quality assessment, but excludes biological or scientific interpretation unless otherwise agreed.

6. Data storage

Raw data (reader print-outs) are saved in folders in the room of the lab technician. In addition, all EIA templates are scanned and saved as pdf files with a name following the template: EIA-5aP-20190219, where 5a-P defines the EIA.
Data evaluation is carried out with *Excel* and the calculated hormone concentrations are saved in *Excel* sheets. From each sheet a link is
given to the corresponding EIA (e.g. EIA-5aP-20190219) to connect the calculated concentrations to the corresponding raw data.
Files are stored for at least 5 years post analysis.

7. Quality management

We use analytical methods that in most cases have been published in peer-reviewed journals including quality criteria and thus have passed critical assessment of external experts.

In addition, each assay is routinely subjected to the following quality management procedures:

1. Sample analyses are carried out in duplicates. In case duplicates deviate more than 5%, the analysis of the extract is repeated.

2. Two quality control samples (controls) are included in each assay. These must always result in the same concentrations, irrespective of the person running the assay. Results are documented over several years in a central data set and are under control of the lab leader.
In case of significant deviations from normal, the assay is repeated. In case of repeated deviations a troubleshooting routine is initiated.

3. Calibrations curves are used for quantification. The calibration curves are compared according to their B10, B20, B50, B80 and B90 values (All our assays are competitive immune assays; B50 is the hormone concentration that is needed to reduce absorption and thus binding of the label by 50%). Dilutions of samples need to be optimized to allow read-outs in the linear part of the curve (B80-B20). B-values are calculated by the Magellan software (Tecan) and must remain constant (CV preferentially < 10%).

8. Ring tests:

Regarding non-invasive monitoring of hormones ring tests are not feasible because every laboratory uses its own unique assays based on a distinct antibody. For non-invasive hormone monitoring, biological (after analytical) validation is the most important prerequisite for generating relevant conclusions.

9. Contact

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