

File No. 16632 Appendix G: Necropsy Protocols

The following are current necropsy protocols and forms. There are separate protocols and forms for the Northwestern Hawaiian Islands (NWHI) and main Hawaiian Islands (MHI).

NWHI HAWAIIAN MONK SEAL NECROPSY PROTOCOL

SAFETY CONSIDERATIONS

Before performing a necropsy, be sure you have read the following documents located in the **Zoonotic Disease** section of your **Master Field Log** and your camp's **Necropsy Manual**:

Occupational Safety: Working with Marine Mammals and Your Health
<http://www.vetmed.ucdavis.edu/whc/mmz/Occupational%20Safety.htm>

Marine Mammal Zoonotic Bacteria
<http://www.vetmed.ucdavis.edu/whc/mmz/bacteria.htm>

Marine Mammal Zoonotic Viruses
<http://www.vetmed.ucdavis.edu/whc/mmz/viruses.htm>

Zoonosis and Quarantine (MARP Manual)

Appendix II: Infectious Agents (Aguirre *et al.*, 1999)

Public Health (Cowan *et al.*, 2001)

Assessment of the Risk of Zoonotic Disease Transmission to Marine Mammal Workers and the Public: Survey of Occupational Risks*
http://swfsc.noaa.gov/uploadedFiles/Divisions/PRD/Programs/Photogrammetry/Marine_Mammal_Zoonoses_Final_Report-2.pdf

*Only available online and on your field computer:

Preventing Disease Transmission

Avoid direct contact with dead seals to prevent transmission of infectious diseases that may be pathogenic to humans.

Persons performing the necropsy must:

1. Cover all surface wounds with a protective dressing before gearing up.
2. Wear protective gear, including latex or vinyl gloves, mask, disposable gowns, and foot covers. Change torn gloves **immediately**.
3. Seek medical attention **immediately** if you get any cuts, punctures or other injuries during the necropsy. Notify the attending physician of the source of the injury.
4. Disposable items such as scalpel blades, needles and biopsy punches **MUST** be disposed of in sharps containers.
5. If possible, pull carcass up the beach to higher ground and bury it after necropsy to avoid attracting scavengers and to minimize the potential for disease transmission.
6. Disinfect all instruments and contaminated equipment after the necropsy has been performed (see Post Necropsy section below).
7. Once the necropsy has been performed and all gear has been cleaned and disinfected, wash thoroughly with soap. Disinfect reusable clothing with bleach solution (see tagging handling protocol) and dispose of all contaminated clothing, gloves, gowns, etc. in a biohazardous waste bag.
8. **DO NOT STORE ANY SPECIMENS IN FREEZERS/REFRIGERATORS USED FOR HUMAN FOOD.**

GENERAL CONSIDERATIONS

A necropsy is a systematic examination of the whole body, organs, and tissues and is a basic tool for investigating disease and for monitoring the health of the Hawaiian monk seal population. **Whenever possible, necropsies should be performed by a trained veterinary pathologist** experienced in recognizing and interpreting lesions and abnormalities.

Necropsy How-To Guides:

For general guidance on the steps in performing a necropsy, please refer to the following resources, but follow the sample collection protocols provided in this document and the most recent version of the Necropsy Report Form.

1. **Field Manual for Phocid Necropsies (specifically *Monachus schauinslandi*)** (FMPN) located in your camp's **Necropsy Manual**
2. **Marine Mammal Necropsy: An Introductory Guide for Stranding Responders and Field Biologists** located on your field computer and also available at: <http://www.bahamaswhales.org/strandings/necropsy.pdf>

Necropsies will have the most scientific value when they are carefully documented. Adherence to this protocol and the **Necropsy Report Form** will assist in the documentation and standardization of information, which may be valuable in determining morbidity and mortality factors within the population and as well as for individual seals.

Things to keep in mind:

1. Record all observations – when in doubt, just describe what you see.
2. The order of the **Necropsy Report Form** follows the sequence of general dissection and examination. If you are skilled and familiar with Hawaiian monk seal necropsies, you may find it easier to use the Necropsy Specimen Checklist, but **be sure to have someone record all observations, photos, measurements, and descriptions of organs on the Necropsy Report Form.**
3. Tissues and organs must be examined in a systematic manner. The precise method used for a necropsy is less important than establishing a routine in which each body system is examined fully.
4. **Once the carcass has been opened, take tissue specimens for virology, bacteriology and toxicology first, then sample for histopathology and parasitology.**
5. Samples of **normal and abnormal** tissue should be collected for laboratory analyses.

The ability to obtain reliable data from necropsies depends on the following:

1. Condition and location of the carcass
2. Adherence to detailed protocols
3. Number of seals necropsied throughout the year
4. Amount of time available to perform a thorough necropsy
5. Care in sample preservation and labeling of specimens
6. Care in shipping and storing specimens

Decomposed carcasses may be unsuitable for histopathology but can be useful for observing gross lesions. Collect brain samples regardless of the state of decomposition. Collect samples from all organs listed, even those that appear normal. In general, tissue specimens must be sufficiently thin (**less than 1 cm thick**) to allow proper fixing of 10 parts 10% buffered formalin: 1 part tissue. For some tissues (e.g. brain and lung), you may need to make parallel cuts (0.5 cm in thickness) in the tissues to allow preservation. After the tissues have been fixed in formalin 24-48 hours, pour off the formalin, rinse the tissues in fresh water, and add fresh formalin solution.

NECROPSY INSTRUCTIONS

Complete a **Hawaiian Monk Seal Necropsy Report Form** for **all** carcasses recovered. Use the **full form** if you perform an internal examination of the carcass, regardless of the condition code. The **partial form** can be used for necropsies where very minimal data is collected. Record "N/A" for any sections that are not applicable, and state what organs/tissues were not examined. At a minimum, describe each organ examined and sample as many organs as possible, prioritizing the following tissues: **brain, lung, liver, kidney, blubber.**

Photograph the exterior for ID (even if tagged), to document injuries or other unusual conditions, and to document body condition. Photograph the seal from all 4 sides (dorsal/ventral/left lateral/right lateral) and a close up of the hind flippers with tags. In addition, take close-ups and a wider view (to show perspective) of injuries and unusual conditions. If possible, include an index card in each frame that notes the following: Seal ID, Date, Size, Sex, and island and a ruler. Record photographs on the Necropsy Report Form.

External examination

Document any specific external lesions, abnormalities, or scar patterns. Examine, describe, and photograph any external lesions or injuries, the anogenital area, scars and other distinguishing characteristics.

Experience has shown that in cases where pinnipeds have drowned, there is often a complete absence of expected gross and histological findings. For this reason, it is imperative to look closely for external indications of entanglement. Findings may

include: bent or missing vibrissae, torn or missing nails, and cuts in and around the nares, mouth, and gums. Closely examine the tips of all extremities to look for line or net cuts. Linear marks on the pelage are also of interest. Photograph any suspected abnormalities with close up/macro images, followed by images that demonstrate the location(s) on the body of each close up image.

Carcass condition codes

Evaluate carcass condition (state of decomposition). Carcass condition is influenced by many factors including disease, body temperature, and environmental temperature. **Rigor mortis** (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. *Rigor mortis* indicates that a carcass may be in good condition (Code 2).

Code 1: just died (*e.g.*, euthanasia)

Code 2: fresh/carcass in good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin and mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas). NOTE: Rigor mortis (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. Rigor mortis indicates that a carcass may be in good condition

Code 3: fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)

Code 4: poor/advanced decomposition (carcass may be intact but collapsed, skin sloughing, strong odor, blubber soft with pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)

Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

Tags

If flipper tags are present, note their condition on the survey form (data type 'T') and tag condition forms. Collect and place them in a whirlpak bag labeled with animal ID, island/atoll, date, and survival factor number. Scan the entire body for PIT tags by holding the PIT tag reader as close to the body as possible. Even if PIT tags are not found, indicate on the survey sheet that a scan was completed and where on the body the scan was performed.

Morphometric measurements

Axillary girth – At the armpit, measure the circumference around the entire body in centimeters.

Standard length – Measure the straight line (not curved) length of the entire seal from the tip of the nose to the tip of the tail in centimeters. If a scale is available, weigh the body and report units. **Record measurements on both the TAGGING/HANDLING CARD and the Necropsy Report Form.**

Swab Collection

Use sterile Dacron swabs. Avoid touching swab tip to anything other than the tissue being swabbed. Immediately place swab in cryovial and break off the end of the plastic applicator against the side of the cryovial container (it should snap easily).

Internal Examination

TAKE INTERNAL PHOTOGRAPHS ONLY WHEN UNUSUAL CONDITIONS ARE NOTED OR

IF YOU ARE UNSURE IF IT IS UNUSUAL. If unusual conditions are noted, include a size reference (*e.g.*, ruler) and label with seal ID, survival factor number, date, and island. Take two photographs, one with the organ *in situ* (in its anatomical position/location) in the body and one with the organ removed from the body and placed on a solid white or light blue surface.

Record complete and thorough observations. Assume more is better when describing and recording information. The rule here is if in doubt, write it down. If unsure whether something is abnormal, state this and succinctly describe. Descriptions should be clear, concise, and without personal interpretation. Appropriate tissue preservation along with YOUR precise description of findings may allow the identification of causes of death in the population.

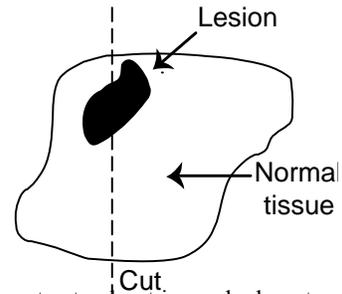
Identify the appropriate descriptors for each organ examined. The descriptions provided herein are NOT an exhaustive list of terms, but rather a list for your reference. **Describe surface, consistency, color, and cut surface of both normal tissues and abnormalities or lesions.**

Descriptors of Organs and Lesions

- Surface: Smooth, rough, shiny, dull, thickened, wrinkled.
- Consistency: Firm, soft, flabby, dry, wet, fluid-filled, sharp-edged, friable (easily pulverized or crumbled).
- Color: Transparent, translucent, opaque; white, cream, green, yellow, brown, pink, red, nutmeg (normal pattern of liver), etc. Use simple colors, do not get complicated. Also comment how color is spread through tissue- homogeneous, speckled, streaked, blotchy, blanched, mottled (i.e., pink with specks of red). Additional descriptors may include bright, pale, dark.
- Cut surface: Slice organ several times appropriately and spread apart to look at internal surface. Be sure to describe color of the cut surface. Descriptors include swollen, bulging, shiny, dull, eroded, glistening, scaly, pitted, oozing
- Size: Record in metric system (mm, cm), measure length, width and depth or diameter of the lesion. Enlarged, (hypertrophied), small (atrophied), normal size.
- Shape: Square, rectangular, triangular, oval, round, cuboidal, spherical, discoid, rhomboid, tear-shaped, wedge-shaped, spindle-shaped, irregular, long, slender, indented, narrow, lace-like, tortuous, branching, speckled (miliary), flat, raised, depressed, shrunken, papillary, cauliflower-like.
- Distribution: Single discrete lesion (focal), multiple lesions in one location (multifocal), or multiple lesions scattered diffusely throughout the organ or body cavity (diffuse); locally extensive, random, even.
- Location: Surface, capsule, wall, dorsal or ventral, caudal or cranial, anterior or posterior, medial or lateral, proximal or distal, internal or external, full or partial thickness of a wall of an organ.
- Fluid: Clear, cloudy, turbid, thick, thin, bloody, mucoid, exudate, dark, tarry
- Consistency: Spongy, granular, gel-like, firm, soft, hard, rock-hard, dense, creamy, buttery, brittle, lumpy, velvety, warty, tenacious, gritty.
- Cut surface: Bulging, engorged, granular, nodular, pitted, oozing
- Odor: None, sweet, sour, rancid, ammonia-like, putrid, fruity, petroleum- like

Collecting necropsy tissues

Each complete necropsy should have two jars containing complete tissue sets of all tissues, and both having the same specimen number. One set should be sub# A and the other sub# B. Tissue set A should be the most complete set, (e.g., if you freeze one eye, tissue set A should have the formalin fixed eye). If there are any unusual lesions in any of the tissues sampled, be sure to include the margin between abnormal and normal tissue in both tissue sets A and B.



Collect samples of ALL LESIONS in formalin. Describe and sample areas that appear to stand out in marked contrast to the main body of tissue. Samples should include the margins between the normal and abnormal tissue and a description (i.e., sharp line versus vague and gradual, circumscribed, encapsulated). Make sure to check the boxes next to the appropriate specimens as collected on the necropsy report form.

Tissues for Toxicology (contaminants and biotoxins): Code 1, 2 ideal. Codes 3, 4, 5 useless.

Toxicological analyses may be performed for heavy metals, organochlorides, selenium, and dioxin. When sampling for toxicology, it is important to use standardized sampling procedures so that even when low levels of contaminants are present, differences may be attributed to biological processes and contaminant exposure and not to variation in the collection process.

For MHI necropsies, follow NIST sample collection protocol to collect toxicology specimens (this has been incorporated into the MHI necropsy form).

Tissues for Microbiology: Code 1 ideal; Codes 2, 3 limited; Codes 4, 5 useless.

Collect by special request only. Specimen collection for bacteriology and virology is determined primarily by the nature of gross pathologic lesions. Samples should be taken aseptically, from external surfaces, body cavities and internal organs as soon as they are exposed. Place swabs in respective transport media and refrigerate at 4 C or place on blue ice immediately and freeze upon arrival to laboratory or field camp. If cryovials are available, ultrafreeze the swabs with tissue samples in liquid nitrogen. Samples for microbiology are worth the time and effort only when tissues are in suitable condition. With an

"aborted fetus", perinatal death, or newborn in main Hawaiian Islands (MHI), collect specimens according to "Fetus" section of MHI Necropsy Form and refrigerate for microbial analysis.

Post Necropsy

1. Review the completed **Necropsy Report Form**, making sure that all boxes have been checked off on the form for all samples collected.
2. Necropsy Report Forms, photos, "List of Specimens Collected", and any other pertinent data should be returned to the NMFS PIFSC Honolulu Laboratory.
3. Refer to the section "Preventing Disease Transmission" #4-8 on page one for post-necropsy clean-up tips. Clean necropsy tools (you may also need to spray them with WD-40 or LPS) and restock necropsy kit so that it is ready for the next necropsy.
4. Change the formalin for all formalin fixed tissues as noted above.
5. Make sure that the tagging/handling card, scar card, and tag condition drawing form are complete. **Necropsy Report Forms**, scar cards, tagging/handling cards, survival factor forms, and photos should be returned to the NMFS Honolulu Laboratory.
6. Record specimens collected on the **Specimen Collection Summary** and assign specimen numbers as outlined in the **Specimen Collection Protocol**.
7. Clean necropsy tools. Before disinfecting, remove all organic matter from instruments by washing them thoroughly with warm (if possible) soapy water. If instruments are not cleaned properly before disinfecting, the remaining organic matter may shield organisms from destruction, and may inactivate the disinfectant. Be sure to wear proper protective gear (gloves, masks, etc.) when washing instruments. To minimize aerosolization, keep instruments below the water line when washing. Disinfect instruments with 70% alcohol or a 1:10 chlorine bleach solution for at least 10 minutes. However, bleach corrodes stainless steel, and may pit the instruments. Regardless of disinfectant used, be sure to thoroughly rinse instruments with fresh water after disinfecting. Air dry all instruments thoroughly before putting them away. You may also need to spray them with WD-40 (or LPS)
8. Restock necropsy kit so that it is ready for the next necropsy.

HAWAIIAN MONK SEAL NECROPSY REPORT FORM (NWHI)

SEAL ID/temp ID _____ DEATH/NEC. # _____ (assign sequential #'s by calendar date for all seals)
Date/time: necropsied _____ found dead _____ last seen alive _____
Island/Atoll _____ Islet _____ Sector _____ Lat _____ Long _____
Beach position _____ Carcass orientation (i.e., horizontal to water line) _____
Size/sex _____ Age (if fetus, refer to pg. 10) _____
Persons performing/assisting with necropsy: _____
RECORDER: _____ **PHOTOGRAPHER:** _____
Photos? Y / N File names: _____

HISTORY

Identifying body markings (take photos): _____

Last live observation(s): _____

Circumstances of death (found dead/euthanized/other-explain): _____

Tags

Record number and condition of any flipper tag(s), photograph, and collect all tags in labeled whirlpak.

R _____ L _____ Notes: _____

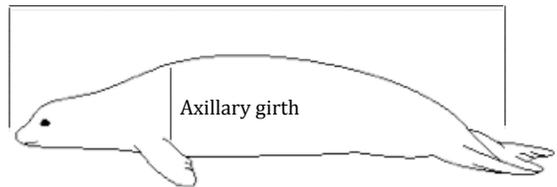
Scan the entire body for pit tags. PIT tag#(s):

R _____ L _____ Location found _____

Morphometrics

Axillary girth _____ (cm)
Straight length _____ (cm, circle: DSL or VSL)
Total body mass _____ (circle: kg/lb)
Measurer _____

Straight Length (tip of nose to tip of tail)



Carcass Condition Code: 1 2 3 4 5

- Code 1: Just died (e.g., euthanasia)
- Code 2: Fresh, good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin/mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas).
- Code 3: Fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)
- Code 4: Poor/advanced decomposition (carcass collapsed, skin sloughing, strong odor, blubber soft w/ pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)
- Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

GROSS NECROPSY EXAMINATION

INSTRUCTIONS

1. Describe all organs, tissues and lesions in the fields provided. If appropriate, circle either NSF, NE or NA at the top of each section. If the tissue examined is abnormal, describe the location, color, size and distribution within the tissue in the blanks provided.

2. **Formalin fixed tissues should be no bigger than 2x2x1cm.**

3. Collect 2 equal Tissue Sets (set "A" and "B") from all organs, to be fixed in formalin.

Tissue Set A = Sp # _____ A

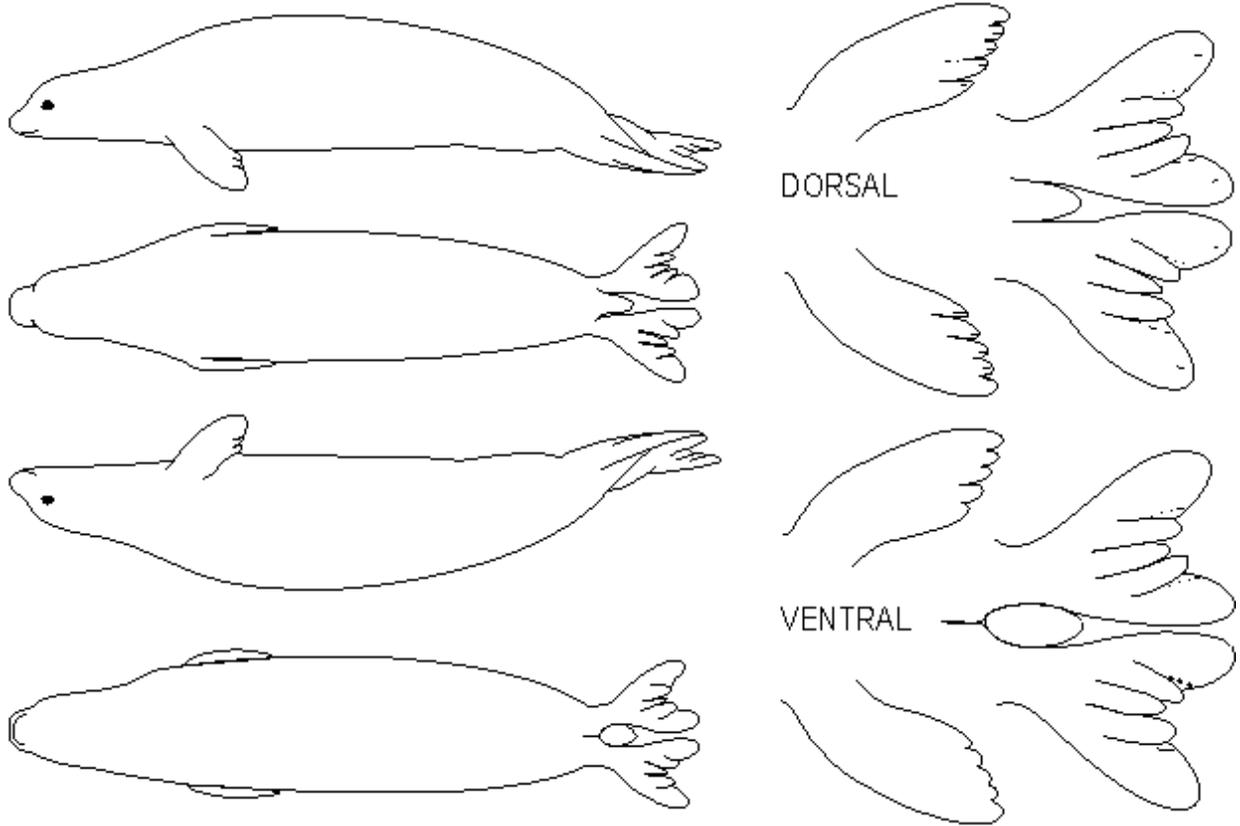
Tissue Set B = Sp # _____ B

4. If possible, remove head and place in cooler on ice while remainder of necropsy is completed, then go back and sample brain after the remainder of the necropsy is complete.

5. Examine all outside surfaces and note abnormalities, discharge, etc. Describe/draw abnormalities below. Photograph with full-frame views of all 4 sides (dorsal/ventral/left lateral/right lateral) as well as close-ups of scars or other distinguishing characteristics, injuries, abscesses, line marks or other abnormalities. Include an index card in the image frame that notes the following: Seal ID, Date, Size, Sex, SF#, Necropsy #, Location (island/atoll).

NOTE: In cases of drowning, there are few clues, if any, so look closely for external indications of entanglement (bent/missing vibrissae, torn/missing nails, cuts in and around the nose, mouth, and gums). Also examine the tips of all extremities to look for line or net marks. Describe/draw and photograph any evidence of entanglement. Collect any gear found on an animal in a ziploc bag and label.

Draw all abnormalities and markings (include bruises, wounds, contusions, old scars, condition of and tears of skin, external parasites):



ABBREVIATIONS	
NSF: no significant finding	DB: DMSO/blue ice
NE: not examined	DR: dry

EXTERNAL PHYSICAL EXAMINATION (Circle all that apply):

GAS/ PUTREFACTION: (Run your hands firmly along the body and feel for bubbles.) NSF / NE / NA

NUTRITIONAL STATE:

Fat/overweight, Normal/average, Thin/poor, Starving/emaciated

Notes: _____

DISCHARGE: NSF / NE / NA

Location: eyes, nose, mouth, genitals, anus Color/Texture: green, white, clear, red, thick, runny

Notes: _____

SWAB COLLECTION: NSF / NE / NA

Use sterile Dacron swabs. Obtain 2 swabs each from: eyes (ES), nasal cavity (NS), oral cavity (OR), genital opening (GS), rectum (RS). Both swabs from the same orifice can be placed into the same cryovial, unless the eyes are abnormal (i.e., you should have a total of five 2.0 ml cryovials with 2 swabs from each orifice in each). However, if one or both eyes are abnormal, store the swabs separately and indicate below which vial contains the abnormal eye.

Sp # _____ Eye swab (LN) Sp # _____ Genital swab (LN) Sp # _____ Oral swab (LN)

Sp # _____ Nasal swab (LN) Sp # _____ Rectal swab (LN)

EYES: NSF / NE / NA

NSF, NE, missing, bulging, deflated, out of socket, foreign body present, penetrating wound

Notes: _____

Aqueous humor: collect using sterile needle and 3cc syringe. If both eyes are normal, aqueous can be combined in one cryovial. If one or both eyes are abnormal, use a clean needle and syringe to collect each sample and place in separate cryovials. Indicate which eye is abnormal above.

Sp # _____ Aqueous humor (LN)

Eyes: Collect both eyes. Fix one eye in Tissue Set A and freeze the other eye in a whirlpak. If an eye is abnormal, it should be fixed rather than frozen. If both eyes are abnormal, fix one in each Tissue Set. To fix an eye, make a 2-3 cm cut in the globe along the interface of the sclera (white part) and the clear cornea at the front of the eye before placing in formalin.

Eye (FM) A **AND** Sp # _____ Eye (LN) **OR** Eye (FM) B

MUCOUS MEMBRANES: NSF / NE / NA

Pink, pale pink, red, yellow, white, purple, brown, other: _____

ORAL CAVITY: NSF / NE / NA

Ulcers, vomitus, blood, foreign body, other: _____

TEETH: NSF / NE / NA

Unerupted, just erupting, fully erupted, missing, broken, worn (describe): _____

LEFT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___

RIGHT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___

VIBRISSAE: NSF / NE / NA

Absent, torn, other: _____

Collect two vibrissae (with roots) and freeze in a cryovial or whirlpak Sp# _____ Vibrissae (LN)

NAILS: NSF / NE / NA

Absent, torn, bleeding, cracked, crushed, other: _____

PERIPHERAL LYMPH NODES: **NSF / NE / NA**

Feel around the point of the shoulder (prescapular LN), and the angle of the jaw (mandibular LN)
Palpable, obvious, unnoticeable, other: _____

HAIR COAT: **NSF / NE / NA**

Hair missing, oil, molting, scruffy, parasites, foreign bodies, fishhooks, scavenging, abrasion, other:

SKIN: **NSF / NE / NA**

Cracking, bleeding, dry, moist, smooth, rough, wounds, masses, vesicles, ulcers, scars, bruising, abscesses, masses, abnormal coloration, other: _____

Collect two 1x1cm flipper tips in a 2mL cryovial pre-filled with 1.8 ml DMSO then freeze. **For the proper ratio of DMSO to tissue, please ensure that samples approximate the size of a pencil eraser.

Sp# _____ A TP (DMSO/LN)

Collect two samples from any masses, ulcers, vesicles or other external abnormalities. Fix one sample each in Tissue Set A and B(if possible collect unusual findings for both sets A and B, if this is not possible, unusual findings should go into at least set A) and freeze the other in a whirlpak. Try to include the junction of normal and abnormal tissue in the sample. Describe lesions sampled: _____

Skin lesion(s) (FM)A (FM)B Sp# _____ Skin lesion(s) (LN)

EXTERNAL GENITALIA & ANUS: **NSF / NE / NA**

Swollen, protruding/prolapsed, diarrhea, ulcerations, masses, plaques, other: _____

Collect and fix anything appearing abnormal. Abnormal genital tissue (FM)A (FM)B

MAMMARY GLANDS: **NSF / NE / NA**

Lactating, swelling, discharge, parasites, other: _____

If lactating, collect as much milk as possible and freeze. Sp # _____ Milk (LN)

OTHER NOTES ON EXTERNAL EXAMINATION:

INTERNAL EXAMINATION:

BLOOD **NSF / NE / NA**

NOTE: Unused PAX gene tubes should be stored at room temperature and not exposed to excessive heat or cold before use.

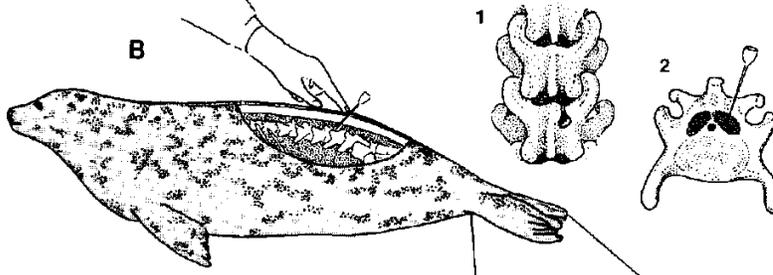
For code 1 carcasses only, attempt to collect blood (1 to 2.5ml) from the epidural venous sinus into a PAX gene tube (see below). Gently rock tube to mix blood and additive. After blood collection, store tube UPRIGHT at ROOM TEMPERATURE for a minimum of 2 hours (longer is ok, just no more than 72 hours). After the 2 hour incubation, transfer sample to cryovial(s) using disposable pipette and place on blue ice (or -20C freezer if available) for 24 hours. After 24 hours on blue ice, transfer cryovials to dewar. If no blue ice is immediately available, tubes may be stored in a refrigerator for up to 5 days before being transferred to blue ice and then to the dewar. Avoid placing samples directly from room temperature storage into the dewar, as this will likely ruin the sample.

Palpate the vertebral column and pelvis and move your fingers cranially 2 or more vertebral spaces, feeling for a “divot” lateral to the spinous processes of the vertebrae. Attach a needle to a 3cc syringe.

Needle choice:

- Pups/weaners: use a 20g or 21g x 1 1/2" needle.
- Adults: use a 3.5" spinal needle. Before insertion, remove the stylet, holding needle from hub only.

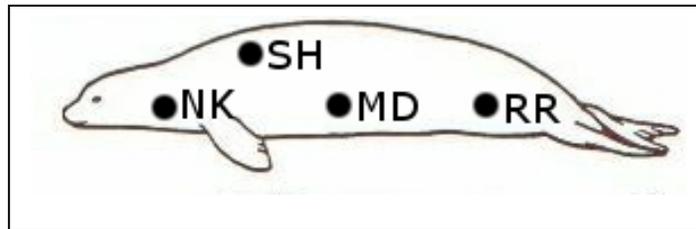
The angle of the needle may vary from a 45 - 90 degree angle to the dorsal surface of the animal. As the needle is inserted, feel it moving through skin, blubber, and muscle until you feel it pop through the membrane of the extradural sinus. Draw back on syringe to collect blood. Put a fresh needle (a small one is fine) on the syringe and push needle through top of PAX gene tube. The vacuum in the syringe should draw the blood into the tube.



BLUBBER

NSF / NE / NA

Thickness (measure on midline of chest between the front flippers): _____ mm



Samples from RR location (either side of the body is ok):

Code 1 and 2 animals only:

Use a 6 mm biopsy punch. **Collect 8 blubber samples** through the **full depth** of the blubber layer, about 2-5 cm, until you reach the muscle layer. Use thumb forceps and/or scissors to retrieve the samples, without damaging or contaminating the blubber tissue. **One punch can be used to collect all 8 samples.** Take great care to prevent contamination of the samples by contact with gloves or other items. **The 4 best full thickness biopsies should go into the 5.0 ml cryovial, and the other 4 should go into the Teflon vial.**

Sp# _____ BB/FA from RR x4 (blubber for fatty acid in cryovial) (LN)

Sp# _____ BB/TX from RR x4 (blubber for toxicology in small Teflon vial) (LN)

All animals:

Collect two 1x1x1cm samples of skin and blubber from the RR location and fix one in each Tissue Set.

Skin/Blubber (FM) A (FM) B

Samples from NK, MD and SH locations (either side of the body is ok):

Collect one **full thickness**, 2 x 2 cm blubber sample from each of the following locations: neck (NK), shoulder (SH) and midsection (MD) locations. Place each sample in separate whirlpak (do not wrap in foil), and label each bag with the body site from which it was collected.

Sp# _____ BL/NK one 2 x 2 cm from NK (fatty acids) (LN)

Sp# _____ BL/MD one 2 x 2 cm from MD (fatty acids) (LN)

Sp# _____ BL/SH one 2 x 2 cm from SH (fatty acids) (LN)

CEREBRAL SPINAL FLUID (CSF)

NSF / NE / NA

Cut through the skin, blubber, nuchal ligament, and neck muscles to the dorsal aspect of the atlantoccipital skull joint (trying to avoid cutting into joint). Slowly insert needle w/ syringe approximately 5-7 mm into the foramen magnum. Collect up to 3mL into a cryovial.

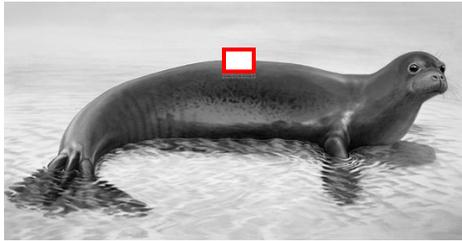
Sp # _____ CSF (LN)

MUSCLE

NSF / NE / NA

Describe abnormalities: _____

Collect the following samples from the middle of the the *longissimus dorsi*, the large long muscle that runs down either side of the spine:



One 2x2x2 inch muscle sample for myoglobin analysis. **Double bag** the sample in whirlpaks and freeze.

Sp# _____ A MU (LN)

One 1x1x0.5cm piece for stable isotope analysis and freeze.

Sp# _____ B MU (LN)

Two 1x1x1cm pieces in formalin and place one in each Tissue Set.

Muscle (FM) A (FM) B

ORO/NASOPHARYNX

NSF / NE / NA

NASOPHARYNX: Look for nasal mites. If observed, please approximate quantity: <10 10-30 30-50 >50

Fluid (Y / N) volume: _____ ml. Describe fluid: _____

TONSILS: enlarged, red, purple, pus, other: _____

Ulcers on tongue, ulcers on gums, ulcers on hard palate, foreign body, vomitus (volume: _____ ml, appearance: _____)

THORACIC CAVITY

Open the chest. Examine external surfaces of lungs and heart *in situ* and note abnormalities in appropriate section. Look for free fluid in the thoracic cavity (around the lungs, pooled at the diaphragm), collect 1-3ml if abnormal (see below) and describe.

Fluid present (Y / N), volume: _____ ml, appearance: purulent (thick/cloudy), serous, fibrinous, yellow, white, green, blood-tinged, frank blood, adhesions, plaques, other: _____

Sp # _____ chest fluid (LN)

Sp # _____ parasites (LN)

SALIVARY GLANDS: NSF / NE abnormalities: _____

THYROID: NSF / NE abnormalities: _____

LARYNX: NSF / NE abnormalities: _____

ESOPHAGUS: NSF / NE dilated, constricted, perforated, ulcerated, hemorrhagic, foreign body, fluid (volume: _____ ml, appearance: _____), other: _____

TRACHEA: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

BRONCHI: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

PARASITES: nematodes, other: _____ location: _____ Severity: <10, 10-20, 20-50, >50

THYMUS: NSF / NE atrophy, prominent, enlarged, other: _____

Note: the thymus shrinks with age, and is not likely be found on adult animals.

Submandibular LN (FM) A (FM) B Thymus (FM) A (FM) B

Tonsil (FM) A (FM) B Esophagus (FM) A (FM) B

Tongue (FM) A (FM) B Trachea (FM) A (FM) B

Thyroid (FM) A (FM) B Bronchus (FM) A (FM) B

BRACHIAL PLEXUS

NSF / NE / NA

If possible, collect the adjacent axillary LN with the brachial plexus and associated vessels and place in formalin.

Notes: _____

Brachial plexus (FM) A (FM) B

CRANIAL and THORACIC LYMPH NODES

NSF / NE / NA

PULMONARY LYMPH NODES: Look around the base of the heart (they are located at the largest end of the blood vessels going to the lungs). Collect paired samples in formalin and freeze one for microbiology.

Pulmonary LN (FM) A (FM) B Sp # _____ PN (LN)

MEDIASTINAL LYMPH NODES: Look around the heart and between the lungs. It is very difficult to determine the specific name of the node but all we are after are any abnormal lymph nodes and a few normal from the chest cavity. Collect paired sampled in formalin and freeze one for microbiology.

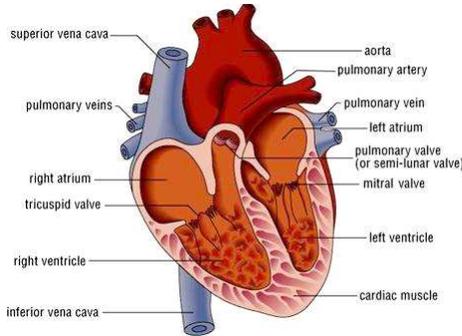
Mediastinal LN (FM) A (FM) B Sp # _____ MN (LN)

OTHER LYMPH NODES: Examine as many additional cranial lymph nodes as possible: mandibular, axillary, prescapular, sternal. Describe any abnormalities below. Suggested descriptors: hemorrhagic, gelatinous, serous fluid, soft, hard, enlarged (mild/mod/severe).

Collect 1x1x1cm samples from abnormal nodes and preferably 2-5 normal nodes, label with a laundry tag and fix one sample from each node in each Tissue Set. Be sure to include abnormal and normal tissue in the fixed samples. For code 2+ carcasses, sample the freshest lymph nodes, label and place one sample from each node in each Tissue Set.

List lymph nodes collected in formalin: _____ (FM) A (FM) B

HEART



PERICARDIUM (heart sac): NSF / NE thickened, plaques on surface, contains fluid (volume: _____ ml, describe: _____)
Collect pericardial fluid if abnormal (>1 or 2 mL):

Sp # _____ HF pericardial fluid (LN)

PULMONARY ARTERIES AND AORTA: NSF / NE thrombi, plaques, rupture, other: _____

VALVES*: NSF / NE diffusely thickened, nodular thickening, vegetative/proliferative lesion (valve(s): _____)

**If valves appear abnormal, place a sample in each Tissue Set.*

LEFT / RIGHT VENTRICLES: NSF / NE thickened, dilated (location _____ thickness: _____ mm)

MYOCARDIUM (heart muscle): NSF / NE pale, tumors, abscess, white foci (location: _____)

ATRIA AND AURICLES: NSF / NE thickened, dilated, pale, tumor, abscess, white foci

Parasites describe:

severity: <10, 10-20, 20-50, >50

Notes: _____

Collect the following and fix one of each in each Tissue Set:

- Section through L. ventricle/Intraventricular septum/R. ventricle* (FM) A (FM) B
- Aorta (FM) A (FM) B
- Pulmonary arteries (FM) A (FM) B
- Section through R. atrium and atrioventricular valve** (FM) A (FM) B
- Section through L. atrium and atrioventricular valve** (FM) A (FM) B

*The intraventricular septum separates the right and left ventricles. The left ventricle is typically thicker-walled than the right ventricle.

**The atrioventricular valves are the valves located between the atrium and the ventricle on each side of the heart (left side = bicuspid; right side = tricuspid).

Next, collect (2) 4x4cm samples of heart tissue, wrap in foil, and freeze.

Sp # _____ A Heart (LN) Sp # _____ parasites (LN)

B Heart (LN)

LUNGS

NSF / NE / NA

Describe: pink, red, purple, mottled, congested, consolidated, abscesses, granulomas, emphysema, masses, interstitial edema

Specify location, distribution, severity: _____

Parasites: none detected 1 2+ 3+ 4+ Describe color, size: _____

Collect two 2x2x1cm pieces of lung and fix one in each Tissue Set. Collect additional samples if abnormalities are observed. Be sure to include both normal and abnormal tissue and describe abnormalities.

Lung (FM) A (FM) B

Collect (2) 4x4cm pieces of lung, wrap in foil, and chill on blue ice.

Sp # _____ A Lung (LN)
B Lung (LN)

ABDOMINAL CAVITY

NSF / NE / NA

Open the abdomen and look for any excess or thickened fluid, collect up to 3ml of fluid in a cryovial and describe it below. Examine external surfaces of abdominal organs *in situ* (before you move them) and note abnormalities in appropriate organ section below. Fluid present (Y / N), volume: _____ ml, appearance: pus, serous (like serum), stringy, yellow, white, green, blood-tinged, blood, fibrous adhesions/scars, plaques, other: _____

Sp # _____ abdominal fluid (LN)

DIAPHRAGM

NSF / NE / NA

Collect two 2x2 cm samples of the diaphragm and fix one in each Tissue Set. Notes: _____

Diaphragm (FM) A (FM) B

ADRENAL GLAND

Right: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____
Left: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____
Region (cortex vs medulla) and distribution of lesions: _____

Adrenal gland (FM) A (FM) B Sp # _____ Adrenal (LN)

GALL BLADDER

NSF / NE / NA

Describe: full, empty, thickened wall, flukes (severity: <10, 10-20, 20-50, >50), other: _____

Bile: thick/chunky, thin/runny, black, dark green, light green, yellow, orange, stones present
If present, collect 1-3ml of bile (code 1-2 carcasses only) and place in a cryovial and whirlpak, wrap in foil to protect from light.

Sp # _____ Bile (LN)

Collect two 2x2x1 cm sections of gall bladder and fix one in each Tissue Set.

Gall bladder (FM) A (FM) B

LIVER

NSF / NE / NA

Describe: enlarged, small, tan, brown, black, yellow, orange, mottled, abscesses, granulomas, masses, cysts, hemorrhage, parasites, other: _____

Collect at least two 2x2x1 cm of liver and fix one in each Tissue Set. Be sure to also include both normal and abnormal liver in each Tissue Set.

Liver (FM) A (FM) B

Collect and freeze: (3) 4x4cm liver samples, two 1x1x1cm samples and one 2x2x1cm sample and freeze.

Sp # _____ A liver 4x4cm (tox) (LN) D liver 1x1x1 (micro) (LN)
B liver 4x4cm (tox) (LN) E liver 1x1x1 (micro) (LN)
C liver 4x4cm (biotox) (LN) F liver 2x2x1 (stable isotopes) (LN)

Collect the hepatic lymph nodes for microbiology, chill on blue ice. Look around the base of the large blood vessel coming from the aorta nearest the liver.

Sp # _____ hepatic lymph nodes (LN)

PANCREAS

NSF / NE / NA

Loss of lobulation, swollen, hemorrhage, abscesses, other: _____

Collect two 2x2x1 cm pices of pancreas and fix one in each Tissue Set.

Pancreas (FM) A (FM) B

SPLEEN **NSF / NE / NA**

Masses, enlarged (mild / moderate / severe), constricted, congested, abscesses, scars, pale, purple, brown, red, other: _____

Collect tissues for 2 sets for histopathology and one for microbiology (1x1x1 cm):

Spleen (FM) A (FM) B Sp# _____ 1x1x1cm spleen A (LN)

STOMACH **NSF / NE / NA**

Erosions, ulcers, perforated ulcers, hemorrhage, loss of rugal folds, swollen rugal folds, other: _____

Mucosa: white, pale pink, red, purple, other: _____

Parasites (ascarids): <10 10-20 20-50 >50 Describe (size, color): _____

Collect representative sample of parasites and freeze. Parasites Sp# _____ (LN)

Stomach contents: empty, dilated with gas, milk, mucus, fish (digested / partially digested / undigested), foreign body, other: _____

Collect stomach contents into whirlpaks or cryovials and chill on blue ice. Stomach contents Sp# _____ (LN)

Collect two 2x2x1 cm sections of stomach tissue and fix one in each Tissue Set. Be sure to also include any abnormal tissue (ulcers, thickened rugal folds, etc.).

Stomach (FM) A (FM) B

INTESTINES **NSF / NE / NA**

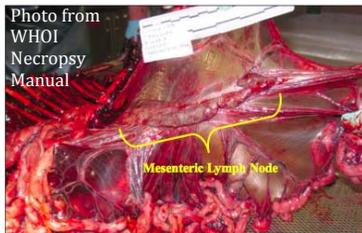
Open up ~4-6" of each section of the gastrointestinal tract, and look for abnormalities in color or thickness. Be sure to include any abnormal tissue as well as normal tissue in the formalin fixed samples. For intestinal samples, take a complete transverse "ring" of tissue ~1-2 cm in width, trying not to touch or disrupt the inside of the ring.

Duodenum (FM) A (FM) B Cecum (FM) A (FM) B

Jejunum (FM) A (FM) B Colon (FM) A (FM) B

Also collect one sample (2 cubic inches) of small intestine for microbiology and freeze. Small intestine Sp# _____ A (LN)

MESENTERIC LYMPH NODE:



Collect samples from the mesenteric lymph node anywhere along the intestinal mesentery, preferably both at the cranial and caudal portions of the abdomen/intestinal tract. Collect 2 sets for tissue set A and B, and freeze one whole for microbiology. Chill on blue ice.

Mesenteric Lymph Node (FM) A (FM) B

Sp # _____ ML (LN)

FECES: Cut colon near anus and squeeze contents from distal intestines directly into container.

FE (feces) Sp# _____

A. Collect sub-sample in pre-filled DMSO vial, fill to the 5 ml line on the vial

Feces A (DMSO)

B. Collect 1-3 g in a whirlpak for ciguatera analysis (FE/CX)

Feces B (whirlpak)

C. Freeze an additional 10-30 g in whirlpaks or wide-mouth cyrovials for hormonal studies (FE/HR) (whirlpak/cryo)

Feces C

D. Freeze 10 g of feces (or meconium if newborn pup) into whirlpaks or cryovials

Feces D (whirlpak/cryo)

URINARY TRACT **NSF / NE / NA**

KIDNEYS:

Right: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis (distended), mass, calculi, emboli, infarct, loss of renule differentiation, other: _____

Size: normal, small, enlarged (mild / moderate / severe), other: _____

Cortex (outside layer): pink, tan, red, purple, other: _____

Medulla (inside layer): pink, tan, red, purple, other: _____

Left: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis (distended), mass, calculi, emboli, infarct, loss of renule differentiation, other: _____

Size: normal, small, enlarged (mild / moderate / severe), other: _____

Cortex (outside layer): pink, tan, red, purple, other: _____

Medulla (inside layer): pink, tan, red, purple, other: _____

Collect kidney in each Tissue Set, as well as two 4x4cm samples for toxicology, and two 1x1x1cm samples for microbiology:

Kidney (FM) A (FM) B

Sp # _____ A Kidney 4x4cm (tox) (LN) C Kidney 1x1cm (micro) (LN)

B Kidney 4x4cm (tox) (LN) D Kidney 1x1cm (micro) (LN)

URETERS: NSF / NE dilated, tumors, abscesses, stones/calculi, hydroureter (distended due to obstruction), other: _____

URINE: Attach a sterile 18 g x1.5" needle to a sterile syringe, insert the needle into bladder, and draw on syringe to collect up to 5 mL.

Amount: _____ ml ; bloody, golden, yellow, pale yellow, clear, cloudy, purulent, other: _____

Sp # _____ Urine (LN)

URINARY BLADDER: empty, full, dilated, thickened, masses, hemorrhagic, ulcerated, necrotic, other: _____

Bladder (FM) A (FM) B

MALE REPRODUCTIVE TRACT **NSF / NE / NA**

PREPUCE: NSF / NE

PENIS: NSF / NE discolored, pustules, mass, torsion, laceration, plaque, other: _____

TESTES

Left: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Right: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Collect paired samples and place one in each Tissue Set. Also collect and fix anything appearing abnormal.

Penis (FM) A (FM) B

Left Testis (FM) A (FM) B

Right Testis (FM) A (FM) B

FEMALE REPRODUCTIVE TRACT **NSF / NE / NA**

VULVA: NSF / NE other: _____

VAGINA: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

UTERUS: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

CERVIX: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

OVARIES:

Left: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Right: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Female Reproductive Tract (FM) A (FM) B

For pregnant females, aborted fetuses, or perinatal pup deaths, examine and collect umbilicus, placenta and fetus:

UMBILICUS (describe): _____

Umbilicus (FM) A (FM) B Sp# _____ Umbilicus (LN)

PLACENTA: Collect four 5cm x 1cm **full thickness** strips (extending through to include both the fetal and maternal side) representative of normal and any abnormal portions of the placenta. Fix one sample in each Tissue set and freeze the other two samples.

Placenta (FM) A (FM) B Sp # _____ Placenta A (LN) B (LN)

FETUS: Perform a complete necropsy if possible and use separate Necropsy Report Form.

A fetus or premature pup "P0" is defined as <75cm straight length; the pelage, whiskers, nails, or oral cavity not fully developed.

Fetus necropsied: Y / N Necropsy # _____ (PIFSC assigns)
Straight length: _____ cm Axillary girth: _____ cm
Mass: _____ kg Sex: M / F

If the fetus appears fresh, take one Dacron virology swab from both the throat and rectum *before* beginning fetal necropsy.

Sp # _____ Throat Swab (LN)

Sp # _____ Rectal Swab (LN)

SPINAL CORD	NSF / NE / NA
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After cutting off the head, the spinal cord will be visible within the spinal canal. Fix one sample in each tissue set and freeze one.

Spinal cord (FM) A (FM) B Sp # _____ Spinal cord (LN)

BRAIN	NSF / NE / NA
--------------	----------------------

Clean away tissue on the skull where the hacksaw will be cutting. Before cutting the skull, examine it carefully, **photograph** any fractures or blunt injuries and collect any injured portions of the skull. Attempt to remove the brain intact and handle gently. **Collect the brain even if it has liquefied.**

Sp # _____ Skeletal (collect injured skulls only) (DR)

If liquefied: Collect liquefied brain in a whirlpak or cryovials and freeze.

Sp # _____ Brain A (LN)

If the brain is whole: there is a tough covering (tentorium cerebellum) separating the cerebrum from the cerebellum that can be cut with scissors or scalpel. Once removed, split the right and left halves of the brain using a new scalpel blade.

Fix a sample from cerebrum, cerebellum and brainstem in each Tissue Set. Freeze two pieces of cerebrum in whirlpaks. Be sure to collect representative samples of both normal and abnormal brain tissue in formalin and whirlpaks.

CEREBRUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

CEREBELLUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

Cerebrum (FM) A (FM) B Sp # _____ Brain A (LN) B (LN)

Cerebellum (FM) A (FM) B

Brainstem (FM) A (FM) B

DURA MATER and SKULL	NSF / NE / NA
-----------------------------	----------------------

Examine the inside of the skull (the side against the brain) for any evidence of fractures or hemorrhage (discrete regions of black/purple discoloration that may look like grape jelly). Thoroughly photograph any that are found. Collect any injured portions of the skull.

SKULL: discolored (describe: _____), pus, hemorrhage, congested, other: _____

The mandible (lower jaw) and all teeth within it should be collected and placed in a whirlpak for **unknown age animals only**.

Sp # _____ Skeletal (mandible) (DR)

Reminder:

24-48 hours after necropsy, pour off formalin from all formalin fixed tissues, rinse the tissues in fresh water, and store them in afresh 10% formalin solution until transporting tissues to Honolulu. Just before transport, all formalin should be poured off and transported in carboys. If necropsy is conducted within the 24-48 hour period, it is ok to transport the tissues in formalin, but be sure to pack them with enough absorbent material to soak up all formalin in case of leakage and clearly label any buckets containing formalin (for more details see "Packing and Shipping" section of Specimen Collection Protocol).

HAWAIIAN MONK SEAL *PARTIAL* NECROPSY REPORT FORM (for code 4 & 5 carcasses only)

If there is **any** doubt whether to use this form of the full form, **USE THE FULL FORM** and do as thorough a necropsy as possible.

Assign a necropsy number to ALL seals found dead in the season, whether or not a necropsy is actually performed.

SEAL ID/temp ID _____ **DEATH/NEC. #** _____ *(assign sequential #'s by calendar date for all seals)*
Date/time: necropsied _____ found dead _____ last seen alive _____
 Island/Atoll _____ Islet _____ Sector _____ Lat _____ Long _____
 Beach position _____ Carcass orientation (i.e., horizontal to water line) _____
 Size/sex _____ Age _____
 Persons performing/assisting with necropsy: _____
RECORDER: _____ **PHOTOGRAPHER:** _____
 Photos? Y / N File names: _____

HISTORY

Identifying body markings (take photos): _____

Last live observation(s): _____

Circumstances of death (found dead/euthanized/other-explain): _____

Tags

Record number and condition of any flipper tag(s), photograph, and collect all tags in labeled whirlpak.

R _____ L _____ Notes: _____

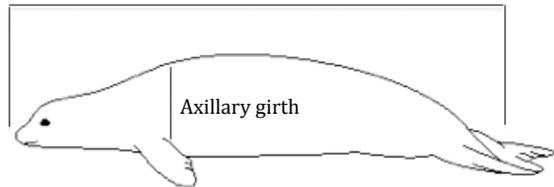
Scan the entire body for pit tags. PIT tag#(s):

R _____ L _____ Location found _____

Morphometrics

Axillary girth _____ (cm)
 Straight length _____ (cm, circle: DSL or VSL)
 Total body mass _____ (circle: kg/lb)
 Measurer _____

Straight Length (tip of nose to tip of tail)



Carcass Condition Code: 4 5

Code 4: Poor/advanced decomposition (carcass collapsed, skin slough
 liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)
 Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

GROSS NECROPSY EXAMINATION

INSTRUCTIONS

1. Describe all organs, tissues and lesions in the fields provided. If appropriate, circle either NSF, NE or NA at the top of each section. If the tissue examined is abnormal, describe the location, color, size and distribution within the tissue in the blanks provided.

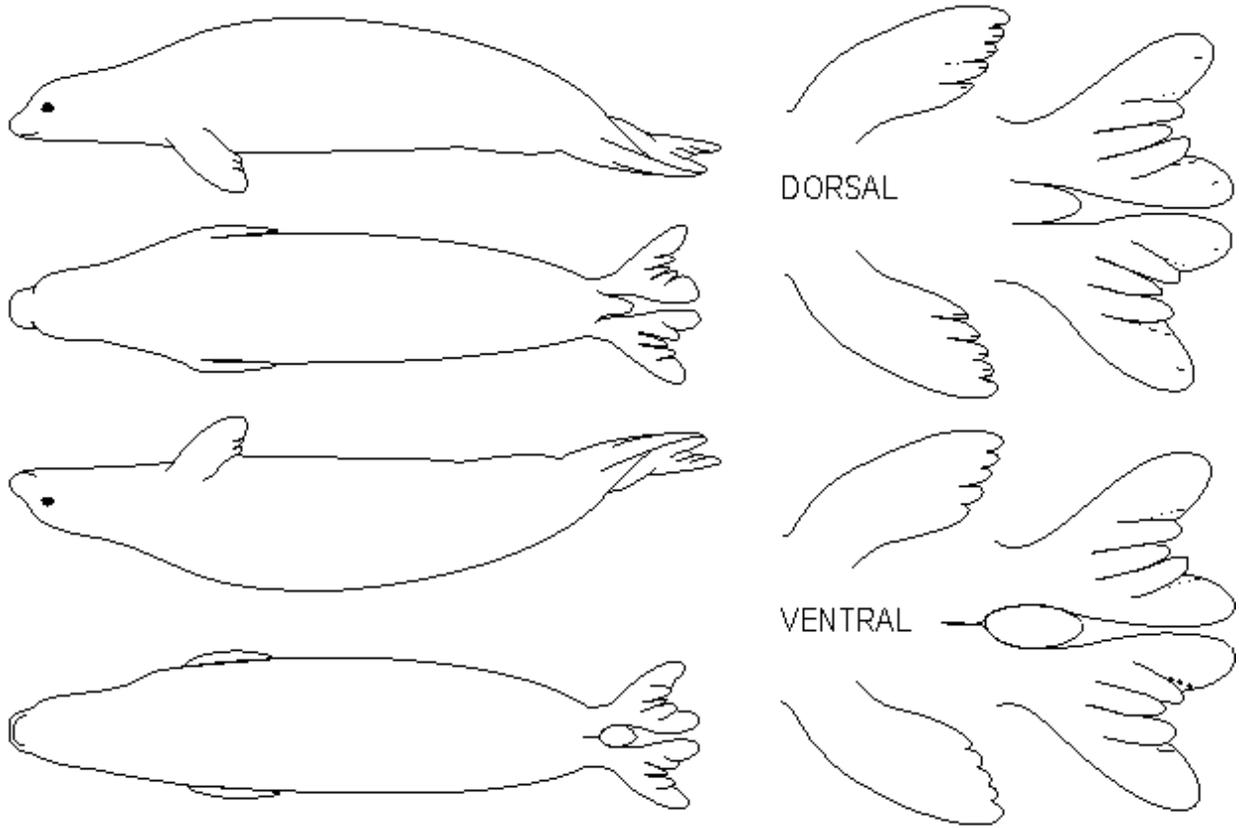
4. A sample of any visible endoparasites should be collected in alcohol per protocol below.

5. If possible, remove head and place in cooler on ice while remainder of necropsy is completed, then go back and sample brain after the remainder of the necropsy is complete.

6. Examine all outside surfaces and note abnormalities, discharge, etc. Describe/draw abnormalities below. Photograph with full-frame views of all 4 sides (dorsal/ventral/left lateral/right lateral) as well as close-ups of scars or other distinguishing characteristics, injuries, abscesses, line marks or other abnormalities. Include an index card in the image frame that notes the following: Seal ID, Date, Size, Sex, SF#, Necropsy #, Location (island/atoll).

NOTE: In cases of drowning, there are few clues, if any, so look closely for external indications of entanglement (bent/missing vibrissae, torn/missing nails, cuts in and around the nose, mouth, and gums). Also examine the tips of all extremities to look for line or net marks. Describe/draw and photograph any evidence of entanglement. Collect any gear found on an animal in a ziploc bag and label.

Draw all abnormalities and markings (include bruises, wounds, contusions, old scars, condition of and tears of skin, external parasites):



ABBREVIATIONS	
NSF: no significant finding	DB: DMSO/blue ice
NE: not examined	DR: dry

EXTERNAL PHYSICAL EXAMINATION (Circle all that apply):

GAS/ PUTREFACTION: (<i>Run your hands firmly along the body and feel for bubbles.</i>) NE / NA	NSF /
---	--------------

NUTRITIONAL STATE: Fat/overweight, Normal/average, Thin/poor, Starving/emaciated Notes: _____
--

DISCHARGE: NE / NA	NSF /
Location: eyes, nose, mouth, genitals, anus Color/Texture: green, white, clear, red, thick, runny Notes: _____	

EYES: NE / NA	NSF /
NSF, NE, missing, bulging, deflated, out of socket, foreign body present, penetrating wound Notes: _____	

MUCOUS MEMBRANES: NE / NA	NSF /
Pink, pale pink, red, yellow, white, purple, brown, other: _____	

ORAL CAVITY: NE / NA	NSF /
Ulcers, vomitus, blood, foreign body, other: _____	

TEETH: NE / NA	NSF /
Unerupted, just erupting, fully erupted, missing, broken, worn (describe): _____ LEFT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___ RIGHT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___	

VIBRISSAE: NE / NA	NSF /
Absent, torn, other: _____ Collect two vibrissae (with roots) and freeze in a whirlpak Sp# _____ Vibrissae <input type="checkbox"/> (LN)	

NAILS: NE / NA	NSF /
Absent, torn, bleeding, cracked, crushed, other: _____	

PERIPHERAL LYMPH NODES: NE / NA	NSF /
<i>Feel around the point of the shoulder (prescapular LN), and the angle of the jaw (mandibular LN)</i> Palpable, obvious, unnoticeable, other: _____	

HAIR COAT: NE / NA	NSF /
Hair missing, oil, molting, scruffy, parasites, foreign bodies, fishhooks, scavenging, abrasion, other: _____	

SKIN: **NSF /**
NE / NA

Cracking, bleeding, dry, moist, smooth, rough, wounds, masses, vesicles, ulcers, scars, bruising, abscesses, masses, abnormal coloration, other: _____

Collect two 1x1cm flipper tips in a 2mL cryovial pre-filled with 1.8 ml *DMSO* then freeze. **For the proper ratio of DMSO to tissue, please ensure that samples approximate the size of a pencil eraser.

Sp# _____ A TP (DMSO/LN)

Collect any skin lesions and freeze in a whirlpak.

Describe lesions:

Skin lesion(s) (LN)

MUSCLE **NSF / NE / NA**

Describe abnormalities: _____

Collect one 1x1x0.5cm piece of muscle for stable isotope analysis and freeze.

Sp# _____ A MU (LN)

OTHER NOTES ON EXTERNAL EXAMINATION:

INTERNAL EXAMINATION:

Thickness (measure on midline of chest between the front flippers): _____ mm

BLUBBER **NSF / NE / NA**

Thickness (measure on midline of chest between the front flippers): _____ mm

STOMACH **NSF / NE / NA**

Stomach contents: empty, dilated with gas, milk, mucus, fish (digested / partially digested / undigested), foreign body, other: _____

Collect stomach contents into whirlpaks or cryovials and chill on blue ice then freeze.

Stomach contents Sp# _____ (LN)

BRAIN **NSF / NE / NA**

Collect the brain even if it has liquefied. Before cutting the skull, follow directions below under the skull section. When opening the skull to collect the brain, be sure to collect the injured portions of the skull without damaging them. To prevent tissue from clogging the teeth on the saw, first clean away any tissue on the skull where the hacksaw blade will be cutting.

Describe: _____

If the brain has decomposed to the point that it has liquefied, collect in a whirlpak or cryovials and freeze.

Sp # _____ A Brain (BI)

B Brain (BI)

SKULL

NSF / NE / NA

Examine the skull carefully and **photograph** any fractures or blunt injuries. Collect any injured portions of the skull. Examine the inside of the skull (the side against the brain) for any evidence of fractures or hemorrhage (discrete regions of black/purple discoloration that may look like grape jelly). Thoroughly photograph any that are found. Collect any portions that appear injured.

SKULL: discolored (describe: _____), pus, hemorrhage, congested, other: _____

The mandible (lower jaw) and all teeth within it should be collected and placed in a whirlpak for **unknown age animals only**.

Sp # _____ Skeletal (mandible) (DR)

Sp # _____ Skeletal (collect injured skulls only) (DR)

If a Fetus is found – follow instructions below**Fetus (Describe):**

If fetus is <25 cm in length, split it in half from chin to pubis and place both halves in formalin. If fetus is >25 cm in length, **perform a complete necropsy and use the full Necropsy Report Form.**

If complete necropsy is performed, use full Necropsy Report Form. Handle tissues in a sterile manner. Take one swab from both the throat and rectum before beginning necropsy. **Priority samples (if available), regardless of condition code: Brain, lung, liver, stomach/stomach contents and placenta.**

If possible, collect several cc's of fluid from the stomach and freeze in liquid nitrogen. *Record this information on the full Necropsy Report Form.*

Ventral Length: _____ cm

Axillary Girth: _____ cm

Mass: _____ kg

Sex: M or F

Condition (Describe):

Fetus Necropsied: Y or N (if Y, see Necropsy # _____)

If Fetus is < 25 cm:

OR

If Fetus is > 25 cm:

Sp # _____ A Fetus (WC) (FM)

Sp # _____ Throat Swab (LN)

B Fetus (WC) (FM)

Sp # _____ Rectal Swab (LN)

Sp # _____ Stomach Fluid (LN)

MHI HAWAIIAN MONK SEAL NECROPSY PROTOCOL

SAFETY CONSIDERATIONS

Before performing a necropsy, read the following documents:

“Appendix II: Infectious Agents” (Aguirre, *et al.*, 1999) at the end of this document and/or in the tagging/handling section of your manual.

“Marine Mammal Zoonotic Bacteria” available in the “Visual Monk Seal” and online at:
<http://www.vetmed.ucdavis.edu/whc/mmz/bacteria.htm#Marine%20Mammal%20Zoonotic%20Bacteria%A0>

“Working with Marine Mammals and Your Health” available in the “Visual Monk Seal” and online at:
<http://www.vetmed.ucdavis.edu/whc/mmz/Occupational%20Safety.htm>

“Assessment of the Risk of Zoonotic Disease Transmission to Marine Mammal Workers and the Public: Survey of Occupational Risks” available in the “Visual Monk Seal” and online at:
http://www.sefsc.noaa.gov/PDFdocs/Marine_Mammal_Zoonoses_Final_Report.pdf

Preventing Disease Transmission

Avoid direct contact with dead seals to prevent transmission of infectious diseases that may be pathogenic to humans.

Persons performing the necropsy must:

9. Cover all surface wounds with a protective dressing before gearing up.
10. Wear protective gear, including latex or vinyl gloves, mask, disposable gowns, and foot covers. Change torn gloves **immediately**.
11. Seek medical attention immediately if you get any cuts, punctures or other injuries during the necropsy. Notify the attending physician of the source of the injury.
12. Disposable items such as scalpel blades, needles and biopsy punches **MUST** be disposed on in the sharps containers.
13. If possible, pull carcass up the beach to higher ground and bury it after necropsy to avoid attracting scavengers and minimize the potential for disease transmission.
14. Disinfect all instruments and contaminated equipment after the necropsy has been performed (see Post Necropsy section, below).
15. Once the necropsy has been performed and all gear has been cleaned and disinfected, wash thoroughly with soap. Disinfect reusable clothing with bleach solution (see tagging handling protocol) and dispose of all contaminated clothing, gloves, gowns, etc in a biohazardous waste bag.
16. **DO NOT STORE ANY SPECIMENS IN FREEZERS/REFRIGERATORS USED FOR HUMAN FOOD.**

GENERAL CONSIDERATIONS

A necropsy is a systematic examination of the whole body, organs, and tissues and is a basic tool for investigating disease and for monitoring the health of the Hawaiian monk seal population. **Whenever possible, necropsies should be performed by a trained veterinary pathologist** experienced in recognizing and interpreting lesions and abnormalities.

Necropsy How-To Guides:

For general guidance on the steps in performing a necropsy, please refer to the following resources, but follow the sample collection protocols provided in this document and the most recent version of the Necropsy Report Form.

3. "Field Manual for Phocid Necropsies (specifically *Monachus schauinslandi*)" (FMPN)
4. “Marine Mammal Necropsy: An Introductory Guide for Stranding Responders and Field Biologists” – available at:
<https://darchive.mblwhoilibrary.org/handle/1912/1823>

Necropsies will have the most scientific value when they are carefully documented. Adherence to this protocol and the Necropsy Report Form will assist in the documentation and standardization of information, which may be valuable in determining morbidity and mortality factors within the population and as well as for individual seals.

Things to keep in mind:

1. Record all observations – when in doubt, just describe what you see.
2. The order of the Necropsy Report Form follows the sequence of general dissection and examination. If you are skilled and familiar with Hawaiian monk seal necropsies, you may find it easier to use the Necropsy Specimen Checklist, but **be sure to have someone record all observations, photos, measurements, and descriptions of organs on the Necropsy Report Form.**
3. Tissues and organs must be examined in a systematic manner. The precise method used for a necropsy is less important than establishing a routine in which each body system is examined fully.
4. **Once the carcass has been opened, take tissue specimens for virology, bacteriology and toxicology first, then sample for histopathology and parasitology.**
5. Samples of **normal and abnormal** tissue should be collected for laboratory analyses.

The ability to obtain reliable data from necropsies depends on the following:

1. Condition and location of the carcass
2. Adherence to detailed protocols
3. Number of seals necropsied throughout the year
4. Amount of time available to perform a thorough necropsy
5. Care in sample preservation and labeling of specimens
6. Care in shipping and storing specimens

Decomposed carcasses may be unsuitable for histopathology but can be useful for observing gross lesions. Collect brain samples regardless of the state of decomposition. Collect samples from all organs listed, even those that appear normal. In general, tissue specimens must be sufficiently thin (**less than 1 cm thick**) to allow proper fixing of 10 parts 10% buffered formalin: 1 part tissue. For some tissues (e.g. brain and lung), you may need to make parallel cuts (0.5 cm in thickness) in the tissues to allow preservation. After the tissues have been fixed in formalin 24-48 hours, pour off the formalin, rinse the tissues in fresh water, and add fresh formalin solution.

NECROPSY INSTRUCTIONS

Complete a Hawaiian Monk Seal Necropsy Report Form for **all** carcasses recovered. Use the full form if you perform an internal examination of the carcass, regardless of the condition code. The partial form can be used for necropsies where very minimal data is collected. Record "N/A" for any sections that are not applicable, and state what organs/tissues were not examined. At a minimum, describe each organ examined and sample as many organs as possible, prioritizing the following tissues: brain, lung, liver, kidney, blubber.

Photograph the exterior for ID (even if tagged), to document injuries or other unusual conditions, and to document body condition. Photograph the seal from all 4 sides (dorsal/ventral/left lateral/right lateral) and a close up of the hind flippers with tags. In addition, take close-ups and a wider view (to show perspective) of injuries and unusual conditions. If possible, include an index card in each frame that notes the following: Seal ID, Date, Size, Sex, and island and a ruler. Record photographs on the Necropsy Report Form.

External examination

Document any specific external lesions, abnormalities, or scar patterns. Examine, describe, and photograph any external lesions or injuries, the anogenital area, scars and other distinguishing characteristics.

Experience has shown that in cases where pinnipeds have drowned, there is often a complete absence of expected gross and histological findings. For this reason, it is imperative to look closely for external indications of entanglement. Findings may include: bent or missing vibrissae, torn or missing nails, and cuts in and around the nares, mouth, and gums. Closely examine the tips of all extremities to look for line or net cuts. Linear marks on the pelage are also of interest. Photograph any suspected abnormalities with close up/macro images, followed by images that demonstrate the location(s) on the body of each close up image.

Carcass condition codes

Evaluate carcass condition (state of decomposition). Carcass condition is influenced by many factors including disease, body temperature, and environmental temperature. **Rigor mortis** (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. *Rigor mortis* indicates that a carcass may be in good condition (Code 2).

Code 1: just died (e.g., euthanasia)

Code 2: fresh/carcass in good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin and mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas). NOTE: Rigor mortis (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. Rigor mortis indicates that a carcass may be in good condition

Code 3: fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)

Code 4: poor/advanced decomposition (carcass may be intact but collapsed, skin sloughing, strong odor, blubber soft with pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)

Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

Tags

If flipper tags are present, note their condition on the survey form (data type 'T') and tag condition forms. Collect and place them in a whirlpak bag labeled with animal ID, island/atoll, date, and survival factor number. Scan the entire body for PIT tags by holding the PIT tag reader as close to the body as possible. Even if PIT tags are not found, indicate on the survey sheet that a scan was completed and where on the body the scan was performed.

Morphometric measurements

Axillary girth – At the armpit, measure the circumference around the entire body in centimeters.

Standard length – Measure the straight line (not curved) length of the entire seal from the tip of the nose to the tip of the tail in centimeters. If a scale is available, weigh the body and report units. **Record measurements on both the TAGGING/HANDLING CARD and the Necropsy Report Form.**

Swab Collection

Use sterile Dacron swabs. Avoid touching swab tip to anything other than the tissue being swabbed. Immediately place swab in cryovial and break off the end of the plastic applicator against the side of the cryovial container (it should snap easily).

Internal Examination

TAKE INTERNAL PHOTOGRAPHS ONLY WHEN UNUSUAL CONDITIONS ARE NOTED OR IF YOU ARE UNSURE IF IT IS UNUSUAL. If unusual conditions are noted, include a size reference (*e.g.*, ruler) and label with seal ID, survival factor number, date, and island. Take two photographs, one with the organ *in situ* (in its anatomical position/location) in the body and one with the organ removed from the body and placed on a solid white or light blue surface.

Record complete and thorough observations. Assume more is better when describing and recording information. The rule here is if in doubt, write it down. If unsure whether something is abnormal, state this and succinctly describe. Descriptions should be clear, concise, and without personal interpretation. Appropriate tissue preservation along with YOUR precise description of findings may allow the identification of causes of death in the population.

Identify the appropriate descriptors for each organ examined. The descriptions provided herein are NOT an exhaustive list of terms, but rather a list for your reference. **Describe surface, consistency, color, and cut surface of both normal tissues and abnormalities or lesions.**

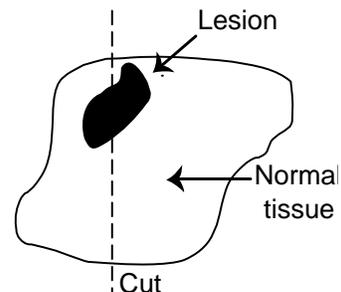
Descriptors of Organs and Lesions

Surface: Smooth, rough, shiny, dull, thickened, wrinkled.
Consistency: Firm, soft, flabby, dry, wet, fluid-filled, sharp-edged, friable (easily pulverized or crumbled).
Color: Transparent, translucent, opaque; white, cream, green, yellow, brown, pink, red, nutmeg (normal pattern of liver), etc. Use simple colors, do not get complicated. Also comment how color is spread through tissue- homogeneous, speckled, streaked, blotchy, blanched, mottled (*i.e.*, pink with specks of red). Additional descriptors may include bright, pale, dark.
Cut surface: Slice organ several times appropriately and spread apart to look at internal surface. Be sure to describe color of the cut surface. Descriptors include swollen, bulging, shiny, dull, eroded, glistening, scaly,

Size:	pitted, oozing Record in metric system (mm, cm), measure length, width and depth or diameter of the lesion. Enlarged, (hypertrophied), small (atrophied), normal size.
Shape:	Square, rectangular, triangular, oval, round, cuboidal, spherical, discoid, rhomboid, tear-shaped, wedge-shaped, spindle-shaped, irregular, long, slender, indented, narrow, lace-like, tortuous, branching, speckled (miliary), flat, raised, depressed, shrunken, papillary, cauliflower-like.
Distribution:	Single discrete lesion (focal), multiple lesions in one location (multifocal), or multiple lesions scattered diffusely throughout the organ or body cavity (diffuse); locally extensive, random, even.
Location:	Surface, capsule, wall, dorsal or ventral, caudal or cranial, anterior or posterior, medial or lateral, proximal or distal, internal or external, full or partial thickness of a wall of an organ.
Fluid:	Clear, cloudy, turbid, thick, thin, bloody, mucoid, exudate, dark, tarry
Consistency:	Spongy, granular, gel-like, firm, soft, hard, rock-hard, dense, creamy, buttery, brittle, lumpy, velvety, warty, tenacious, gritty.
Cut surface:	Bulging, engorged, granular, nodular, pitted, oozing
Odor:	None, sweet, sour, rancid, ammonia-like, putrid, fruity, petroleum-like

Collecting necropsy tissues

Each complete necropsy should have two jars containing complete tissue sets of all tissues, and both having the same specimen number. One set should be sub# A and the other sub# B. Tissue set A should be the most complete set, (e.g. if you freeze one eye, tissue set A should have the formalin fixed eye). If there are any unusual lesions in any of the tissues sampled, be sure to include the margin between abnormal and normal tissue in both tissue sets A and B.



Collect samples of ALL LESIONS in formalin. Describe and sample areas that appear to stand out in marked contrast to the main body of tissue. Samples should include the margins between the normal and abnormal tissue and a description (i.e., sharp line versus vague and gradual, circumscribed, encapsulated). Make sure to check the boxes next to the appropriate specimens as collected on the necropsy report form.

Tissues for Toxicology (contaminants and biotoxins): Code 1, 2 ideal. Codes 3, 4, 5 useless.

Toxicological analyses may be performed for heavy metals, organochlorides, selenium, and dioxin. When sampling for toxicology, it is important to use standardized sampling procedures so that even when low levels of contaminants are present, differences may be attributed to biological processes and contaminant exposure and not to variation in the collection process.

1. Samples must be collected **less than 24 hours** from time of death. **Freeze at lowest temperature** available ASAP.
2. Tissue samples will be taken in duplicate (2 of each, except when noted).
3. Use a new, sterile blade for each organ sampled. Any stainless steel instruments used in contact with tissues should be cleaned with distilled water and rinsed with isopropyl alcohol before using if possible. **Each specimen should be rinsed with distilled water (if possible), wrapped in aluminum foil (with dull side touching specimen), placed in whirlpaks or ziplocks, then frozen.**
4. If carcass is fresh dead (code 1, 2), collect whole, heparinized blood. Use sterile syringe and needle to collect **uncoagulated** (not clotted) blood and place in GTT (green-top, heparinized vacutainer).
5. Avoid salt water, tobacco smoke, bug sprays, and other aerosolized foreign materials during collection.
6. **Tissues should be collected as rapidly as possible** after opening the body cavity to prevent contamination and deterioration.

Tissues for Microbiology: Code 1 ideal; Codes 2, 3 limited; Codes 4, 5 useless

Collect the following by special request only. Specimen collection for bacteriology and virology is determined primarily by the nature of gross pathologic lesions. Samples should be taken aseptically, from external surfaces, body cavities and internal organs as soon as they are exposed. Place swabs in respective transport media and refrigerate at 4 C or place on blue ice immediately and freeze upon arrival to laboratory or field camp. If cryovials are available, ultrafreeze the swabs with tissue samples in liquid nitrogen. Samples for microbiology are worth the time and effort only when tissues are in suitable condition. With an "aborted fetus", perinatal death, or newborn, collect specimens according to "Fetus" section of Necropsy Form for freezing and later microbial analysis.

Post Necropsy

9. Review the completed Necropsy Report Form, making sure that all boxes have been checked off on the form for all samples collected.
10. Necropsy Report Forms, photos, "List of Specimens Collected", and any other pertinent data should be returned to the NMFS PIFSC Honolulu Laboratory.
11. Refer to the section "Preventing Disease Transmission" #4-8 on page one for post-necropsy clean-up tips. Clean necropsy tools (you may also need to spray them with WD-40 or LPS) and restock necropsy kit so that it is ready for the next necropsy.
12. Change the formalin for all formalin fixed tissues as noted above.
13. Make sure that the tagging/handling card, scar card, and tag condition drawing form are complete. Necropsy Report Forms, scar cards, tagging/handling cards, survival factor forms, and photos should be returned to the NMFS Honolulu Laboratory.
14. Record specimens collected on the Specimen Collection Summary and assign specimen numbers as outlined in the **Specimen Collection Protocol**.
17. Clean necropsy tools. Before disinfecting, remove all organic matter from instruments by washing them thoroughly with warm (if possible) soapy water. If instruments are not cleaned properly before disinfecting, the remaining organic matter may shield organisms from destruction, and may inactivate the disinfectant. Be sure to wear proper protective gear (gloves, masks, etc.) when washing instruments. To minimize aerosolization, keep instruments below the water line when washing. Disinfect instruments with 70% alcohol or a 1:10 chlorine bleach solution for at least **10 minutes**. However, bleach corrodes stainless steel, and may pit the instruments. Regardless of disinfectant used, be sure to thoroughly rinse instruments with fresh water after disinfecting. Air dry all instruments thoroughly before putting them away. You may also need to spray them with WD-40 (or LPS)
18. Restock necropsy kit so that it is ready for the next necropsy.

HAWAIIAN MONK SEAL NECROPSY REPORT FORM (MHI)

SEAL ID/temp ID _____ NEC. DATE/ TIME _____ NEC. # _____ (PIFSC assigns)
Date/time found dead _____ Date/time last seen alive _____
Island _____ Location _____ Lat _____ Long _____
Size/sex _____ Age (if fetus, see pg. 19) _____
DVM/ PROSECTOR(S): _____
RECORDER: _____ **PHOTOGRAPHER:** _____
Photos? Y / N File names: _____
X-rays? Y / N Findings: _____

HISTORY

Identifying body markings (take photos): _____

Last live observation(s): _____

Circumstances of death (found dead/euthanized/other-explain): _____

Tags

Record number and condition of any flipper tag(s), photograph, and collect all tags in labeled whirlpak.

R _____ L _____ Notes: _____

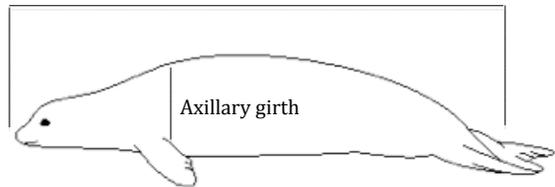
Scan the entire body for pit tags. PIT tag#(s):

R _____ L _____ Location found _____

Morphometrics

Axillary girth _____ (cm)
Straight length _____ (cm)
Total body mass _____ (circle: kg/lb)

Straight Length (tip of nose to tip of tail)



Carcass Condition Code: 1 2 3 4 5

Code 1*: Just died (eg., euthanasia)

Code 2*: Fresh, good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin/mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas).

Code 3: Fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)

Code 4: Poor/advanced decomposition (carcass collapsed, skin sloughing, strong odor, blubber soft w/ pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)

Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

***For Code 1 and 2 carcasses:** Follow instructions below to collect blubber, liver and kidney for NIST. It is imperative that you adhere to the NIST Sampling Protocol and AVOID TOUCHING THESE TISSUES with regular gloves until NIST samples are collected.

If not sampled for NIST, explain: _____

GROSS NECROPSY EXAMINATION

INSTRUCTIONS

1. Describe all organs, tissues and lesions in the fields provided. For descriptive terms, refer to Necropsy Protocol page 2. If appropriate, circle either NSF, NE or NA at the top of each section. If the tissue examined is abnormal, describe the location, color, size and distribution within the tissue in the blanks provided.
2. Formalin fixed tissues should be no bigger than 2x2x1cm.
3. Collect 2 equal Tissue Sets (set "A" and "B") from all organs, to be fixed in formalin.

Tissue Set A = Sp # _____ A Tissue Set B = Sp # _____ B

- 4. All visible endoparasites should be collected and chilled on blue ice.
- 5. If possible, remove head and place in cooler on ice while remainder of necropsy is completed.

ABBREVIATIONS		
NSF: no significant finding	BI: blue ice	AA=95% ethyl alcohol
NE: not examined	FM:10% formalin	AL=70-75% alcohol

EXTERNAL PHYSICAL EXAMINATION (Circle all that apply):

GAS/ PUTREFACTION: (Run your hands firmly along the body and feel for bubbles.) NSF / NE / NA

NUTRITIONAL STATE:
Fat/overweight, Normal/average, Thin/poor, Starving/emaciated
Notes: _____

DISCHARGE: NSF / NE / NA
Location: eyes, nose, mouth, genitals, anus Color/Texture: green, white, clear, red, thick, runny
Notes: _____

SWAB COLLECTION: NSF / NE / NA
Use sterile Dacron swabs. Obtain 2 swabs each from: eyes (ES), nasal cavity (NS), oral cavity (OR), genital opening (GS), rectum (RS). Both swabs from the same orifice can be placed into the same cryovial, unless the eyes are abnormal. If one or both eyes are abnormal, store the swabs separately and indicate below which vial contains the abnormal eye.

Sp # _____ Eye swab (BI) Sp # _____ Genital swab (BI) Sp # _____ Oral swab (BI)
Sp # _____ Nasal swab (BI) Sp # _____ Rectal swab (BI)

MUCOUS MEMBRANES: NSF / NE / NA
Pink, pale pink, red, yellow, white, purple, brown, other: _____

ORAL CAVITY: NSF / NE / NA
Ulcers, vomitus, blood, foreign body, other: _____

TEETH: NSF / NE / NA
Unerupted, just erupting, fully erupted, missing, broken, worn (describe): _____
LEFT (top/bottom): # incisors __/__, # canines __/__, # post-canines __/____
RIGHT (top/bottom): # incisors __/__, # canines __/__, # post-canines __/____

VIBRISSAE: NSF / NE / NA
Absent, torn, other: _____
Collect two vibrissae (with roots) and freeze in a whirlpak Sp# _____ Vibrissae (BI)

EYES: NSF / NE / NA
NSF, NE, missing, bulging, deflated, out of socket, foreign body present, penetrating wound
Notes: _____

Aqueous humor: collect using sterile needle and 3cc syringe. If both eyes are normal, aqueous can be combined in one cryovial. If one or both eyes are abnormal, use a clean needle and syringe to collect each sample and place in separate cryovials. Indicate which eye is abnormal above.

Sp # _____ Aqueous humor (BI)

Eyes: Collect both eyes. Fix one eye in Tissue Set A and freeze the other eye in a whirlpak. If an eye is abnormal, it should be fixed rather than frozen. If both eyes are abnormal, fix one in each Tissue Set. To fix an eye, make a 2-3 cm cut in the globe along the interface of the sclera (white part) and the clear cornea at the front of the eye before placing in formalin.

Eye (FM) A **AND** Sp # _____ Eye (BI) **OR** Eye (FM) B

NAILS: _____ **NSF / NE / NA**

Absent, torn, bleeding, cracked, crushed, other: _____

PERIPHERAL LYMPH NODES: _____ **NSF / NE / NA**

Palpate around the point of the shoulder (prescapular LN), and the angle of the jaw (mandibular LN)

Palpable, obvious, unnoticeable, other: _____

HAIR COAT: _____ **NSF / NE / NA**

Fur missing, oil, molting, scruffy, parasites, foreign bodies, fishhooks, scavenging, abrasion, other:

SKIN: _____ **NSF / NE / NA**

Cracking, bleeding, dry, moist, smooth, rough, wounds, masses, vesicles, ulcers, scars, bruising, abscesses, masses, abnormal coloration, other: _____

Collect two 1x1cm flipper tips in a 2mL cryovial pre-filled with 1.8 ml DMSO. For the proper ratio of DMSO to tissue, please ensure that samples approximate the size of a pencil eraser.

Sp# _____ A TP (DMSO)

Collect 2 samples from any masses, ulcers, vesicles or other external abnormalities. Fix one sample in Tissue Set A and freeze the other in a whirlpak. Try to include the junction of normal and abnormal tissue in the sample. Describe lesions sampled: _____

Skin lesion(s) (FM) Sp# _____ Skin lesion(s) (BI)

EXTERNAL GENITALIA & ANUS: _____ **NSF / NE / NA**

Swollen, protruding/prolapsed, diarrhea, ulcerations, masses, plaques, other: _____

Collect and fix anything appearing abnormal. Abnormal genital tissue (FM) A (FM) B

MAMMARY GLANDS: _____ **NSF / NE / NA**

Lactating, swelling, discharge, parasites, other: _____

If lactating, collect as much milk as possible and freeze. Sp # _____ Milk (BI)

INTERNAL EXAMINATION:

BLOOD: _____

For code 1 and code 2 animals ONLY, collect whole blood from extradural vein at least one (up to four) SST tubes. If unsuccessful, return to this step when you get to the heart. In the heart, look for a pale, tan, gelatinous “chicken fat clot” and separate it into RTTs. Centrifuge, aliquot serum and freeze as soon as possible.

Sp # _____ Whole blood or serum (CODE 1 and 2 animals only)

For code 1 carcasses ONLY, collect 2.5mL whole blood from extradural vein into a PAX gene tube. Gently rock tube to mix blood and additive. After blood collection, store tube UPRIGHT at ROOM TEMPERATURE for a minimum of 2 hours (longer is ok, just no more than 72 hours). After the 2 hour incubation, transfer sample to cryovial(s) using disposable pipette and place in -20C freezer for 24 hours. After 24 hours in -20C freezer, samples can be transferred to -80C freezer if desired. If no freezer is immediately available, tubes may be stored in a refrigerator for up to 5 days. Avoid placing samples directly from room temperature storage into the -80 freezer, as this will likely ruin the sample. NOTE: Unused PAX gene tubes should be stored at room temperature and not exposed to excessive heat or cold before use.

Sp # _____ Whole blood PAX gene tube (CODE 1 animals only)

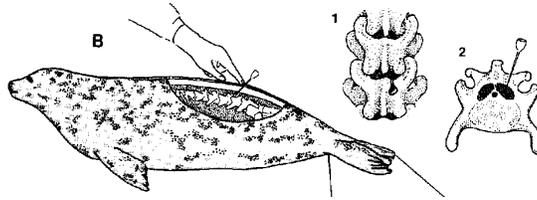
Extradural vein blood collection:

Palpate the vertebral column and pelvis and move your fingers cranially 2 or more vertebral spaces, feeling for a “divot” lateral to the spinous processes of the vertebrae. Attach a needle to a 3cc syringe.

Needle choice:

- Pups/weaners: use a 20g or 21g x 1 1/2” needle.
- Adults: use a 3.5” spinal needle. Before insertion, remove the stylet, holding needle from hub only.

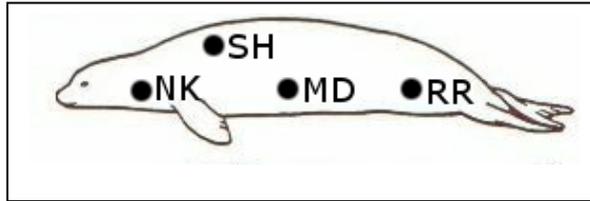
The angle of the needle may vary from a 45 - 90 degree angle to the dorsal surface of the animal. As the needle is inserted, feel it moving through skin, blubber, and muscle until you feel it pop through the membrane of the extradural sinus. Draw back on syringe to collect blood. Put a fresh needle (a small one is fine) on the syringe and push needle through top of PAX gene tube. The vacuum in the syringe should draw the blood into the tube.



BLUBBER

NSF / NE / NA

Thickness (measure at sternum or chest between the front flippers): _____ mm



NIST BLUBBER SAMPLING: For **code 1 and 2** animals only, using NIST provided materials (tyvek lab coat, **vinyl gloves**, **Teflon bags**, zip ties and data sheets) and stainless steel instruments, collect 300-350 g of blubber. Place in Teflon bag and seal with zip ties, label, and place on ice until it can be processed.

Sp# _____ NIST Blubber sample 300-350 g in Teflon bag (BI)

Code 1 and 2 animals only:

Using a twisting motion, insert a sterile 6 mm biopsy punch through the skin at the lateral aspect of the seal’s pelvic girdle, approximately 5-15 cm cranial to the wing of the ileum on either side of the body (RR, below). **Collect 8 blubber samples** through the **full depth** of the blubber layer, about 2-5 cm, until you reach the muscle layer. Use thumb forceps and/or scissors to retrieve the samples, without damaging or contaminating the blubber tissue. **One punch can be used to collect all 8 samples.** Take great care to prevent contamination of the samples by contact with gloves or other items. **The 4 best full thickness biopsies should go into the 5.0 ml cryovial, and the other 4 should go into the Teflon vial.**

Sp# _____ BB/FA from RR x4 (blubber biopsy for fatty acid in cryovial) (BI)

Sp# _____ BB/TX from RR x4 (blubber biopsy for toxicology in small Teflon vial) (BI)

All animals:

Collect two 1x1x1cm samples of skin and blubber from the RR location and fix one in each Tissue Set.

Skin/Blubber (FM) A (FM) B

Collect one **full thickness**, 2 x 2 cm blubber sample from each of the following locations: neck (NK), shoulder (SH) and midsection (MD) locations. Place each sample in separate whirlpak (do not wrap in foil), and label each bag with the body site from which it was collected.

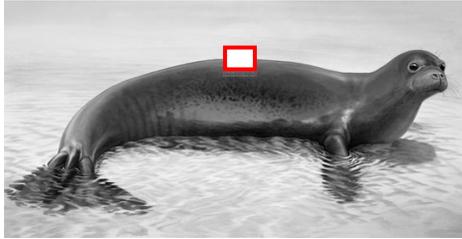
Sp# _____ BL/NK one 2 x 2 cm from NK (fatty acids) (BI)

Sp# _____ BL/MD one 2 x 2 cm from MD (fatty acids) (BI)

Sp# _____ BL/SH one 2 x 2 cm from SH (fatty acids) (BI)

MUSCLE **NSF / NE / NA**

Describe abnormalities: _____
 Collect the following samples from the middle of the the *longissimus dorsi*, the large long muscle that runs down either side of the spine:



One 2x2x2 inch muscle sample for myoglobin analysis. **Double bag** the sample in whirlpaks and freeze.

Sp# _____ A MU (BI)

One 1x1x0.5cm piece for stable isotope analysis and freeze.

Sp# _____ B MU (BI)

Two 1x1x1cm pieces in formalin and place one in each Tissue Set.

Muscle (FM) A (FM) B

CEREBRAL SPINAL FLUID (CSF) **NSF / NE / NA**

Cut through the skin, blubber, nuchal ligament, and neck muscles to the dorsal aspect of the atlantoccipital skull joint. Maintaining sterile technique, slowly insert needle w/ syringe approximately 5-7 mm into the foramen magnum. Collect up to 5ml and freeze.

Sp # _____ CSF (BI) sterile/ non-hemolyzed sample

Sp # _____ CSF (BI) contaminated/ hemolyzed sample

ORO/NASOPHARYNX **NSF / NE / NA**

NASOPHARYNX: Look for nasal mites. If observed, collect in alcohol. Sp # _____ nasal mites (AL)

Fluid (Y / N) volume: _____ ml. Describe fluid: _____

TONSILS: enlarged, red, purple, pus, other: _____

Ulcers on tongue, ulcers on gums, ulcers on hard palate, foreign body, vomitus (volume: _____ ml, appearance: _____)

THORACIC CAVITY

Open the chest and look for free fluid in the thoracic cavity (around the lungs, pooled at the diaphragm), collect 1-3ml if indicated (veterinary discretion) and describe below. Examine external surfaces of lungs and heart *in situ* and note abnormalities in appropriate section.

Fluid present (Y / N), volume: _____ ml, appearance: purulent, serous, fibrinous, yellow, white, green, blood-tinged, frank blood, adhesions, plaques, other: _____

Sp # _____ chest fluid (BI)

SALIVARY GLANDS: NSF / NE abnormalities: _____

THYROID: NSF / NE abnormalities: _____

LARYNX: NSF / NE abnormalities: _____

ESOPHAGUS: NSF / NE dilated, constricted, perforated, ulcerated, hemorrhagic, foreign body, fluid (volume: _____ ml, appearance: _____), other: _____

TRACHEA: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

BRONCHI: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

PARASITES: nematodes, mites, other: _____ location: _____ Severity: <10, 10-20, 20-50, >50

THYMUS: NSF / NE atrophy, prominent, enlarged, other: _____

Note: the thymus shrinks with age, and is not likely be found on adult animals.

Submandibular LN	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Thymus	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B
Tonsil	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Esophagus	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B
Tongue	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Trachea	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B
Thyroid	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Bronchus	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B

BRACHIAL PLEXUS **NSF / NE / NA**

If possible, collect the adjacent axillary LN with the brachial plexus and associated vessels and place in formalin.

Notes: _____

Brachial plexus (FM) A (FM) B

CRANIAL and THORACIC LYMPH NODES **NSF / NE / NA**

PULMONARY LYMPH NODES: Collect one sample in formalin and and freeze one for microbiology. Look around the base of the heart (they are located at the largest end of the blood vessels going to the lungs).

Pulmonary LN (FM) A (FM) B Sp # _____ PN (BI)

MEDIASTINAL LYMPH NODES: Collect one sample in formalin and freeze one for microbiology. Look around the heart and between the lungs. It is very difficult to determine the specific name of the node but all we are after are any abnormal lymph nodes and a few normal from the chest cavity

Mediastinal LN (FM) A (FM) B Sp # _____ mediastinal lymph node (BI)

OTHER LYMPH NODES: Examine as many additional cranial lymph nodes as possible: mandibular, axillary, prescapular, sternal. Describe any abnormalities below. Suggested descriptors: hemorrhagic, gelatinous, serous fluid, soft, hard, enlarged (mild/mod/severe).

Collect 1x1x1cm samples from abnormal nodes and preferably 2-5 normal nodes, label with a laundry tag and fix one sample from each node in each Tissue Set. Be sure to include abnormal and normal tissue in the fixed samples. For code 2+ carcasses, sample the freshest lymph nodes, label and place one sample from each node in each Tissue Set.

List lymph nodes collected in formalin: _____ (FM) A (FM)

HEART

PERICARDIUM: NSF / NE thickened, plaques on surface, contains fluid (volume: _____ ml, describe: _____)

Collect pericardial fluid if abnormal (veterinary discretion): Sp # _____ HF pericardial fluid (BI)

PULMONARY ARTERIES AND AORTA: NSF / NE thrombi, plaques, rupture, other: _____

VALVES*: NSF / NE diffusely thickened, nodular thickening, vegetative/proliferative lesion (valve(s): _____)

**If valves appear abnormal, place a sample in each Tissue Set.*

LEFT / RIGHT VENTRICLES: NSF / NE thickened, dilated (location _____ thickness: _____ mm)

MYOCARDIUM (heart muscle): NSF / NE pale, tumors, abscess, white foci (location: _____)

ATRIA AND AURICLES: NSF / NE thickened, dilated, pale, tumor, abscess, white foci

Parasites type: _____ severity: <10, 10-20, 20-50, >50

Notes: _____

Collect the following and fix one of each in each Tissue Set:

Section through L. ventricle - IVS - R. ventricle (FM) A (FM) B

Aorta (FM) A (FM) B

Pulmonary arteries (FM) A (FM) B

Section through R. atrium – AV valve (FM) A (FM) B

Section through L. atrium – AV valve (FM) A (FM) B

Next, collect 3 samples of heart tissue, wrap in foil, and freeze. REVISE FOR APPROPRIATE TOXICOLOGY NEEDS

Sp # _____ A Heart (BI)

B Heart (BI)

C Heart (BI)

LUNGS

NSF / NE / NA

Describe: pink, red, purple, mottled, congested, consolidated, abscesses, granulomas, emphysema, masses, interstitial edema

Specify location, distribution, severity: _____

Parasites: none detected 1 2+ 3+ 4+ Describe color, size: _____

Collect two 2x2x1cm pieces of lung and fix one in each Tissue Set. Collect additional samples if abnormalities are observed. Be sure to include both normal and abnormal tissue and describe abnormalities.

Lung (FM) A (FM) B

Collect 3 pieces of lung, 100 g each, wrap in foil, and chill on blue ice. REVISE FOR APPROPRIATE TOXICOLOGY NEEDS

Sp # _____ A Lung (BI)

B Lung (BI)

C Lung (BI)

ABDOMINAL CAVITY **NSF / NE / NA**

Open the abdomen and look for free fluid, collect 1-3ml if indicated (veterinary discretion) and describe below. Examine external surfaces of abdominal organs *in situ* and note abnormalities (adhesions, intussusceptions, etc.) in appropriate section.

Fluid present (Y / N), volume: _____ ml, appearance: purulent, serous, fibrinous, yellow, white, green, blood-tinged, frank blood, adhesions, plaques, other: _____

Sp # _____ abdominal fluid (BI)

DIAPHRAGM **NSF / NE / NA**

Collect two 2x2 cm samples of the diaphragm and fix one in each Tissue Set.

Diaphragm (FM) A (FM) B

LIVER **NSF / NE / NA**

NIST SAMPLING: For **codes 1 and 2** animals only, using NIST provided materials (tyvek lab coat, vinyl gloves, Teflon bags, zip ties and data sheets) and using stainless steel instruments, collect **300-350 g** of liver. Place in Teflon bag and seal with zip ties, label, and place on ice until it can be processed.

Sp# _____ NIST liver sample 300-350 g in Teflon bag (BI)

Describe: enlarged, small, tan, brown, black, yellow, orange, mottled, abscesses, granuomas, masses, cysts, hemorrhage, parasites, other: _____

Collect at least two 2x2x1 cm of liver and fix one in each Tissue Set. Be sure to also include both normal and abnormal liver in each Tissue Set.

Liver (FM) A (FM) B

Collect and freeze: three 100g liver samples, two 1x1x1cm samples and one 2x2x1cm sample and freeze.

Sp # _____	A liver 100 g (tox)	<input type="checkbox"/> (BI)	D liver 1x1x1 (micro)	<input type="checkbox"/> (BI)
	B liver 100 g (tox)	<input type="checkbox"/> (BI)	E liver 1x1x1 (micro)	<input type="checkbox"/> (BI)
	C liver 100 g (biotox)	<input type="checkbox"/> (BI)	F liver 2x2x1 (stable isotopes)	<input type="checkbox"/> (BI)

Collect the hepatic lymph nodes for microbiology, chill on blue ice. Look around the base of the large blood vessel coming from the aorta nearest the liver.

Sp # _____ hepatic lymph nodes (BI)

GALL BLADDER **NSF / NE / NA**

Describe: full, empty, thickened wall, flukes (severity: <10, 10-20, 20-50, >50), other: _____

Bile: thick/chunky, thin/runny, black, dark green, light green, yellow, orange, stones present

If present, collect 1-3ml of bile (code 1-2 carcasses only) and place in a cryovial and whirlpak.

Sp # _____ Bile (BI)

Collect two 2x2x1 cm sections of gall bladder and fix one in each Tissue Set.

Gall bladder (FM) A (FM) B

PANCREAS **NSF / NE / NA**

Loss of lobulation, swollen, hemorrhage, abscesses, other: _____

Collect two 2x2x1 cm pices of pancreas and fix one in each Tissue Set.

Pancreas (FM) A (FM) B

SPLEEN **NSF / NE / NA**

Masses, enlarged (mild / moderate / severe), constricted, congested, abscesses, scars, pale, purple, brown, red, other: _____

Collect tissues for 2 sets for histopathology and for 2 for microbiology (1x1x1 cm):

Spleen (FM) A (FM) B

Sp# _____ A spleen (BI)

B spleen (BI)

STOMACH **NSF / NE / NA**

Erosions, ulcers, perforated ulcers, loss of rugal folds, swollen rugal folds, other: _____

Mucosa: white, pale pink, red, purple, other: _____

Parasites (ascaris): <10 10-20 20-50 >50 Describe (size, color): _____

Collect representative sample of parasites and freeze.

Parasites Sp# _____ (BI)

Stomach contents: empty, dilated with gas, mucus, fish (digested / partially digested / undigested), foreign body, other: _____

Collect stomach contents into whirlpaks or cryovials and chill on blue ice.

Stomach contents Sp# _____ (BI)

Collect two 2x2x1 cm sections of stomach tissue and fix one in each Tissue Set. Be sure to also include any abnormal tissue.

Stomach (FM) A (FM) B

INTESTINES **NSF / NE / NA**

Open up ~4-6" of each section of the gastrointestinal tract, and look for abnormalities in color or thickness. Be sure to include any abnormal tissue as well as normal tissue in the formalin fixed samples. For intestinal samples, take a complete transverse "ring" of tissue ~1-2 cm in width, trying not to touch or disrupt the inside of the ring.

Duodenum (FM) A (FM) B

Jejunum (FM) A (FM) B Cecum (FM) A (FM) B Colon (FM) A

(FM) B

Also collect samples (2 cubic inches) of small intestine for microbiology, chill on blue ice:

Small intestine Sp# _____ A (BI) B (BI) C (BI)

MESENTERIC LYMPH NODES:

Collect anywhere along the mesentery, preferably both at the cranial and caudal portions of the abdomen/intestinal tract. Collect 2 sets for tissue set A and B, and freeze several whole for microbiology. Chill on blue ice.

Mesenteric Lymph Node (FM) A (FM) B Sp # _____ ML (BI)

FECAL SAMPLES: Cut colon near anus and squeeze contents from distal intestines directly into container.

FE (feces-Intestinal Contents) Sp# _____

E. Collect sub-sample in pre-filled DMSO vial, fill to the 5 ml line on the vial Feces A (DMSO)

F. Collect 1-3 g in a whirlpak for ciguatera analysis (FE/CX) Feces B (whirlpak)

G. Freeze an additional 10-30 g in whirlpaks or cryovials for hormonal studies (FE/HR) Feces C (whirlpak/cryo)

H. Freeze 10 g of feces (or meconium if newborn pup) into whirlpaks or cryovials Feces D (whirlpak/cryo)

ADRENAL GLAND

Right: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____

Left: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____

Region (cortex vs medulla) and distribution of lesions: _____

Adrenal gland (FM) A (FM) B Sp # _____ Adrenal (BI)

URINARY TRACT **NSF / NE / NA**

NIST SAMPLING: For **codes 1 and 2** animals only, using NIST provided materials (tyvek lab coat, vinyl gloves, Teflon bags, zip ties and data sheets) and using stainless steel instruments, collect 300-350 g of kidney. Place in Teflon bag and seal with zip ties, label, and place on ice until it can be processed.

Sp# _____ NIST Kidney sample 300-350 g in Teflon bag (BI)

Right: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis, mass, calculi, emboli, infarct, loss of renule differentiation, other: _____

Size: normal, small, enlarged (mild / moderate / severe), other: _____

Cortex (outside layer): pink, tan, red, purple, other: _____

Medulla (inside layer): pink, tan, red, purple, other: _____

Left: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis, mass, calculi, emboli, infarct, loss of renule

differentiation, other: _____
 Size: normal, small, enlarged (mild / moderate / severe), other: _____
 Cortex (outside layer): pink, tan, red, purple, other: _____
 Medulla (inside layer): pink, tan, red, purple, other: _____

Collect kidney in each Tissue Set, as well as two 100g samples for toxicology, and two 1x1x1cm samples for microbiology: Kidney

(FM) A (FM) B

Sp # _____ A Kidney 100g (tox) (BI) C Kidney (micro) (BI)

B Kidney 100g (tox) (BI) D Kidney (micro) (BI)

URINE: Attach a sterile 18 g x 1.5" needle to a sterile syringe, insert the needle into bladder, and draw on syringe to collect up to 12 ml.
 Amount: _____ ml; bloody, golden, yellow, pale yellow, clear, cloudy, purulent, other: _____

Sp # _____ Urine (BI)

URINARY BLADDER: empty, full, dilated, thickened, masses, hemorrhagic, ulcerated, necrotic, other: _____

Bladder (FM) A (FM) B

URETERS: NSF / NE dilated, tumors, abscesses, stones/calculi, hydroureter (distended due to obstruction), other: _____

MALE REPRODUCTIVE TRACT

NSF / NE / NA

PREPUCE: NSF / NE

PENIS: NSF / NE discolored, pustules, mass, torsion, laceration, plaque, other: _____

TESTES

Left: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Right: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Collect a 2x2x1cm section of each tissue and place one in each Tissue Set. Also collect and fix anything appearing abnormal.

Penis (FM) A (FM) B

Left Testis (FM) A (FM) B

Right Testis (FM) A (FM) B

FEMALE REPRODUCTIVE TRACT

NSF / NE / NA

VULVA: NSF / NE other: _____

VAGINA: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

UTERUS: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

PREGNANT: Y / N Field number of fetus: _____ (see instructions below)

CERVIX: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

OVARIES:

Left: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Right: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Female Reproductive Tract (FM) A (FM) B

For pregnant females, aborted fetuses, or perinatal pup deaths, examine and collect umbilicus, placenta and fetus:

UMBILICUS (describe): _____

Umbilicus (FM) A (FM) B

Sp # _____ Umbilicus (BI)

PLACENTA: Collect four 5cm x 1cm **full thickness** strips (extending through to include both the fetal and maternal side) representative of normal and any abnormal portions of the placenta. Fix one sample in each Tissue set and freeze the other two samples.

Placenta (FM) A (FM) B Sp # _____ A Placenta (BI)

B Placenta (BI)

FETUS:

A fetus or premature pup "P0" is defined as <75cm straight length; the pelage, whiskers, nails, or oral cavity not fully developed.

Complete a new Necropsy Report Form. Fetus necropsied: Y / N Necropsy # _____ (PIFSC assigns)

If complete necropsy is performed, use separate Necropsy Report Form.

Straight length: _____ cm

Axillary girth: _____ cm

Mass: _____ kg
Sex: M or F
Condition (Describe): _____

Take one swab from both the throat and rectum before beginning necropsy. If fetus is fresh, collect fluid from the stomach and freeze.

Sp # _____ Throat Swab (BI)

Sp # _____ Rectal Swab (BI)

Sp # _____ Stomach Fluid (BI)

Swabs are collected in an attempt to identify any bacterial/viral related to abortion. A swab of secondary bacterial overgrowth is not useful. If the carcass appears fresh (absence of autolysis, maggots, etc.) collect bacteriology swabs.

SPINAL CORD	NSF / NE / NA
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Using a Sawzall, cut through the ventral vertebral column through to the epaxial muscles, transecting the vertebrae, exposing intervertebral disks and spaces, spinal column, and spinal cord (medulla spinalis). Inspect all vertebral joints for abnormalities and collect 2 sets of spinal cord tissue (3-5 cm long) from the upper, middle, and lower spinal cord.

Spinal cord (FM) A (FM) B Sp # _____ A Spinal cord (BI)
B Spinal cord (BI)

BRAIN	NSF / NE / NA
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Collect the brain even if it has liquefied. Before cutting the skull, examine it carefully and photograph any fractures or blunt injuries. When opening the skull to collect the brain, collect the injured portions of the skull without damaging them. To prevent tissue from clogging the teeth on the saw, first clean away any tissue on the skull where the hacksaw blade will be cutting. Attempt to remove the brain intact and handle gently.

CEREBRUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

CEREBELLUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

Collect a 2x2x1cm sample from the cerebrum, cerebellum and brainstem in each Tissue Set. If any region appears abnormal, place a sample of the abnormal region in Tissue Set A as well.

Remaining portions should be wrapped in foil then frozen, same specimen # as #2, sub-number B

Brain (FM) A (FM) B Sp # _____ A Brain (BI)
B Brain (BI)

If the brain has decomposed to the point that it has liquefied, instead of collecting the above samples, collect brain in a whirlpak or cryovials and freeze.

Liquefied Brain Sp# _____ A (BI)

DURA MATER and SKULL	NSF / NE / NA
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Examine the skull for any fractures and thoroughly photograph any that are found. Examine the inside of the skull (the side against the brain). Collect any injured portions of the skull.

SKULL: discolored (describe: _____), pus, hemorrhage, congested, other: _____

The mandible (lower jaw) and all teeth within it should be collected and placed in a whirlpak for unknown age animals only. Sp

_____ Skeletal (**circle one:** injured skull/ mandible) (BI)