
# Guidelines for health monitoring of the Balkan lynx

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# Introduction

Wildlife disease monitoring, prevention and control are crucial factors for safeguarding biodiversity and public and animal health worldwide (https://wwww.woah.org/en/home). In the context of free-roaming Eurasian lynx (*Lynx lynx*), the impact of infectious agents on most occasions may be limited due to the rather low level of social interaction among individuals. Consequently, disease prevalence is anticipated to be low. However, it is also important to emphasise that limited scientific data is available on the prevalence of pathogens in lynx populations across Europe. For sure, challenges can arise when infections occur in small and fragmented populations, where disease transmission can have a more significant impact, for instance, due to a limited gene pool (Van Mulders & Marti, 2024). Disease surveillance and confinement are therefore mandatory, as in the Balkan lynx, the spread of an epidemic could have detrimental effects on population persistence.

Based on an overview on pathogens potentially affecting or carried by Carpathian lynx (Ryser-Degiorgis et al., 2021), the main disease of concern was sarcoptic mange, typically observed in lynx in geographical areas where mange affects the local fox population (Munson et al., 2010). No specific bacteria was of particular concern but feline viruses were considered a potential threat, taking into account their significance in both domestic and wild felids (Lutz, 2005; Meli et al., 2010). To our knowledge no information about health screenings of *Lynx lynx balcanicus* are available. Between 2010 and 2024, a total of 25 Balkan lynx have been examined (11 dead recoveries and 14 live-captured individuals). So far, no health issues have been detected, with the exception of one case of sarcoptic mange in 2019 in North Macedonia (Fig. 1). But there has been no specific protocol in place for disease screening. There is a clear need for uniform health monitoring throughout the range of the Balkan lynx. Systematic health monitoring of Balkan lynx consists of pathological examination of all individuals found dead, either by chance or through radio-telemetry, the clinical examination of live lynx captured as well as the testing of fresh faeces found during fieldwork for pathogen presence. These guidelines for the health monitoring of the Balkan lynx were developed based on experiences built up in other teams/projects, namely KORA, FIWI, LIFE Luchs Pfälzerwald, LIFE Lynx, Linking Lynx.

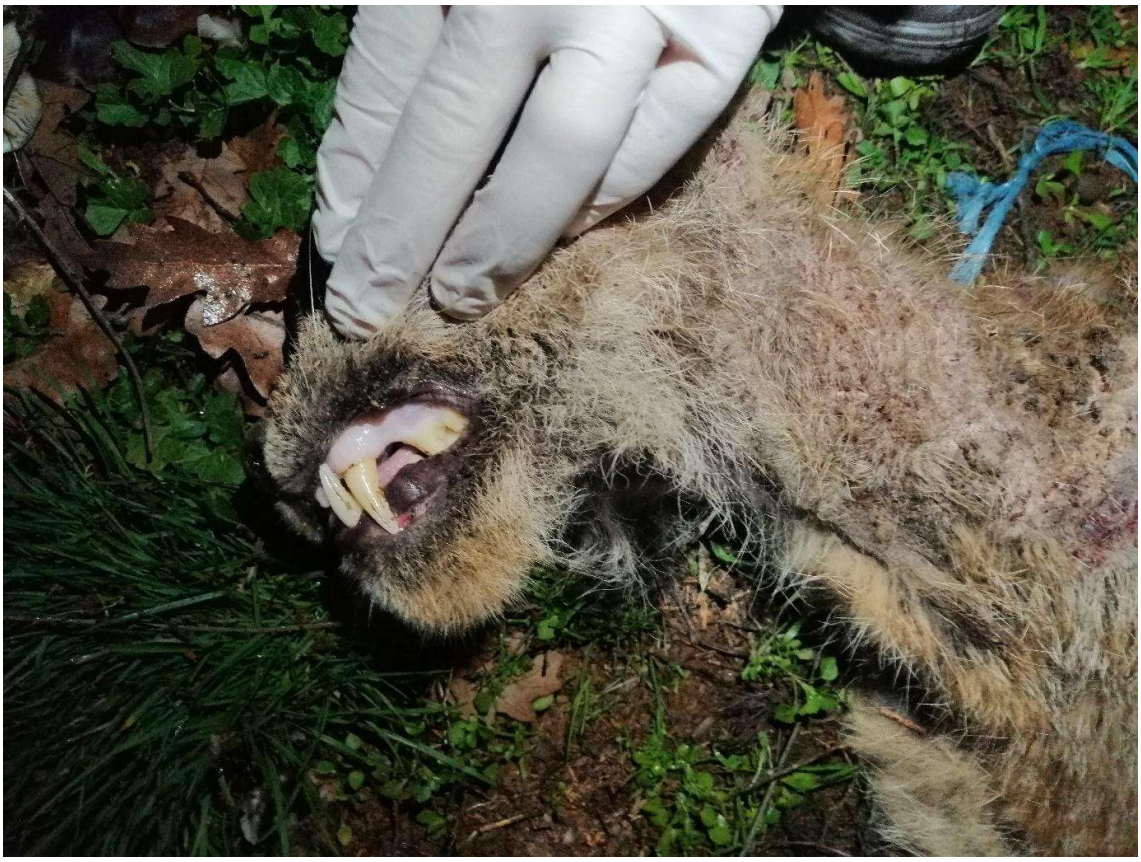

**Fig. 1.** Lynx with severe dermatitis caused by *Sarcoptes* sp. *Photo*: D. Melovski

## Post-mortem examination

Lynx are photographed on both sides to record the individual coat pattern to identify individual animals for comparison with camera trapping data. Sex and body condition are determined, the body weight is recorded, and standard morphological measurements are taken (Marti & Ryser-Degiorgis, 2018). Age is estimated mainly based on dentition, tooth wear and body size but also considering maturity of genital organs and season. Photographs of dentition are taken. All animals are systematically radiographed to search for foreign bodies such as ammunition fragments and for skeletal anomalies (Morend et al., 2022).

Necropsy is performed by a veterinarian, according to a standardised protocol (see Appendix 1). Photographs and measurements of organs are taken according to the protocol and pathological findings. Multiple organ samples are fixed in 4% buffered formalin for histological examination. Additional native samples (blood, selected organs) are stored frozen at both -20°C and -80°C for

archive purposes. See Chapter 5 for instructions on preserving blood and tissue samples for genetic analyses. Samples of hair (both guard hairs and underfur, plucked, cut or shaved, at least 0,2 g) should be stored in paper envelopes and archived in a dry place, for future toxicology analysis.

Due to the high prevalence of heart diseases observed in Central European lynx populations (Ryser-Degiorgis et al., 2020), special attention should be paid to macroscopic examination of the heart to detect any anomalies. The entire heart can be conserved in formalin for later analysis.

Samples of the muscle on the lower forelimb (m. flexor carpi ulnaris) and diaphragm (tongue and/or masseter muscle) should be tested for *Trichinella* sp. (Frey et al., 2009). Entire digestive system (stomach and intestines) and faeces from the rectum are submitted to parasitological examination. If the analysis cannot be effectuated immediately, both the digestive system and the faeces can be frozen and analysed later.

If necessary, bacteriological, virological or toxicological analyses are initiated to determine health status and/or the cause of death.

Eventually, the entire skeleton (especially the skull) can be cleaned and archived for future research. See Appendix 1 for further details on lynx necropsy.

# Live capture

External examination is performed. The animal's coat should be maintained in good grooming condition. A poorly cared for coat, characterised by grease, plucked areas, or matting hair, may signal various underlying issues. These issues can span from physical challenges in grooming of the coat, such as musculoskeletal pain, to discomfort originating from oral problems, obesity, or a general sense of illness, irrespective of the specific underlying cause (Van Mulders & Marti, 2024).

The skin should be evaluated thoroughly for wounds (post-fighting), dermatitis and/or alopecia related to parasitic (*Sarcoptes* spp., *Notoedres* spp.), fungal (*Trichophyton* spp., *Microsporum* spp.) and secondary bacterial agents. If pathologies are found, photographs and dermatological samples should be taken for further analyses. A prophylactic treatment with ivermectin is recommended in any case. Moreover, the clinician must be aware of the potential occurrence of neoplastic lesions such as squamous cell carcinomas or other skin tumours. Female lynx typically have bite wounds on the neck if examined during or shortly after the mating period (Van Mulders & Marti, 2024).

Evaluation of general **body condition** (muscles, skeleton) and palpation of **lymph nodes**. Any irregularities can be indicative of a problem. The **nails** and **footpads** should be examined for any

signs of wounds, swelling, or warmth. Although hyperthyroidism seems rare in lynx in comparison to other Felid species, **thyroid palpation** can be executed by gently stroking both fingers along the trachea, starting from the larynx and moving towards the thoracic inlet. Normally, the thyroid glands should not be palpable. However, if you detect any symmetrical or asymmetrical enlargement or nodule, it should raise concerns about potential underlying thyroid disease. The **ears** should be inspected externally for signs of scratching (redness, crusts) or extensive amounts of cerumen. Otoscopic examination can be of additional value to assess the external ear canal for signs of inflammation as this may indicate external otitis, which may have different causes including parasitic (*Otodectes* spp.), bacterial and yeast infections (Van Mulders & Marti, 2024).

When examining the **eye**, in healthy lynx the conjunctiva of the eyelids is not readily visible and has a pale pink colour. The conjunctival membranes become red and swollen in cases of conjunctivitis potentially in combination with ocular discharge and may warrant further testing (FHV, FCV, chlamydia, mycoplasma). Recently, cases of blindness have been observed on one eye or both in free-ranging lynx (Quintard, pers. comm.). Therefore, it is recommended to take photos of lynx eyes during capture (front and lateral view). If possible, an ophthalmoscope view is preferable to see all the organs of the eye. We also recommend taking samples from the eye or lacrimal region for checking for the presence of the eyeworm *Thelazia callipaeda*. Samples are taken from the area above the eye, the third eyelid, and the lacrimal sacs of the eye using sterile Q-tips. Generally, the eyeworm is visible on the retina as they are 15–20 mm long and very shiny.

The **nasal plane** should have a slightly moist texture. There should be no nasal discharge present, and there should be no signs of chronic nasal discharge, such as reddening and hair loss under the nostrils. When nasal discharge is observed, the type (serous, mucous, blood, …) must be described, as this may warn for additional sampling (FHV, FCV, chlamydia, mycoplasma, …).

It is essential to conduct a comprehensive examination of the **teeth, gingiva, and oral cavity** to identify any abnormalities. Special attention should be given to the identification and management of canine fractures or dental wear for age determination according to Marti and Ryser-Degiorgis (2018). In instances where the dental pulp is exposed, prompt and appropriate treatment should be implemented, given that this is a painful condition and to prevent the development of dental root abscesses (see Appendix XI of Linking Lynx protocols linking-lynx.org/?action=get_file&id=24&resource_link_id=144).

During **cardiac auscultation**, it is relevant to evaluate both the heart rate and rhythm. Any irregularities in the heart rhythm should be carefully noted and documented. Additionally, any abnormal heart sounds (murmurs), should be described in terms of their intensity, the timing of occurrence (systolic, diastolic, or continuous), and their location (left or right side, heart apex, or base). It is useful to have a stethoscope that can record sounds, so the recording can be analysed later. Any abnormal rhythm (including brady/tachycardia) or auscultated murmur (> 2/6) may indicate further electrocardiographic and/or echocardiographic examination. For further details see the protocol on cardiac screening (Appendix VIII of Linking Lynx protocols linking-

lynx.org/?action=get_file&id=24&resource_link_id=142). It's important to keep in mind that certain irregular rhythms (such as 2nd degree AV-blocks), bradycardia, and minor valvular murmurs might be induced or exacerbated by the use of anaesthetics, particularly alpha-2-agonists.

A point-of-care test (i.e., a fast field test) for feline immunodeficiency virus (FIV) antibody and feline leukaemia virus (FeLV) antigen detection validated for domestic cats (SNAP FIV/FeLV Combo Test, IDEXX, Switzerland) should be done already in the field. Although experiences with testing for FIV in Switzerland have shown that this fast field test may deliver false negative results, experiences in the Iberian lynx suggest that the test can detect progressive FeLV infections that might end fatally (Ryser-Degiorgis et al., 2021). Lynx with a positive SNAP test result should be taken to captivity. If a progressive infection is confirmed by laboratory testing, they should be extracted from the population (Meli et al., 2010). Otherwise, the lynx is released at the capture site.

Reference values for **temperature** in lynx are not well established. Therefore, it may be appropriate to adapt normal rectal temperature ranges from those observed in domestic cats, typically ranging from 37.5–39°C. However, note that the upper limit for lynx may not be as high. It is important to consider factors such as pre-anaesthetic excitement and stress, as they can potentially induce hyperthermia. Additionally, in healthy animals, it's common for body temperatures to slightly decrease during the course of anaesthesia (Van Mulders & Marti, 2024).

**Blood samples** are collected for:

1. Complete Blood Count (CBC): • Hemoglobin (Hb) • Hematocrit (Hct) • Red Blood Cell Count (RBC) • White Blood Cell Count (WBC) • Platelet Count • Mean Corpuscular Volume (MCV) • Mean Corpuscular Hemoglobin (MCH) • Mean Corpuscular Hemoglobin Concentration (MCHC) • Differential White Blood Cell Count (neutrophils, lymphocytes, monocytes, eosinophils, basophils)

2. Blood Chemistry Panel: • Blood Glucose • Blood Urea Nitrogen (BUN) • Creatinine • Total Protein • Albumin • Globulin • Alanine Aminotransferase (ALT) • Aspartate Aminotransferase (AST) • Alkaline Phosphatase (ALP) • Creatine Kinase (CK) • Total Bilirubin • Thyroid Function Test (tT4) in senior and geriatric animals

3. Electrolytes and others: • Calcium (total) • Phosphorus • Sodium • Potassium • Chloride

Use EDTA tubes for PCR and haematology and plain tubes for serum. Let serum tubes clot 30–60 min, then centrifuge and transfer the clear serum. If centrifugation isn’t available, let the blood sit cool for up to 1–2 days; clot will form and serum will separate naturally. Transfer the clear serum to a clean labelled tube as soon as possible. Store all samples in a cool box (4°C) and freeze at -20°C or -80°C if not processed within 1–2 days. Label everything with ID, date, and type, and avoid repeated freeze-thaw cycles. The collection of 8 ml EDTA and 8 ml serum should be sufficient for the analyses and the retention of reserve blood (Marti, pers. comm.). Additionally, blood can be

archived using blood-stain cards and these samples can be used to analyse various biomolecules and for the isolation of the DNA.

**Faecal examination**: A standard faecal examination for routine screening should include the following tests:

A. Parasitology: • Sedimentation/floatation for *Toxocara* spp., *Toxascaris* spp., cestodes, *Cystoisospora* spp., *Eucoleus* spp., … • Specific Ag testing for *Giardia* spp. • Baermann's funnel technique for *Aelurostrongylus* spp. and other lungworms in endemic areas.

B. Bacteriology: Note that felids in human care frequently shed Salmonella spp., seldom linked to the development of diarrhoea.

Swabs of eye, nose, pharynx and anus are taken for virology analysis, preferably two swabs of each opening. Use sterile dry cotton swabs in a tube (https://www.puritanmedproducts.com/25-806-1pc-bt.html) and store in the freezer (Fig. 2).

Sample of hair (both guard hairs and underfur, plucked, cut or shaved, at least 0.2 g) should be stored in paper envelopes and archived in a dry place, for future toxicology analysis.

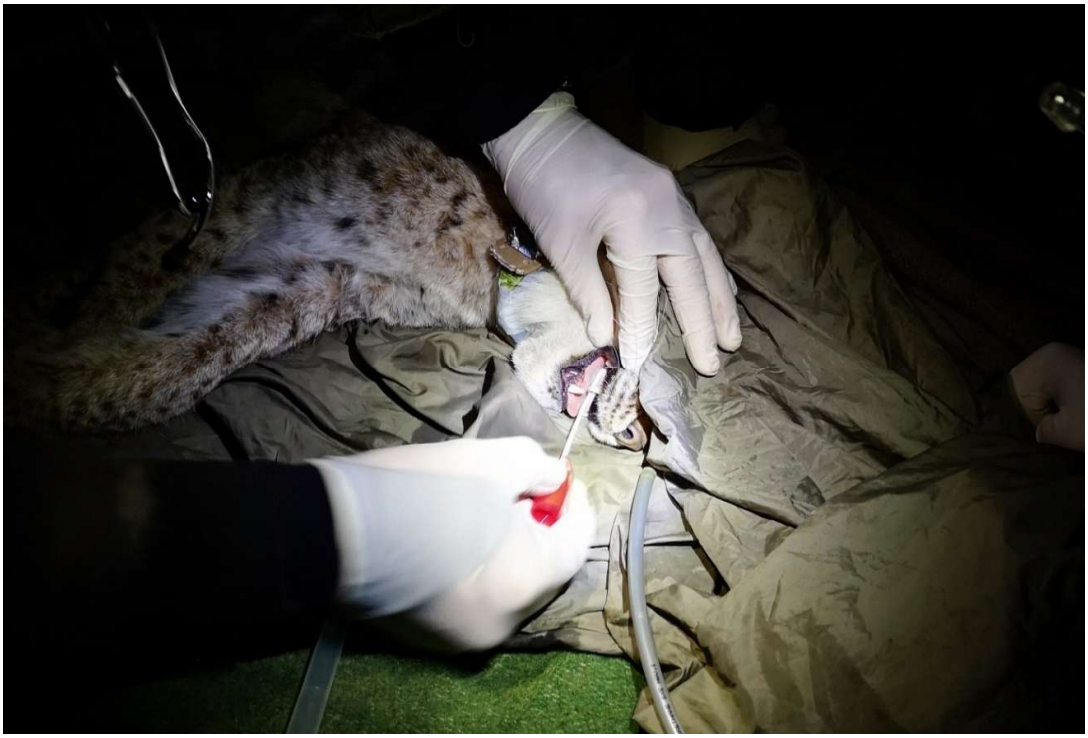

**Fig. 2.** Checking mouth cavity and taking swab. *Photo*: Špela Sokolovič

**Pathogen testing** is mandatory for feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV), feline calicivirus (FCV), feline herpes virus (FHV), *Chlamydia felis*, feline panleukopenia virus (FPV) and canine distemper virus (CDV), and highly recommended for feline heartworm (FHW), *Leptospira interrogans* spp., *Toxoplasma gondii*, *Cytauxzoon felis*, haemotropic mycoplasmas, feline corona virus (FCoV), *Dirofilaria immitis*, SARS-CoV-2, avian influenza and *Echinoccocus* spp., tick-borne pathogens *Babesia* spp., *Ehrlichia* spp., *Anaplasma* spp. (Van Mulders & Marti, 2024).

Health screening checklist in live captures:

- Sex, body condition, measurements and weight are recorded
- SNAP test for FIV and FeLV
- Blood sampling for FIV
- Blood sampling for FeLV
- Blood sampling for Complete Blood Count, chemistry, genetic and pathogen analysis
- Blood sampling for storage (backup)
- Hair sample for toxicology
- Photo of both sides of the body
- Photo of teeth (age determination)
- Photo of eyes (front and lateral view) to detect glaucoma or other eye diseases
- Antibiotics for wound treatment
- Ivermectin for treatment of sarcoptic mange (or general parasite treatment)
- Heart examination

# Faeces analysis

All fresh scats discovered in the field are collected for genetic analyses, parasitology (see above) and pathogens. Scats are considered fresh if they have a strong smell, look moist and possibly slimy. For genetic analysis, a sample is collected from the surface of the scat, if possible from a part that is not in contact with the ground (Fig. 3). That part of the scat is the first to dry out, which conserves the DNA. If the end of the scat is seen (the conical “tuft” or “tail” sticking out from one end), we should try collecting a sample from there. If the sample was exposed to heavy rain, we try to take a sample from the least exposed part. If there is mucus on the sample, we should try to collect it since it contains a lot of target DNA. We collect a pea-sized sample of the scat (Fig. 4) and put it in the flask with scat conservation liquid (Skrbinšek, 2017). Check with the laboratory which will do the analyses of what liquid to use. Record date, coordinates etc. and send it to the

laboratory for DNA extraction as soon as possible. Of the remaining scat, collect half of it for the health screening (see above).

**Fig. 3.** DNA sampling from faeces. *Photo*: A. Molinari-Jobin

**Fig. 4.** We collect only a pea-sized piece for it to be fully emerged in conserving liquid (ethanol). *Photo*: A. Molinari-Jobin

# Genomic analysis

For doing a more comprehensive genetic health screening that involves identifying defective or maladaptive genes, quantifying genetic load and other fitness-related measurements, it is advisable to obtain samples that provide enough DNA for whole genome sequencing, which is either fresh blood (collected in whole blood tubes during live capture or post-mortem examination), or a tissue sample from a deceased individual, as it is the most invasive method of obtaining samples. They are stored in appropriate buffers (TES buffer for blood, NAS buffer for tissue), and treated following the protocol adopted by the Iberian lynx team (Godoy, pers. comm.):

Blood in TES buffer:

1. Prepare in advance two 15 ml Falcon tubes containing 8 ml of TES Buffer (Appendix 2)
2. Add 2 ml of blood collected with a syringe or Vacutainer (Heparin or EDTA) to each tube. Mix gently until completely homogenised.
3. Label the tubes with the sample code using a permanent marker.
4. Fill in the corresponding data sheet for each sample.
5. Store at 4°C when possible; if not, at room temperature and in the dark.
6. Ship or transport at room temperature.

Tissue in NAS buffer:

1. With a clean scalpel or disposable blade (do not use for more than one sample) cut the tissue into pieces of no more than 3 $mm^3$.
2. Transfer to tube containing NAS buffer (Appendix 3). Maintain a ratio of at least 1:10 of sample to buffer (e.g. max 150 mg of tissue in 1.5 ml of buffer, or 1.5 g in 15 ml of buffer)
3. Label the tubes with the corresponding code.
4. Leave at least a couple of hours at room temperature to allow the buffer to penetrate the tissue
5. Fill in the corresponding data sheet for each sample
6. Freeze when possible; better at -80°C, at least at -20°C.
7. Ship or transport at room temperature.

The buffers can be made in-house using the preparation recipes in Appendix 2 and 3 of this document (Godoy, pers. comm.).

It is imperative that all samples are labelled with a unique identifier, written with a permanent marker or pencil on the sample tube. If alcohol is used, it is advisable to use a pencil and/or protect the text with clear tape. All samples must be accompanied by a descriptive sheet containing all relevant metadata, such as: identity, coordinates, date of collection and sex of individual.

For simplicity and avoiding logistic difficulties of shipping biological samples internationally, DNA extraction of the samples can be done in the country of sample origin, in a molecular biology lab, following any established DNA extraction protocol (an organic extraction using a standard phenol-chloroform protocol is recommended for maximum DNA yield).

Library construction and whole genome sequencing is usually outsourced to a sequencing facility (either a private company or a genomic lab at a university). Prior to shipping, all samples are checked for quality and quantity as per the sequencing facility's recommendations. All tubes that will be shipped need to be closed well and sealed with Parafilm to prevent spillage and contamination, placed in large envelopes or boxes wrapped in bubble wrap or similar material to prevent transport damages. Samples preserved in TES buffer, NAS buffer or ethanol can be transported at room temperatures, while frozen samples are required to be shipped with excess dry ice (minimum 5 kg for transports shorter than two days) in insulated containers.

# Acknowledgements

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# Appendix 1. Lynx Necropsy Protocol[1]

**1. General overview of workflow**

The post-mortem examination of submitted lynx is carried out according to the following steps:

- □ Case registration in clinic system
- □ Radiography (latero-lateral projection whole body; additional projections in case of abnormalities)
- □ External examination
    - O Photo documentation of the exterior (general overviews for photo-ID, abnormalities, dentition)
    - O Age estimation based on dentition
    - O Morphometrics
- □ Necropsy - internal examination with preservation of skeletal and pelt integrity:
    - O Photo documentation of organs (heart, kidneys, uterus, stomach content, any abnormalities)
    - O Measurements: Heart, adrenal gland, thyroid gland, testicles
    - O Sample collection

        Organs (biobank, virology)

        Histopathology

        Parasitology

        Genetics
- □ Processing post-necropsy:
    - O Initial description of macroscopic examination
    - O Transfer images and lynx data sheet to Balkan lynx team
    - O Histology: sectioning and description

[1] FIWI version 2024

## 2. Explanations

### 2.1 Case Registration

Case registration according to institute rules. The submitted paperwork should be checked for completeness and correctness, specifically complete anamnesis and exact location.

### 2.2 Radiography

#### 2.2.1. Radiology request submission

The main inquiries include age estimation, evidence of metal-dense fragments and skeletal changes. The radiology department is contacted by phone to confirm a time slot.

#### 2.2.2. Radiography

A standard latero-lateral whole body overview radiograph is performed for each case.

In case of abnormalities (metal-dense fragments, skeletal changes, etc.), a second projection (ventro-dorsal or dorso-ventral) is made.

### 2.3 External examination

The external examination is carried out according to standard pathology procedure. In addition, the animal's body is measured and photographed.

#### 2.3.1 Morphometrics

Bodyweight (g) without bags or other transport materials.

BL-Physiological head-to-torso length: The lynx is placed on its right side in a natural body position (like an anaesthetised animal). The length from the tip of the nose to the base of the tail (sacrococcygeal joint) is measured along the natural body contour.

BL-Stretched head-to-torso length: The animal is stretched at the edge of the table. The distance from the tip of the nose to the base of the tail is measured projected onto the straight edge of the table.

Ear length (Breitenmoser method): Inner ear length from the antihelix to the ear excluding the fur tuft.

Maxillar/mandibular intercanine distance (ICD): Measured from canine tip to canine tip.

Shoulder height (SH): Physiological posture from the plantar paw to the dorsal scapula.

Hind foot length (HFL) right & left: Measured from the distal end of the longest paw pad to the tuber calcanei.

Anal-genital distance (AGD): Centre of rectum to centre of penis/vulva.

### 2.3.2 Age estimation

The age is estimated according to the Marti & Ryser 2018 assessment scheme.

### 2.3.3 External photodocumentation / Lynx-ID

The following photos are taken:

- Full body lateral overview, both sides: The limbs must be offset so that the coat pattern is visible on the medial surfaces of the limbs
- Back
- Head, both sides
- Dentition: Frontal and both lateral views. The mouth should be opened as wide as possible for the view of maxillary M1.
- Teats

## 2.4 Internal examination / necropsy

The necropsy should retain cadaver quality for eventual taxidermy. If specific questions arise which would require further damage to the cadaver integrity, the responsible authorities are informed. The cadaver is opened from the sternum to the pelvis along the *linea alba* and ventrally in the throat area (thyroid gland). The removal of the organs follows the standard necropsy procedure as far as possible.

### 2.4.1. Photodocumentation of inner organs

The following organs are photographed:

- Heart
- Uterus opened longitudinally (focus on placental scarring)

- Kidneys
- Stomach contents
- Foetus, including SSL
- Any observed changes to the body or organs

### 2.4.2 Organ measurements

The following organs are weighed, measured and noted on the 'Lynx data sheet':

- Heart: weight in g (blood clots removed), wall thickness in mm
- Adrenal gland (Li, Re): weighed using the mg scale
- Thyroid gland: length x width in mm

### 2.4.3 Focal points and deviations from routine necropsy protocols

**Ears:** Documentation of any deformations of pinnae. Assess ear canals by inspection. In case of increased cerumen, examine the material microscopically for mites. Describe the presence/absence of mites on the diagnostic report.

**Heart**: The macroscopic examination is performed as follows:

Remove the heart and lungs in one piece

Take photos of the outer sides (facies auricularis, facies atrialis)

Make an incision of the heart according to specifications and assessment with OA

Separate the heart from the lung with at least 2 cm of exposed aorta

Take photos of the heart chambers/outflow tracts (left ventricle AV & aortic valve, right ventricle AV valves, right ventricle pulmonary valves)

Measure wall thickness (right ventricle, septum, left ventricle) and weight (blood clots removed)

Fixation for histology: entire heart in small individuals, transverse slice of all walls at mid-heart level in larger individuals, longitudinal section of left outflow tract)

**Uterus:** Open the uterine horns longitudinally and check for placental scars.

### 2.4.4. Sample collection for archiving

Sampling is carried out according to the protocol. Labelling includes animal species, sampling date, sample type and purpose noted in each case.

**Biobank:**

- 4 $cm^3$ pieces of the following organs: Lung, liver, spleen, kidney, muscle, reproductive organs
- (1 testis/1 ovary with uterine horn)
- Several Eppendorf's of EDTA whole blood (n=3) and serum (as many as possible)
- A small amount of faeces from the rectum (approx. hazelnut to walnut-sized piece)
- A hair sample (incl. hair root)

**Blood samples:**

- The serum tube (10 ml) is centrifuged at 3500 RPM, at 4°C for 20 min. The serum is pipetted into appropriately labelled Eppendorf tubes.
- The EDTA blood is transferred to the appropriately labelled Eppendorf tubes.

After centrifugation, the blood samples can be stored in the freezer.

**Virology:**

- Spleen
- Lymph nodes (mesenteric > mandibular, others)
- several Eppendorf tubes EDTA whole blood (n=3)

After the dissection, this bag is stored at -80°C.

**Histology:**

The following organs are sampled and trimmed for histopathological examination:

- Lungs (4x)
- Heart (cross-section of the three walls at mid-height, aorta outflow tract)
- Liver (2x)
- Spleen
- Kidney, both sides
- Urinary bladder
- Skeletal muscle (psoas major > quadriceps > diaphragm)
- Testicles or part of uterus with ovary
- Thyroid gland, incl. trachea
- Stomach, duodenum & pancreas, ileum, large intestine
- Lymph nodes (mesenteric > mandibular > other, localisation should be noted in the description)
- All other organs with notable changes

**Further details on histology**

Trimming: The formalin samples are cut as a standard after 24 hours.

Heart: The three heart walls and a longitudinal section through the aortic valve should be cut into 2 capsules. A Van Giessen staining is ordered directly.

Asservation: After case closure, the formalin container with samples is placed for asservation. Store the samples permanently after sealing.

### 2.4.5 Parasitology

The following 2 samples are submitted to the parasitology department:

Native faeces (rectal faeces) for gastrointestinal parasites and lungworms

Musculature (base of tongue, diaphragm, at least 20 g) for *Trichinella sp.*

### 2.4.6 Genetics

One piece each of **spleen** and **muscle** are asserved in the same plastic bag. This is stored at -20°C.

If **foetuses** are present, pack a piece of spleen and muscle from each foetus separately. For small foetuses, remove each foetus completely and pack separately.

### 2.4.7 Further examinations

Further examinations (bacteriology, virology, molecular genetic testing) are carried out if necessary.

### 2.4.8 Carcass

The entire carcass is labelled and stored in the freezer. This is noted on the lists of the respective freezer.

## 2.5 Post-processing

The post-processing includes the following:

The sender is informed of the main findings by telephone.

Images and ‘lynx data sheet are sent to team within two working days.

Organs are cut for histopathological examination (see Histology), assessed and described. This usually includes a case discussion.

The provisional report is prepared.

Case closure after final adaptions.

Diagnostic checklist, lynx data sheet and sample collection sheet are inserted in computer.

## 3.1. Final report

The entire provisional report is first sent to the pathologist. The pathologist forwards the report to the field team.

# Appendix 2. TES buffer preparation

**Final concentrations:** 100mM Tris pH 7,5, 100mM EDTA, 2% SDS

**Source**: LGD -NCI, e.g. Haag T, Santos AS, De Angelo C, et al. (2009) Genetica 136, 505-512

Buffer is stored at room temperature (20-25 °C).

| **From stock solutions** | **100 ml** | **1 L** |
|---|---|---|
| 0.5M Tris HCl pH7.5 | 20 ml | 200 ml |
| 0.5M EDTA | 20 ml | 200 ml |
| 20% SDS | 10 ml | 100 ml |
| Sterile distilled water | 50 ml | 500 ml |

| **From reagents** | **100 ml** | **1 L** |
|---|---|---|
| Tris base (MW=121.2) | 1.2 g | 12 g |
| EDTA-$Na_2$ (MW= 372.2) | 3.7 g | 37 g |
| SDS | 2.0 g | 20 g |
| Sterile distilled water | Up to 100ml | Up to 1000ml |

Adjust pH to 7.5-8.0.

# Appendix 3. NAS buffer preparation

**Final concentrations:** EDTA disodium salt dihydrate

0.019 M, sodium citrate trisodium dihydrate salt 0.018 M, ammonium sulfate 3.8 M

| **From reagents** | **1 L** |
|---|---|
| Sodium citrate trisodium dihydrate salt (MW = 294.1) | 7.07 g |
| EDTA-$Na_2$ (MW = 372.24)* | 5.29 g |
| Ammonium sulfate (MW = 132.14) | 502.13 g |
| Sterile distilled water (miliQ) | Up to 1000ml |

1. Stir with low to moderate heat until the ammonium sulfate is completely dissolved, which usually takes hours.
2. Cool to room temperature, then adjust the pH to 5.2 with $H_2SO_4$.
3. Store at room temperature or keep refrigerated until divided into aliquots.
4. Dispense 1.5 ml aliquots of buffer into 2 ml screw cap tubes for preservation of up to 150 mg of cut tissue.
5. Dispense 10 ml aliquots into 15 ml Falcon tubes for preservation of up to 1 g of cut tissue.

Store at room temperature (20–25°C).