

3.18 NECROPSY PROCEDURES

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NECROPSY LOCATION or ROOM

Procedure

- Protective gear i.e. overalls, gloves, boots and aprons should be used. **In field situations, if level 3 or 4 biosecurity measures are suspected to be needed, contact the** Great Ape Health Monitoring Unit or WCS field veterinary staff for further advice
- A disinfectant foot bath should be at the entrance to the post mortem room area.
- After each necropsy, the kit is washed and stored, the table is cleaned down and disinfected and the floor is hosed down, washed and disinfected each time
- The Necropsy room should be well stocked at all times with items in the checklist.
- A full set of Necropsy instruments should be available and in good condition.
- Labels, pens, labelling pens should be available.
- When cleaning the room, carcasses should not be mixed with non biodegradable material. They should be separated and disposed of accordingly.

NECROPSY SAFETY PRECAUTIONS

Personal Safety

Because some diseases of primates can cause serious illness or death in humans, all carcasses should be handled as if they were harbouring potentially dangerous diseases and precautions for personal safety should be exercised. Minimal protective clothing includes coveralls, gloves and a mask that covers the nose and mouth, rubber boots.

Overalls with a washable rubber apron and rubber boots is recommended with face mask, coveralls, and double gloves.

Samples Handling and Carcase Handling and Disposal

Diseased wildlife also should be handled to minimise exposure of other people and other animals.

There is a duty of care to ensure that observers know of the hazards and are provided with protective clothing (especially if they are asked to participate.) All sample containers, swabs, blood samples should be handled by the dissector only and placed in a plastic box for transportation. The dissector should ensure that no leakage occurs.

The carcase maybe required for further research. The carcase should be doubled bagged in large heavy duty plastic bags for transportation. Any tissues not required should be buried in a deep hole or incinerated and not left unattended.

Cleaning

The examination table and floors should be thoroughly washed and then disinfected.

All contaminated waste paper or plastic materials should be incinerated or disinfected and bagged for removal.

All instruments should be cleaned with soap and warm water to remove blood and tissues and then they should be disinfected

Necropsy boots, apron and overalls should be washed and cleaned

The external surfaces of any containers with samples should be washed and disinfected.

NECROPSY INSTRUMENTS

Most damage to instruments sustain relates to cleaning, particularly delayed cleaning.

- Clean with a soft brush (open joints and/or disassemble).
- Soak in detergent
- Rinse
- Dunk in Surgical milk
- Place on padded surface to drain
- Instruments are ready to use
- Do not use abrasives or steel wool on instruments
- Avoid metal to metal contact
- Avoid over loading, e.g. cutting tissues that are too hard or thick with scissors

CHECKLIST FOR NECROPSY

Protective Clothing

- Rubber Gloves
- Rubber Boots
- Rubber Apron
- Overalls
- Mask (to cover mouth and nose) and eye goggles/ face shield

Necropsy Equipment

- Curved knife for skinning
- Straight pointed knife for dissection
- Sharpening stone or steel
- Dissecting scissors (small and large)

- 15-20cm rat tooth forceps
- 15-20cm pointed forceps
- Scalpel handle and blades
- Hack saw or bone saw
- Small and large shears
- Chisel and mallet/hammer
- Panga for removing spinal cord
- Chopping board
- Alcohol for sterilizing instruments
- Oscillating saw (in the future)

Specimen Containers and Sampling Equipment

- 5ml and 10ml syringes
- 20G needles
- Many 3-500ml wide mouth tight fitting containers or plastic jars for tissues
- Serum vacutainer tubes
- CPT vacutainer tubes
- Microscope slides
- Microscope slide container – small
- Sterile culture swabs with transport medium
- Small universal bottles for fluid samples, urine or for parasites
- Permanent marker pens and pencils
- Labels, labelling tape and tags
- String
- Plastic ruler or measuring tape
- Plastic zip-lock bags
- Aluminium foil
- Plastic pipettes
- Absorbent towel

Transport Materials

- Insulated cooler box with ice blocks
- Plastic box for transporting containers
- Sterile buffered glycerine (50%)

Fixatives

- 10% buffered formalin
- (Make by diluting 100ml 37% formalin with 900ml water. Add 4g sodium phosphate monobasic and 6.5g sodium phosphate dibasic)
- 100% acetone for cytology
- 70% ethyl alcohol for parasites
- Decal Solution (Mix 500mls formic acid (88-91% pure) with 4.5L buffered formalin).
- Bouins Solution (if necessary)

Disposal, Cleaning And Disinfecting Materials

- Plastic container or large thick plastic bags with string to hold carcase or parts of the carcase to move to Medical Waste Hole
- Rubbish bags
- Sharps containers
- Plastic bucket and brush
- Nailbrush, soap and towel
- Disinfectant - dettol
- Sodium hypochlorite (0.5%)
- 70% ethyl alcohol for disinfecting instruments

Documentation

- Camera and film
- Notebook and necropsy worksheets
- Necropsy procedures and protocols
- Pen and pencils

PERFORMING A PRIMATE NECROPSY

Introduction

- This procedure should be read with the necropsy sampling procedures below before the necropsy is performed
- All primates that die should be necropsied as soon after death as possible to establish the cause of death and to generate reference material for later study. It is vital that infectious diseases are recognised promptly and appropriate action taken to safeguard any in-contact primates.
- All necropsies should be done in a thorough, consistent and systematic way.
- All equipment should be available (see checklist) and all samples containers are labelled and readily accessible.
- Normally one or two people help with the dissection because a thorough dissection may take 3 hours.
- Store the body refrigerated at 4°C until necropsy where this can be performed within 72hrs of death. If a fridge is not available keep the body as cool as possible.
- Do not freeze as ice formation within tissues considerably reduces the value of subsequent histological examination.
- When it is not possible to carry out a necropsy within 72hrs, the body should be frozen to arrest decomposition. Although histological examination then becomes difficult, at least gross lesions will still be identifiable once the body is thawed.
- In many cases bacterial cultures of tissue samples will still have value. Once thoroughly frozen at -20°C or below, the body will be preserved for many months. Thawing may take longer than one might expect - allow at least 1 day for an adult female and up to 2 days for an adult male. Carrying out a necropsy on a frozen and thawed body is far from ideal and all attempts should be made to perform the necropsy before freezing is necessary.
- Photographs and or video recording of the necropsy is important for future reference. Photographs should be taken with a blue non reflective back ground and the sample/body should be labelled (to include date, species, ID, comparative measurement)
- All tissues sampled should be taken in duplicate as a minimum for possible future use. One set is sent for histopathology and the other set is kept for future reference. Representative samples of all tissues are collected and this includes apparently normal tissues and any abnormalities particularly with adjacent normal tissue.
- Any abnormalities should be described in the present tense using full sentences. The following criteria should be used
 - Location
 - Number and distribution
 - Colour
 - Shape

- Size
- Consistency and texture

History

- Note any accounts of the longevity of any illness.
- Read medical notes if available
- Note the ambient temperature and recent weather conditions
- Note any signs of struggle

External Examination

- Weigh the animal and record.
- Note any wounds. If present, look for bruising and bleeding in the tissues near the wounds which would indicate that they occurred before the animal died. Note any broken bones, broken or missing teeth
- Estimate the time of death and record.
- Note the muscle mass and condition of the chimpanzee and possible hydration status.
- A thorough visual examination is made of the entire body including all body orifices and any changes noted. The oral cavity should be examined and the condition of the teeth should be recorded. The condition of the skin and hair is noted and any signs of injuries/trauma to the head and body
- The extent of any external parasites should be noted and samples collected in 70% alcohol.

Internal Examination

- The animal is placed on its back (supine position) on the examination table and all four limbs are disarticulated by severing the muscular attachments in the axilla and inguinal regions.
- Viscera can be weighed and measured.
- A ventral midline incision is made from the pubic region to the mandibular symphysis. Note mammary glands, prepuce, penis and testes.
- Reflect the skin to the level of the backbone on right and left sides.
- Open the abdominal cavity along the ventral midline.
- Remove the entire ventral abdominal wall musculature from the lateral aspects of the lumbar vertebrae, dissecting carefully and take note of any abnormalities associated with the abdominal viscera. Take culture samples at this stage and any smears
- Using bone shears open the chest cavity along the backbone and working towards the cranial chest cavity and then continuing on the other side but this time working cranially - caudally. If a cosmetic necropsy be required then the dissection can continue at the costochondral junction on each side or along one side of the sternum being careful not to damage or contaminate the thoracic viscera.

- Remove the ventral chest wall, cutting through the diaphragm and take note of any abnormalities associated with the thoracic viscera. Bacterial cultures should be taken at this stage. Blood from the right side of the heart for later serology and for blood culture should be done at this stage.
- Locate the entry of the oesophagus into the stomach and ligate with string twice. Cut between the two ligatures.
- Remove the stomach and intestines as a unit by detaching the mesenteries from the intestines. Examine and sample any lymph nodes in the mesenteries.
- The pancreas and spleen remain attached to the stomach-intestine unit.
- Cut across the rectum ensuring no faeces fall into the abdominal cavity.
- Separate the bones of the larynx and dissect out the tongue, larynx and trachea and oesophagus and continue to work caudally towards the thoracic inlet. The entire length of the trachea and oesophagus unit and the lungs and heart to the attachment of the vessels and oesophagus as they go through the diaphragm are removed. Ligate the great vessels as they enter the diaphragm if necessary.
- The trachea is dissected from the larynx caudally to the end of the bronchi.
- The cervical and thoracic oesophagus is opened its entire length
- The heart is examined and weighed. Note amount of fat around the heart. The heart is dissected to examine all chambers and all the great vessels are opened to check for atheroma deposits
- Mediastinal lymph nodes are located, examined and sampled.
- The lungs are examined and dissected. Bacterial culture samples are taken from any abscesses and smears made.
- The head is disarticulated from the vertebral column and examined. The eyes, ears and nasal cavity are examined.
- The skin is removed from the head and the temporal muscles removed so that it is easier to remove the cranium. The lines to cut are outlined in order to remove the brain with minimal disturbance. The cranium is removed carefully to reveal the meninges and brain. The surfaces are examined and bacterial cultures and smears are taken immediately. The dura mater is removed and the tentorium cerebelli is cut. The brain stem is severed and the cerebellum and the cranial nerves are severed using gravity to help reduce damage to the brain. The brain is removed and cut mid sagittally; one half is taken for histopathology and the other half can be taken for toxicology/virology.
- The skull is sawed mid-sagittally to examine the nasal cavity, turbinate bones and sinuses. The pharynx is also examined and a clearer view of the tonsils and teeth can be obtained.
- The obturator foramina are located and are dissected. The ischial bone is then sawed on both sides into the obturator foramina. This piece of bone is removed and the pelvic canal is fully exposed. This allows full access to the reproductive tract which can be removed and examined.
- The mucosal and serosal surfaces of the reproductive tract are examined. Samples are taken.
- The adrenal glands are located and examined and transverse samples are taken. The kidneys are located and examined and samples taken of the cortex, medulla and pelvis.
- Joints are examined particularly the stifle and hip joints.

- The spleen is removed from the stomach and examined by slicing across in many sites
- The liver is detached and examined by cutting across in several sites. The hepatic lymph nodes are located and examined. The gall bladder is opened along the length of the bladder and into the bile ducts
- Skeletal muscle is taken from several places if possible including the thigh.
- A long bone preferably the femur is dissected and cracked open. The bone is placed in "decal" solution and the bone marrow is removed.
- Finally, the stomach and intestines are examined. Note the amount of ingesta in stomach and intestinal tract. The pancreas is located, examined, and sampled.
- Then the mesentery is cut along its entire length to allow the intestinal tract to be stretched out and examined more thoroughly. The stomach is opened and the surfaces examined. Note and describe the contents of the stomach and intestinal tract. Some of the contents may be kept for toxicology.
- The entire length of the intestinal tract is opened and examined. Samples are taken at various sites and parasites are removed and stored in 70% alcohol.

DEATH AND POST MORTEM EXAMINATION SUBMISSION FORM
PLEASE COMPLETE ALL SECTIONS BELOW

Free range/ large enclosure/ small enclosure:	Permanent I.D. :	Other I.D. :
Species :	Sex :	
DOB or Approx. age:	Enclosure I.D.:	
Number of this species remaining in enclosure/ area: (record as estimate if necessary)	Any other species in enclosure/ area: If yes what are they?	
Circumstances of death or reason for euthanasia:		
Date died/euthanased: / / Found dead? Euthanased? Predated?		
Previous health status (tick one): 1. Previously healthy? 2. Under treatment? 3. Long term health problems? 4. Disease / deaths in group?		
Breeding history (tick one): 1. Offspring produced before 2. Mated but never produced offspring 3. Opportunity to but never seen mating 4. No opportunity for mating 5. Unknown		
Other Helpful Comments (PTO if required): Any recent husbandry changes or problems? (new introductions / moved / feeding / supplements / lighting / heating)?		

GROSS PATHOLOGY

Pathology Case Number:

Specimen Died:

Submitted by:

Death was spontaneous/ following acute illness/ following chronic illness

Enclosure/ location:

Clinical History:

Prosector:

Necropsy Date:

Death to Necropsy Interval: 0-6 hours, 6-24 hours, > 24 hours (estimate time delay)

Necropsy Location:

Carcass Disposition: incineration/ burial – be as specific as possible:

AGE: Newborn/ baby/ juvenile/ adult / old adult weight :

MORPHOMETRICS:

STATE OF PRESERVATION: Good/ Fair/ Autolysed (Mild)/ Autolysed (Severe)

SYSTEMS

SKIN / APPENDAGES / EXTERNAL EXAM:

SENSORY:

MUSCULAR:

SKELETAL:

DIGESTIVE:

LIVER:

RESPIRATORY:

CARDIOVASCULAR:

LYMPHO RETICULAR:

URINARY:

ENDOCRINE:

REPRODUCTIVE:

NERVOUS:

Comment

Gross PM Key words (in order of importance): (Lesion/disease process, Topography, Topography/lesion/process modifiers, Severity, Chronicity)

I.E- Distribution: Organ(s) – unilateral/ bilateral; focal/ multifocal; locally extensive/ diffuse

Whole Body – Localised/ generalises

Time: peracute/ acute/ subacute/ chronic/ chronic active

Severity: minimal/ slight/ moderate/ severe/ marked

Cause: Verminous/ bacterial/ chemical/ viral/ traumatic/ protozoal/ mycotoc/ toxic etc/

Type: Croupous/ Haemorrhagic/ Purulent/ Necrotic/ Fibrinous/ Fibrinopurulent etc.

Laboratory tests requested/to follow: E.G Bacteriology and Parasitology

Samples Stored: E.G in formalin, frozen and as blocks/slides

NECROPSY SAMPLING PROCEDURES (refer to Section 3.17)

Histopathology

Soft tissues

- Representative samples are taken from all major organs and any abnormal tissues. Two sets of tissues are collected.
- Samples should be no larger than 10mm thick so that they can fix properly.
- Samples should be grasped carefully at the edges. Sections from hollow viscera or skin can be stretched flat on paper before being placed in the fixative container with the paper which can be labelled.
- Most tissues are placed in a common container in 10% buffered formalin. Samples of lymph nodes can be placed in separate containers and labelled.
- All tissues should be submerged in at least 10 times the volume of formalin as the volume of tissues collected.

Tissues contained bone

- Tissues that contain bone should be added to 10% formalin and then decalcified in "Decal Solution"
- Tissues should be submerged in "Decal Solution" in at least 20 times the volume of tissues collected.
- Renew the solution every third day and cut specimens into smaller pieces when possible.
- Place into 10% buffered formalin when tissues are readily sectioned with a scalpel blade.
- The container should be labelled with the Sanctuary ID, animals ID, age, sex, and date using a permanent marker pen or label.

Bacteriology

- Attempt to take samples without contaminating them. Take samples before touching the tissues and use sterile instruments (usually immersed in alcohol and flame them until red hot using a burner).
- Samples can be taken using a culture swab or sterile syringe and needle and placing contents into transport media or by placing a large tissue sample in a sterile container.
- Take samples from the edge of a potentially infected area where the bacteria are more likely to be alive.
- If there are no obvious signs of infection then samples from standard locations are suitable ie lung, liver, tonsil, kidney, spleen and intestines.
- Blood can be taken using a syringe and needle and removing any blood from the right side of the heart and dropping the blood onto the tip of the culture swab.
- Keep all samples moist using transport media, sealed and kept cool. If refrigeration is not possible then samples can be kept in buffered glycerine.
- Smears of pus or infected tissues are useful and can be air-dried, fixed in methanol, labelled and sent with other culture samples to the laboratory.

Virology

- Small segments of tissue ie liver, lung, heart, kidney, and any tissues with suspected lesions can be wrapped in aluminium foil, labelled and frozen.
- Whole blood can be stored in CPT tubes.
- Tissue samples can be stored in RNA Later

Toxicology

- Small segments of tissue ie liver, lung, heart, kidney, and any tissues with suspected lesions can be wrapped in aluminium foil, labelled and frozen.
- Contents of stomach can be kept in a small zip lock bag, labelled and stored frozen.

Serology

- Blood can be taken from the right side of the heart. If not clotted yet, then it can be allowed to clot and then spun down to remove the serum which is kept in a labelled plastic vial. If no centrifuge is available then the blood can be allowed to settle and the serum/plasma removed using syringe and needle and transferred into an EDTA tube or serum tube.
- Serum from whole blood can be removed by turning the vacutainer container upside down until it clots and the clot attaches to the rubber stopper. The vacutainer is then turned the correct way up and the rubber removed with the clot leaving serum in the tube which can be decanted to smaller vials and labelled.

Parasitology

- Make three blood smears and air dry
- Collect helminths in 70% alcohol or 10% buffered formalin
- External parasites can be collected in 70% alcohol.
- Faeces is stored in 10% buffered formalin

Cytology

- Make a clean cut of the tissue required and take a sample
- Grasp the sample and blot on a paper towel until no blood or clots are noticeable
- Gently touch the blotted surface on clean slides several times.
- Air dry slides and label

FIXED TISSUE CHECKLIST

Preserve the following tissues and samples of all lesions in 10mm segments in 10% buffered formalin.

1. Skin - full thickness abdominal skin, lip
2. Mammary gland
3. Salivary gland
4. Oral pharyngeal mucosa and tonsil
5. Tongue
6. Trachea
7. Larynx including air sac
8. Thyroid gland
9. Parathyroid gland
10. Lymph nodes - cervical, mediastinal, bronchial, mesenteric, popliteal, axillary, inguinal
11. Thymus
12. Lung - sections from several lobes and bronchi
13. Heart - Section including atrium, ventricle and valves from R & L heart, pericardium
14. Great vessels - especially pulmonary artery and coronary artery
15. Liver - sections from 3 different areas
16. Gall bladder and bile ducts
17. Spleen - cross section including capsule
18. Oesophagus - 3 cm long section
19. Stomach - 3 cm long section
20. Duodenum - 3 cm long section
21. Ileum - 3 cm long section
22. Caecum - 3 cm long section
23. Colon - 3 cm long section
24. Rectum - 3 cm long section
25. Omentum - 3cm square
26. Pancreas - section from two areas
27. Adrenal gland - transverse incision
28. Kidney - cortex, medulla and pelvis of each kidney
29. Bladder, ureters, urethra - cross section of bladder and sections of ureter and urethra
30. Uterus, ovary, cervix, vagina
31. Testes - transversely cut section
32. Prostate - transversely cut section
33. Eye
34. Brain - cut longitudinally along the midline
35. Spinal cord - sections from various sites
36. Diaphragm
37. Skeletal muscle - cross section of thigh muscle
38. Opened rib or longitudinally section of femur - marrow must be exposed for proper fixation

- 39. Joint tissues
- 40. Nerve tissue - brachial plexus
- 41. Neonate - umbilical stump.
- 42. Long bone - 1/2 of a femur including growth plate unless skeleton is required for other purposes.

Tissue Checklist for Microbiology and Toxicology

Tissue	Microbiology	Toxicology
Brain	X	X
Fat		X
Kidney	X	X
Stomach contents		X
Hair	X	X
Liver	X	X
Whole blood	X	X
Lymph nodes	X	X
Tonsils	X	X
Spleen	X	X
Abscesses and granulomas	X	