	NATIONAL CENTRE FOR ANIMAL HEALTH **LABORATORY SERVICES UNIT**	**Second edition**
	STANDARD OPERATING PROCEDURE	**Version 2018.1**
	POSTMORTEM	

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TITLE: Histopathological sample collection

PREPARED BY: Post-mortem Section

REVISED BY: Post-mortem Section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. Purpose
The purpose of this SOP is to describe proper procedures for collecting, preserving, identifying, and processing tissue specimens during necropsy for evaluation by light microscopic histopathological evaluation.

2. General information/responsibility
It is the responsibility of all staff/lab technicians responsible for the collection, preservation, identification, and processing of specimens for histopathological processing, with an aim to prevent post-mortem deteriorative change, or the formation of artefacts, and preserve the integrity of this data.

3. Equipment/materials
3.1 Scalpel with blade
3.2 Scissors
3.3 HP vial
3.4 Sharp and blunt tip scissors
3.5 Cryo vial Rack
3.6 Cryo vials with VTM

4. Reagents, solution and buffer
4.1 10% Buffered Neutral Formalin or
4.2 10% buffered formalin

5. Procedure
5.1 The portion of normal tissue adjacent to the characteristic lesions should be included.
5.2 Fresh unsqueezed tissues should be placed in fixative immediately after they have been removed from an animal.
5.3 The tissue samples meant for the histopathological examination should be less than 5mm thick to ensure thorough fixation.
5.4 The volume of 10% formalin should be minimum 10-20 times the total volume of all the tissues collected.

5.5 The size of the container should be appropriate to the specimen (i.e. no tissue squashing).
5.6 This should be forwarded to laboratory with letter giving detail clinical history, post-mortem report and other general information.
5.7 Do not over pack the container and do not freeze the tissue.
5.8 If tissues could not be submitted to the lab for more than 2 weeks, change the fixative every 2 weeks.

Recommendations for optimal tissue fixation

5.9 Tissue specimens should be placed in fixative immediately after they have been removed from an animal carcass and the fixative should be changed after the first hour of fixation.
5.10 Tissue section should be cut such that the thinnest dimension is no greater than 4-5mm in thickness.
5.11 Initial fixation should be at room temperature since the penetration of formalin is related to the temperature of the solution.
5.12 The formalin should be gently shaken before use to avoid a concentration gradient in the bottle.
5.13 Tissues and organs should be fixed (depending on their size) for 2 hours to a maximum of 24 hours.
5.14 Once fixed, tissues can be removed from formalin and, as long as they are kept moist and protected (e.g. by wrapping in formalin-soaked paper towels, then sealed in screw-capped jars), they can be forwarded to the laboratory without formalin.

6. SAFETY

Flammable liquid and vapour, toxic if swallowed, toxic in contact with skin.

Formalin is considered hazardous and should be handled only after reviewing the ***MSDS***, while wearing gloves, and under a fume hood. Formalin vapour is an irritant to the eyes and nasal passage and prolonged exposure to the skin can cause dermatitis.

7. TROUBLE SHOOT

NA

8. REFERENCES

Bancroft, J.D. and Stevens, A.: theory and practice of histological techniques ed.3, Churchill livingstone inc. 1990. Edinburgh. London, Melbourne and New York.

USAID PREDICT. Marcela Uhart, University of California, Davis (2016) Livestock Sampling Methods: Cattle, Sheep, Goats, Camels, and Swine.

University of South Florida. (2002) Standard Operating Procedures. Histopathology. SOP#: 019.1

9. APPENDIX

A. 10% Buffered Neutral Formalin

100ml Formalin (37-40% stock solution)
900ml Water
4g/L NaH2PO4 (monobasic)
6.5g/L Na2HPO4 (dibasic/anhydrous)

Formaldehyde is a pungent, colourless gas which dissolves **in water and reaches saturation at 37-40% formaldehyde. This can therefore regarded as 100% formalin**. Usually with 10-15% methanol added to prevent polymerization to a solid (paraformaldehyde). This commercial preparation is regarded as 100% formalin and the 10% solution contains approx. 4% formaldehyde.

The final stock solution required is (in ml) =

$$\frac{\textbf{\textit{Required concentration (\%) X Final diluted solution (ml)}}}{\textbf{\textit{Stock solution concentration (\%)}}}$$

Recommendation for 37-40% stock solution

If a solution of formaldehyde is clear, colorless and has no precipitate, and has been stored at room temperature in a tightly sealed bottle that has not been exposed to sunlight, it is considered good, however it is not recommended for using a stock solution *older than 1 year*.

Bottles of 37% formaldehyde that are already opened should *not be used more than six months*. Hence, it is recommended to procure formaldehyde more frequently in smaller quantities than large volume jars.

B. 10% buffered formalin

The formaldehyde has a greater chance for oxidation in this concentration of tissue fixative and eventually the solution will start to drop in pH, in spite of the buffer.

It is recommended that 10% buffered formalin solutions be used no longer than 3 months after they were initially mixed. The solution should be clear, colorless, with no precipitate and the pH should not be below 6.5.

TITLE: Necropsy and post mortem examination in animals

PREPARED BY: Post-mortem section

REVISED BY: Post-mortem section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. PURPOSE

1.1 To outline the proper procedures for performing a necropsy and post mortem examination in livestock.

1.2 Necropsy is to be performed for diagnostic purpose, disease outbreak, research and also vetero-legal cases.

2. RESPONSIBILITY

2.1 Necropsy must be performed by Veterinarians, Veterinary para-professionals on cadavers died due to disease or on research animals.

2.2 Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a herd or flock especially in the farms.

3. APPARATUS

3.1 Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
3.2 Knives – large and small, with sharpening steel.
3.3 Scissors (various sizes) and saws; bone cutters
3.4 A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
3.5 Sterile swabs in test tubes
3.6 Alcohol cleaned slides for smears, preferably in a rack or box
3.7 Spatula
3.8 Plastic bags for specimens
3.9 Petri dishes
3.10 Labels
3.11 Soap, water, disinfectant and towel.

4. REAGENTS, SOLUTION AND BUFFER

4.1 10% Formalin or Buffer Neutral Formalin
4.2 70% alcohol for wetting and disinfecting the skin,
4.3 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
4.4 Normal saline (0.85% NaCl) with a pipette (for parasitological examination),

5. PROCEDURE

5.1 Preparation

5.1.1 Animal necropsy should be performed in areas specifically designated for that purpose in the laboratories or identify an ideal location in the farms or the areas.

5.1.2 Proper personal protective equipment and attire must be worn when performing necropsy. Disposable gloves, shoe covers, and gown must be worn when conducting a necropsy.

5.1.3 Full protective clothing must be worn when handling animals infected with biohazardous materials or chemical carcinogens and includes double gloves, cap, disposable gown, shoe covers, mask (or respirator if required), and eye protection.

5.1.4 Obtain and record the animal and/or herd history.

5.1.5 Make sure appropriate disinfectant is available.

5.1.6 Carefully note external abnormalities, Check orifices, genitals and mammary glands. Make note of any injuries, wounds, parasites. Observe the general appearance of carcass: rigor mortis, nasal and anal tissues, wounds, enlargements, eyes, skin lesion, condition of flesh and visible mucous membranes.

5.2 Necropsy procedure

5.2.1 Ruminants, Swine, Equines

5.2.1.1 Cattle, sheep, goats, and pigs are best positioned on their left side. Horses should be positioned on their right side.
5.2.1.2 Make an external examination and place ectoparasites in 70% ethanol. Conduct a thorough external examination by reviewing the body surface and orifices for abnormalities. Palpate for superficial swellings, or for enlarged organs or masses within body cavities.
5.2.1.3 Collect nasal swabs and skin lesions or swabs, if required. Place in transport or growth nutrient media, and refrigerate.
5.2.1.4 To prevent contamination, disinfect the skin or use clean instruments to open body cavities. Open the abdominal and thoracic cavities carefully so as to prevent contamination from the outside or from a cut organ.
5.2.1.5 Observe, but do not disturb, organ placement, noting any abnormalities. Examine organs and tissues *in situ* before dissecting or collecting tissues and record any abnormalities.
5.2.1.6 With a syringe, aseptically collect a specimen of any abnormal body fluid.

5.2.1.7 Aseptically collect specimens of liver, kidney, spleen, and lymph node (gastrohepatic node for swine).
5.2.1.8 Aseptically collect specimens of lung and heart.
5.2.1.9 Remove the tongue, open the pharynx, and collect the tonsil (swine).
5.2.1.10 Remove the trachea, lung, and heart. Collect tracheal and bronchial swabs, if appropriate. Examine the respiratory tract and heart.
5.2.1.11 Tie off and remove a 3" section of ileum just anterior to the ileocecal valve. Double ligate to prevent spillage of intestinal contents. Do not tie off intestinal segments to be placed in formalin because the fixative should infiltrate the lumen of the organ.
5.2.1.12 Complete the examination of the abdominal cavity. The entire digestive tract should be opened.
5.2.1.13 Decapitate the animal, remove the brain and collect specimens, noting any abnormalities.
5.2.1.14 Indicate on the necropsy form which tissues are collected or sampled that are to be submitted for other tests.
5.2.1.15 All lesions that were observed during the examination, or that are observed during the necropsy must be recorded on the appropriate necropsy form, and include a complete description (e.g., size, number, color, shape, texture, severity, and weight or volume as appropriate) as far as possible.

5.2.2 Dogs & Cats

General procedures of necropsy in Dogs and Cats are same as above. In addition, the procedure for removal of brain has been detailed below:

5.2.2.1 Make an incision through the temporal muscles on each side, on a line from the centre of the foramen magnum, through a point 1/2 inch above ear canal and onto the frontal bone.
5.2.2.2 Saw just through the bone along these lines, being careful not to damage the brain.
5.2.2.3 Now saw transversely through the frontal bone just above the orbit, meeting the two lateral incisions, Lift off the top and remove the brain.
5.2.2.4 Examine the meninges and the brain itself.

6. RISK ASSESSMENT

6.1 If it is suspected for anthrax or rabies as the cause of death, or in the cases of unexplained sudden death, the carcass must never be opened. If anthrax is suspected, a blood slide taken from a cut on the ear is sent to the labs for confirmation. If rabies is suspected – the head should be removed intact and sent to the lab in a sealed container. For small animals the whole carcass can be sent.
6.2 In both cases, protective clothing, including boots, gloves, face mask and goggles must be worn. The carcasses should be burned or deep buried under stones with quick-lime.

6.3 All carcasses should be handled with care especially if they were known to present risk of zoonotic disease.
6.4 No eating, drinking, grooming, or other activities that are a means of exposure are permitted in necropsy areas.
6.5 To limit the risk of unexpected or unknown exposure, all workers handling carcass must be offered vaccination for rabies (in endemic areas).
6.6 Transport unfixed tissues in leak-proof containers.

7. WASTE DISPOSAL

7.1 Decontaminate instruments before cleaning them.
7.2 Clean and disinfect all work surfaces or the premises where necropsy was conducted.
7.3 Decontaminate self (e.g., disinfect and remove boots, gloves, and coveralls).

8. REFERENCES

University of Minnesota, Veterinary Diagnostic Laboratory, Standard Operating Procedure (2016).

University of Queensland, School of veterinary Science, Standard Operating Procedures (2017).

University of South Florida, Standard Operating Procedure, Necropsy and Post Mortem Examination (2012). SOP#: 018.2

USDA, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, Guidelines for Necropsy

TITLE: Necropsy and post mortem examination in poultry

PREPARED BY: Post-mortem section

REVISED BY: Post-mortem section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. **PURPOSE**
   - 1.1 To outline the proper procedures for performing a necropsy and post mortem examination in poultry.
   - 1.2 Necropsy is to be performed for diagnostic purpose, disease outbreak, and research in poultry.

2. RESPONSIBILITY
   - 2.1 Veterinarians, Veterinary para-professionals conduct necropsies and post mortem examinations on cadavers died due to disease or on research birds.
   - 2.2 Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a flock especially in the farms.

3. APPARATUS
   - 3.1 Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
   - 3.2 Knives – large and small, with sharpening steel.
   - 3.3 Scissors (various sizes) and saws; bone cutters
   - 3.4 A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
   - 3.5 Sterile swabs in test tubes
   - 3.6 Alcohol cleaned slides for smears, preferably in a rack or box
   - 3.7 Spatula
   - 3.8 Plastic bags for specimens
   - 3.9 Petri dishes
   - 3.10 Labels
   - 3.11 Soap, water, disinfectant and towel.

4. REAGENTS, SOLUTION AND BUFFER
   - 4.1 10% Formalin or Buffer Neutral Formalin
   - 4.2 70% alcohol for wetting and disinfecting the skin,
   - 4.3 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
   - 4.4 Normal saline (0.85% NaCl) with a pipette (for parasitological examination),

5. PROCEDURE

5.1 Preparation

5.1.1 Full protective clothing must be worn when handling birds infected with zoonotic infection especially (avian influenza) and include double gloves, cap, disposable gown, shoe covers, mask (or respirator if required), and eye protection.

5.1.2 Small birds are wetted and plucked, all other birds should be wetted with 2% virkon on phenol before the necropsy. This is done to allow better visualisation of the skin, to part the feathers to permit incision of the skin and to prevent loose feathers from irritating or harming the personal (zoonosis) or contaminating the viscera.

5.2 External Examination

5.2.1 Examine the skin of the head, body and legs for lice, mites, injury, moulting, swelling and Marek's disease.

5.2.2 Examine the head, including eyes, nostrils, comb, wattles, mouth and beak.

5.2.3 Infected eyes and conjunctivitis are seen in chronic respiratory disease, sinusitis, infectious coryza, infectious laryngo-tracheitis, infectious bronchitis and Raniket disease.

5.2.4 Kerato-conjunctivitis with ulcers in the cornea suggests ammonia burns.

5.2.5 Swollen sinuses are seen in infectious coryza, fowl cholera and Infectious bronchitis.

5.2.6 Swollen wattles are signs of chronic fowl cholera.

5.2.7 Scales on comb, eyelid and wattles suggest fowl pox; differentiate from scars due to injury and vitamin deficiency.

5.2.8 White patches on the head may be due to flavus (fungal infection).

5.2.9 Injury on the head, neck, breast, or vent may be due to cannibalism or predators.

5.2.10 Swelling with erosion on the breast indicates breast blisters, especially in broilers.

5.2.11 Crusty lesion on the mouth and eyes suggest Vitamin A deficiency or pox.

5.2.12 Poor feathering is a sign of metabolic nutritional disorder.

5.2.13 Examine the joints for swelling. Swollen joints suggest Mycoplasma infection or viral arthritis.

5.2.14 Dropping abdomen suggests hernia, ascites or avian leukosis.

5.2.15 Faecal smeared vent indicates diarrhoea due to various etiological agents (bacteria, parasites, toxic etc.)

5.3 Necropsy procedure

5.3.1 Live sick birds should be killed by dislocating the neck.

5.3.2 Moisten or wet the birds with water before conducting a post-mortem examination.

5.3.3 Place the bird on the post-mortem table with its back on the table.

5.3.4 The legs are abducted (separated) by breaking open the hip joints.

5.3.5 Cut down between the abdomen and the legs.
5.3.6 Remove the skin over the sternum and the abdomen.
5.3.7 Free the sternum by lateral incision along the costo-chondral junction of the ribs and clavicle (dislocate the shoulder join by bone cutter). Remove the entire breast muscle cage as one piece.
5.3.8 Examine the visceral organs as they appear chiefly, liver, heart, spleen, air sacs and examine the general condition of the organs.
5.3.9 Remove the liver along with the gall bladder.
5.3.10 Remove the spleen.
5.3.11 Remove the intestine and proventriculus starting from the interior part of the oesophagus, till the rectum in one piece. For later examination tie both ends of the intestine.
5.3.12 Re-examine the bursa of fabricius laying dorso-ventral to the cloaca, incise it with a scalpel or scissors and examine internally for any exudates etc.
5.3.13 Remove the ovaries from their base, and the oviduct is separated from its attachment till the cloacal aperture.
5.3.14 The heart is opened in situ by incising the wall of the right ventricle near the apex, continue the incision anteriorly up the through the pulmonary artery and laterally up through the right auricle. For the left ventricle an incision is given at apex through the aorta and up through the left auricle. The heart is then remove from its base.
5.3.15 Free the lungs from their thoracic attachment by blunt dissection and then remove by cutting through the trachea immediately anterior to the syrinx.
5.3.16 Cut across the upper beak, at its base to expose the nasal cavities and sinuses, then the mouth is opened by cutting through one corner (left side); continue the incision through the pharynx, oesophagus to open the crop.
5.3.17 Open the trachea till its full length.
5.3.18 Expose the brachial plexus (nerves supplied to the wings), and, sciatic nerve (supplied to the legs) on both sides expose the vagus nerve along the neck on both sides.
5.3.19 Examine the kidneys in situ; incise the kidneys for gross examination.
5.3.20 Examine all major joints.

5.4 Examination of organs and systems after post-mortem

Digestive and Respiratory system examination:

5.4.1 Examine the beak, mouth, oesophagus and crop.
5.4.2 White plaques in the mouth, oesophagus or crop may be due to capillarial worms, candidiasis, or Vitamin A deficiency.
5.4.3 If the crop is enlarged and full, it may be an impacted or a sour crop.
5.4.4 Examine the soft palate, larynx and trachea.
5.4.5 Wet diphtheritic lesions can be seen on the roof of the mouth and on the larynx in pox.
5.4.6 Congestion and mucus in the trachea are seen in chronic respiratory disease and Infectious bronchitis.

5.4.7 Haemorrhage and blood clots in the trachea in Infectious laryngo-tracheitis, Ranikhet disease, fowl plague and poisoning. Also examine the caeca for haemorrhages in Ranikhet disease.
5.4.8 Haemorrhages or mucoid enteritis indicates spirochetosis, Ranikhet disease, sulpha drug toxicity or acute bacterial infection.
5.4.9 Examine thoracic and abdominal organs.
5.4.10 If the air sacs are cloudy suspect Infectious bronchitis, E-coli infection or early chronic respiratory disease.
5.4.11 In hard and fibrinous liver suspect chronic respiratory disease or E. coli infection.
5.4.12 White crystals or chalky smearing on heart, liver, kidneys and other organs indicate gout.
5.4.13 Greenish hard nodules in lungs and plague like formation on the serious membranes indicate Aspergillosis.
5.4.14 Nodules in small intestine indicate tuberculosis, tapeworm infection, leucosis, or *E. coli* infection.
5.4.15 Caecal haemorrhage indicates Coccidiosis.
5.4.16 Examine the digestive tract for various kinds of diarrhoea (non specific, haemorrhagic, ulcerative), parasites (rounds worms, capilleria worms, coccidia and caecal worms), just erosions could be due to injury.
5.4.17 Blood droppings, haemorrhages in the intestine, ballooning of the entire intestine indicates haemorrhagic enteritis, differentiate from other intestinal diseases.
5.4.18 Green stained faeces in the digestive tract indicate a starved bird.
5.4.19 A large, yellow liver may be normal, but when the whole carcass is yellowish or jaundice, with petecheal haemorrhage in the intestine and other organs, inspect for fatty liver syndrome.
5.4.20 In young chicks nasal discharge and coughing; wet and swollen eyes are indicative of Coryza or Tuberculosis.
5.4.21 In older birds gasping, tracheal sounds and coughing is premonitory indication of Infectious bronchitis. In these birds nasal discharge may not be noticed. These symptoms are usually noticed in the night hence observe the flock during night time.
5.4.22 The remarkable symptoms of Infectious laryngo-tracheitis are gasping, respiratory rates and coughing. Many birds will be depressed, sitting on the nests or floor and would be gasping with the head extended and the beaks open. A typical whistling sound may be heard when the bird breaths.
5.4.23 Tumours in different visceral organs may be due to Marek's disease, lymphoid leukosis or due to other varieties of tumours.
5.4.24 Ascites may be seen as a result of liver diseases or from ingestion of toxic material and high altitude sickness.
5.4.25 In young birds, examine the lungs and air sacs for white foci or plaques caused by fungus (*Aspergillus flavus*).
5.4.26 In baby chicks, look for yolk sac infection, peritonitis (omphalitis). In chick the abdomen is swollen wet and discoloured.

5.4.27 Weak chicks would have just died because they don't learn how to eat (starve cuts) or due to dehydration.
5.4.28 Birds that die suddenly (heart attack), heat stroke, or suffocation have congested lungs and full digestive tract.

5.5 Circulatory, Muscular and Immune System

5.5.1 Birds that die of anaemia are pale, weak and the blood is watery.
5.5.2 Widespread petechial Haemorrhage in the tissue may be seen due to sulfa poisoning or *E. coli* infection.
5.5.3 Swelling and congestion in the spleen and lymphoid tissue suggests septicaemia (fowl cholera, fowl typhoid) or viraemia (Ranikhet Disease also affects the lymphoid tissue of the gut).
5.5.4 Lymphoid tumours in Marek's disease and leukosis except for bursa of fabricius which is only seen in lymphoid leukosis.
5.5.5 Mottled spleen is seen in spirochaetosis.
5.5.6 Bursa of Fabricius enlarged and filled with exudates, indicates the infectious bursal disease (Gumboro disease).

5.6 Reproductive system

5.6.1 Uterus containing an egg ready to lay may have died from hypocalcaemia.
5.6.2 Hard or swollen testes indicate bacterial infection.
5.6.3 Oviduct congested with exudates indicates bacterial infection.

5.7 Examination of skin, integument, muscles (external musculature) bones and joints

5.7.1 Examine the eye for de-pigmentation due to ammonia burns.
5.7.2 Examine the eye for spotty de-pigmentation, bluish grey fading of the iris on both sides, and irregular pupil for eye form of lymphomatosis.
5.7.3 Discoloured, hard and granular skin indicates skin form of Marek's disease.
5.7.4 Muscular degeneration may be due to Vitamin E and selenium deficiency.
5.7.5 Examine bones and joints for abnormality and deformity for rickets, gout, osteomalacia and calcium deficiency etc.
5.7.6 Examine the vertebral column for any twisting or deformity (kinky back).
5.7.7 Examine for slipped tendon (gastronemius) due to nutritional or genetic problem.
5.7.8 In young birds, cheekbones, beaks, which if weak and pliable with beaded ribs, indicate calcium deficiency or Vitamin E deficiency.
5.7.9 Curly toe indicates riboflavin deficiency.
5.7.10 Cracked feet and skin lesions of the foot may be due to pantothenic acid or biotin deficiency.
5.7.11 Arthritis (inflammation of the joint with exudates) in the joints suggests infectious synovitis or viral arthritis.

5.8 Nervous System

5.8.1 Nervous symptoms due to disturbances of the nervous system may cause in coordination, staggering, paralysis, walking backwards, tremors, star gazing etc.
5.8.2 In case of lameness, examine the sciatic nerves; brachial plexus, which would, if swollen, and loss of striation indicate Marek's disease.
5.8.3 Soft, with dark areas in the cerebellum indicate Vitamin E (Avian encephalomalacia) deficiency. However, in birds exhibiting tremors examine the brain histologically.

5.9 Excretory system

5.8.1 Ureters packed with urates indicate Gout.
5.8.2 Swollen and congested kidneys indicate bacterial infection.
5.8.3 Tumours in the kidney indicate lymphoid leucosis complex.

6. RISK ASSESSMENT

6.1 All carcasses should be handled with care especially if they were known to present risk of zoonotic disease like Avian influenza.
6.2 In such cases, protective clothing, including boots, gloves, face mask and goggles must be worn. The carcasses should be burned or deep buried under stones with quick-lime.
6.3 No eating, drinking, grooming, or other activities that are a means of exposure are permitted in necropsy areas.
6.4 Transport unfixed tissues in leak-proof containers.

7. WASTE DISPOSAL

7.1 Decontaminate instruments before cleaning them.
7.2 Clean and disinfect all work surfaces or the premises where necropsy was conducted.
7.3 Decontaminate self (e.g., disinfect and remove boots, gloves, and coveralls).

8. REFERENCES

University of Minnesota, Veterinary Diagnostic Laboratory, Standard Operating Procedure (2016).

University of Queensland, School of veterinary Science, Standard Operating Procedures (2017).

USDA, Animal and Plant Health Inspection Service, National Veterinary Services Laboratories, Guidelines for Necropsy.

University of South Florida, Standard Operating Procedure, Necropsy and Post Mortem Examination (2012).

TITLE: Necropsy and post mortem examination in Fish

PREPARED BY: Post-mortem section

REVISED BY: Post-mortem section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. **PURPOSE**

To outline the procedures for performing a diagnostic necropsy in fish.

2. RESPONSIBILITY

2.1 Fish health professionals conduct necropsies and post mortem examinations on dead fish died due to disease.

2.2 Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a fish ponds.

3. APPARATUS

3.1 Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
3.2 Knives – small, with sharpening steel.
3.3 Scissors (various sizes) and saws; bone cutters
3.4 A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
3.5 Sterile swabs in test tubes
3.6 Alcohol cleaned slides for smears, preferably in a rack or box
3.7 Spatula
3.8 Plastic bags for specimens
3.9 Petri dishes
3.10 Labels
3.11 Soap, water, disinfectant and towel
3.12 Scalpel blade with handle

4. REAGENTS, SOLUTION AND BUFFER

4.1 10% Formalin or Buffer Neutral Formalin
4.2 70% alcohol for wetting and disinfecting the skin,
4.3 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
4.4 Normal saline (0.85% NaCl) with a pipette (for parasitological examination),

5. PROCEDURE

5.1 **Visual Assessment** of condition of each fish to observe for an ulcer, reddening underneath operculum or of junction between gills and body or tissue necrosis is detected.

5.2 If necropsy is unable to be performed immediately, **place fish into a plastic bag in an ice slurry**. This is to slow autolytic changes if there is a slight delay in the necropsy.

5.3 **Morphometric Measurements** of fish to include fork length, width, height and weight of individual fish. Note any physical malformations

5.4 **Gill Tissue:** Dissect out 1st gill arch and prepare gill scrape and view under a compound microscope. Dissect out 2nd gill arch and initially view under dissecting scope then prepare a slide using a section of this material and view under compound microscope. These methods will detect for presence of hyperplasia, epitheliocystis, gill fluke or blood fluke eggs.

5.5 **Skin Scraping:** Use the back of a scalpel blade to remove a section of mucus from the skin especially near skin lesions. Place onto a microscope slide with a cover slip and a drop of water, and observe under compound microscope. Look for the movement of live skin parasites.

5.6 **Sampling Organs for Histology:** Dissect out each organ including tissue from the heart, liver, spleen, gonads, kidney, muscle, stomach, hindgut and brain. Look for obvious abnormalities. Take weights of the liver and gonads (if mature) and visually assess their condition.

5.7 **Histology Examination:** Preserve tissues extracted from **Step f** in 10% neutrally buffered formalin and send preserved tissues for histological analyses and subsequent report.

5.8 **Examine Records of Water Quality and Husbandry Activities:** Fluctuating water quality parameters and handling may be a trigger for mortality, especially if there are underlying factors such as high bacterial loads in the culture water.

6. RISK ASSESSMENT

NA

7. WASTE DISPOSAL

7.1 Decontaminate instruments before cleaning them.
7.2 Clean and disinfect all work surfaces or the premises where necropsy was conducted.
7.3 Dispose the carcass as per the waste management guidelines.

8. REFERENCES

Curtin University, standard operating procedures. (2015). Fish dissection and biopsy collection.

TITLE: Necropsy and post mortem examination in reptiles

PREPARED BY: Post-mortem section

REVISED BY: Post-mortem section

APPROVED BY: Head, LSU

DATE: 11.06.2018

1. **PURPOSE**

To outline the procedures for performing a necropsy in reptiles.

2. RESPONSIBILITY

2.1 Wild life veterinarians conduct necropsies and post mortem examinations on dead reptiles died due to diseases.

2.2 Necropsies must be performed by investigators and staff when unexpected morbidities or mortalities are observed in a reptile population.

3. APPARATUS

3.1 Rubber boots, protective clothing, rubber apron or lab coat, rubber gloves.
3.2 Knives – Big & small, with sharpening steel.
3.3 Scissors (various sizes) and saws; bone cutters
3.4 A number of sterile, open-mouthed screw top jars of different sizes for specimen collection
3.5 Sterile swabs in test tubes
3.6 Alcohol cleaned slides for smears, preferably in a rack or box
3.7 Spatula
3.8 Plastic bags for specimens
3.9 Petri dishes
3.10 Labels
3.11 Soap, water, disinfectant and towel
3.12 Scalpel blade with handle

4. REAGENTS, SOLUTION AND BUFFER

4.1 10% Formalin or Buffer Neutral Formalin
4.2 70% alcohol for wetting and disinfecting the skin,
4.3 96-100% ethyl alcohol (for fixing specimens suspected of having gout, and 100mg/1ml for PCR testing),
4.4 Normal saline (0.85% NaCl) with a pipette (for parasitological examination),

5. PROCEDURE

5.1 External Examination

Before you make any incisions, examine the exterior of the animal and document any irregularities. Use a checklist incorporating the following elements to ensure your examination is complete:

5.1.1 **Skin**: look for scars, irregular pigmentation, and wounds.
5.1.2 **Extremities**: check for regenerated tail or missing limbs and digits.
5.1.3 **Ectoparasites**: mites and ticks often hide in compact areas like the corners of the mouth, in and around the cloaca, the tympanic membrane, and the joints of the legs.
5.1.4 **Muscle condition:**
5.1.5 **Eyes**: a sunken eye is a likely sign of severe dehydration or malnourishment.
5.1.6 **Oral Cavity**: look for any signs of disease or inflammation. In reptiles, an infection (abscess) might appear like cottage cheese or curds, unlike any pus or liquid substance you would typically find in similar infections in mammalians. Inspect the teeth, trachea, and tongue. Abnormal fluid or material found on the roof of the mouth, or choanal slit, might be an indicator of an airway infection. Collect any lesions with adjacent normal tissue.
5.1.7 **Skeletal structure**: the specimen should be carefully palpated for evidence of trauma, fractures, or other anomalies.

5.2 Internal Examination

5.2.1 The animal should be placed flat on their backs.
5.2.2 Using a suitable cutting instrument (scalpel, scissors, knife), make an incision along the ventral midline, or belly, from the anal region/cloaca to just under the lower jaw. A large vein, the ventral abdominal vein, is directly beneath the ventral midline. Shift the incision slightly to the left or right away from this vein to avoid excessive blood spill.
5.2.3 Turn back the skin and muscles on each side while cutting to reveal the body cavity.
5.2.4 When you reach the rib cage, cut through the cartilage on one side of the sternum and pull back the ribs to see the heart and liver.
5.2.5 Fat pads: The size and condition of a reptile's fat pads can be useful indicators of the body condition and diet of the animal (large fat pad indicates animal is getting sufficient amount of food). Usually they are found in the body cavity, and some fat bodies may also be present near the heart.
5.2.6 Heart: Carefully remove this membrane to observe the three chambers.
5.2.7 Liver: A relatively large, lobed, mahogany color or light brown organ just under the heart.
5.2.8 Gallbladder: The gall bladder can usually be found near the pancreas and spleen, or, in some species, inside the liver. This organ contains bile that gives it a color in between green and yellow.
5.2.9 Lungs: They should be palpated carefully to feel for any nodules or abnormalities. In addition, a small incision should be made to see if there are any parasites or exudates.

5.2.10 Digestive system: Examine the gut contents either by cutting open the digestive tract or by simply squeezing the contents out of the tract and into a collection container through either the esophagus or the large intestine.

5.2.11 Urinary genital system: Examine for abnormalities in kidneys and reproductive organs.

6. RISK ASSESSMENT

NA

7. WASTE DISPOSAL

7.1 Immediately after the completion of the necropsy, put the animal in a plastic bag for storage and freeze or refrigerate it until it can be properly disposed of in a manner that is compliant with waste management guidelines.

7.2 The table, clothing, and equipment used should be first washed in hot water and detergent, then disinfected with a cleaning agent such as Virkon or phenol.

7.3 All disposable items used (scalpel blades, needles, and latex gloves) should be discarded appropriately in a biohazard or sharps container.

8. REFERENCES

University of Florida (2016). Necropsies of Reptiles: Recommendations and techniques for examining invasive species.

CATEGORIZATION FORE POST MORTEM EXAMINATION

Sl No	Procedure / SOP	DVL	SVL/ TVH	RLDC/ NVH	NCAH
1	Post mortem - Poultry - Ruminants - Canines/Felines - Equines - Swine - Wild (reptiles & fish)	X	X	X	X