

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

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### CETACEAN *Brucella* spp ALERT:

*Brucella* spp. that differ from the recognized species within this genus have been isolated from a number of pinnipeds and cetaceans in the United Kingdom, the United States and more recently in Canada (Ross et al, 1996; Foster et al, 1996; Nielsen et al, 2001; Van Bressema et al, 2001a). Antibodies to *Brucella* spp have been identified in killer whales with no attendant pathology (Jepson et al, 1997; Raverty et al, 2004). Additionally, there are many more species of marine mammals that have been seropositive to *Brucella* spp, but from which *Brucella* has not been isolated. The name *Brucella maris* initially was proposed for all marine strains, but more recently, *B. pinnipediae* and *B. cetaceae* were been proposed for pinniped and cetacean strains, respectively (Cloeckert et al, 2001).

Infection by *Brucella* has been documented to cause placentitis and abortion in captive bottlenose dolphins and blubber abscesses and meningoencephalitis in wild striped dolphins (Ewalt et al, 1994; Gonzalez et al, 2002). The clinical and pathologic significance of infection by these organisms in killer whales and other cetaceans is unknown. In terrestrial vertebrates this pathogen has been associated with fetal and perinatal loss as well as reduced fertility (Gall et al, 2000). Gross observations are inadequate for the diagnosis of infection by *Brucella* spp. and to further resolve the possible contribution of these bacteria to morbidity in stranded killer whales attempted culture and isolation of *Brucella* spp. should be routinely undertaken with each stranded animal. Samples should include multiple sections of reproductive tract, brain, lung, gastrointestinal contents, and lymph nodes. To ensure optimum bacterial recovery, samples obtained at the time of necropsy should be frozen at -70 C (without dry ice) as soon as possible. This bacterium is fastidious and initial isolation can be difficult. As a result, serology of post mortem heart blood by competitive enzyme linked immunosorbant assay (ELISA) and florescent polarization assay (FPA) is recommended (Gall et al, 2000). Due to the sensitivity of these assays, samples may be collected for analysis from carcasses even with moderate to advanced autolysis (hemolysis). Follow up polymerase chain reaction (PCR) to amplify *Brucella* nucleic acid may be considered and if indicated by histopathology, immunohistochemistry with monoclonal or polyclonal antibodies specific to *Brucella* may prove a valuable adjunct to confirm infection.

**Zoonosis Warning:** Marine *Brucella* spp. has been reported to cause infection in a laboratory worker after occupational exposure (Brew et al, 1999) and neurobrucellosis with granuloma formation has been documented in two additional individuals with no known history of exposure (Sohn et al, 2003). The virulence of these strains to humans is currently unknown and appropriate public health and safety precautions at the time of necropsy are warranted.

### Contacts for *Brucella* analysis:

For c-ELISA testing of postmortem heart blood: Ole Nielson, Department of Fisheries and Oceans, 204-983-5126; E-mail: [NielsenO@dfo-mpo.gc.ca](mailto:NielsenO@dfo-mpo.gc.ca)

For bacterial culture of organs and lymph nodes: Darla Ewalt, National Veterinary Services Laboratory (515) 663-7347; E-mail: [darla.ewalt@aphis.usda.gov](mailto:darla.ewalt@aphis.usda.gov)

For PCR: Stephen Raverty, (604) 556-3003; E-mail: [Stephen.Raverty@gems3.gov.bc.ca](mailto:Stephen.Raverty@gems3.gov.bc.ca)

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### CETACEAN MORBILLIVIRUS ALERT:

Porpoise and dolphin morbilliviruses are antigenically and genetically similar and are now generally considered strains of the same viral species, cetacean morbillivirus (Kennedy, 1998). This virus has caused large-scale epizootics in several odontocetes species (Van Bresseem et al, 1991; Duignan et al, 1995; Van Bresseem et al, 2001b) and detection of antibodies in a subadult killer whale recently captured in the northwest Pacific Ocean that succumbed to bacterial pneumonia (A. Mironova, per comm.) suggests that killer whales have been exposed and are presumably susceptible to cetacean morbillivirus. Although no morbillivirus antibodies nor gene sequences have been detected in stranded cetaceans in the northeastern Pacific Ocean to date, this virus is likely endemic in multiple small cetaceans from around the world (Van Bresseem et al, 2001b). Because of the virulence of this virus and its potential to cause large-scale mortality in small killer whale populations, the presence of this virus should be ruled out during all killer whale necropsies. Because of the lack of detectable antibodies (immunologically naïve), it is anticipated that should this virus be inadvertently introduced, an explosive outbreak may occur. Continued surveillance for antibodies to cetacean morbilliviruses in antemortem serum or post mortem heart blood samples by indirect enzyme linked immunosorbant assay (iELISA) or virus neutralization is strongly recommended.

Cetacean morbillivirus is pantropic (infects a variety of cell types) and potential gross necropsy findings include skin ulcerations, stomatitis, pneumonia, and generalized signs of sepsis such as edema of internal organs and accumulation of serosanguinous fluid in the pleural and peritoneal cavities (Lipscomb et al, 1994). Gross lesions are not specific of morbillivirus infection but microscopic lesions are highly characteristic (Domingo et al. 2002). Microscopic lesions commonly seen with morbillivirus infection such as syncytia and acidophilic inclusions in cytoplasm and nuclei of epithelial cells can be widespread, focal, or obscured by severe necrosis caused by opportunistic bacterial and fungal infections. Microscopic examination and laboratory testing are essential to confirm morbillivirus infection. The tests used include immunohistochemistry, reverse transcriptase polymerase chain reaction (RT-PCR), and virus isolation on Vero cells or bovine fetal lung cells (Domingo et al. 1990; Van Bresseem et al. 1991; Barrett et al. 1993; Van Bresseem et al. 1999; Saliki et al, 2002). Potential sequela to cetacean morbillivirus infection includes opportunistic bacterial and fungal infections, as well as toxoplasmosis (Lipscomb et al, 1994; Schulman and Lipscomb, 1999).

#### Contacts:

For serology: Dr. Jeremiah Saliki, Oklahoma State University, 405-744-6623; E-mail: [jsaliki@okstate.edu](mailto:jsaliki@okstate.edu)

For PCR: Stephen Raverty, (604) 556-3003; E-mail: [Stephen.Raverty@gems3.gov.bc.ca](mailto:Stephen.Raverty@gems3.gov.bc.ca)

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### PREVIOUSLY REPORTED PATHOGENS:

There is a profound lack of infectious pathogens reported from free-ranging or captive killer whales (Gaydos et al., 2004). It is anticipated that with more comprehensive necropsies and ancillary diagnostics of stranded killer whales the number of recognized pathogens should increase.

Table 1: Infectious diseases or antibodies to pathogens reported in free-ranging killer whales

Pathogen	Reference	Ocean
<b><u>Bacteria</u></b>		
<i>Brucella</i> spp.	Jepson et al., 1997 Raverty et al, 2004	Northeastern Atlantic and Pacific
<i>Edwardsiella tarda</i>	Ford et al., 2000	Northeastern Pacific
<b><u>Viruses</u></b>		
Cetacean pox like virus (Orthopoxvirus)	Van Bressemer et al., 1999	Not Reported

Table 2: Infectious diseases or antibodies to pathogens reported in captive killer whales

Pathogen	Reference
<b><u>Bacteria</u></b>	
<i>Burkholderia pseudomallei</i>	Hicks et al., 2000
<i>Clostridium perfringens</i>	Walsh et al., 1994
<i>Erysipelothrix rhusiopathiae</i>	Bossart et al., 1988
<i>Nocardia asteroides</i>	Sweeney et al., 1976
<i>Nocardia otitidiscaviarum</i>	Dunn et al., 2001
<i>Salmonella</i> sp.	Ridgway, 1979
<i>Streptococcus</i> sp., beta-hemolytic	Greenwood and Taylor, 1985
<b><u>Viruses</u></b>	
Hepatitis-B like virus	Bossart et al., 1990
Influenza (suspected; no virus isolated)	Ridgway, 1979
Cutaneous papilloma-like virus	Bossart et al., 1996
<b><u>Fungi</u></b>	
<i>Aspergillus fumigatus</i>	Reidarson et al., 1999
<i>Candida albicans</i>	Greenwood and Taylor, 1985; Ridgway, 1979; Sweeney et al., 1976
<i>Saksenaea vasiformis</i>	Reidarson et al., 1999

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Table 3: Endoparasites identified in killer whales

Parasite	Reference
<b><u>Acanthocephala</u></b>	
<i>Bolbosoma niponicum</i>	Heptner et al. 1976
<i>Bolbosoma physeteris</i>	Heptner et al. 1976
<b><u>Cestoda</u></b>	
<i>Phyllobothrium sp.</i>	Dailey and Brownell, 1972
<i>Trigonocotyle spasskyi</i>	Dailey and Brownell, 1972
<b><u>Nematoda</u></b>	
<i>Anasakis simplex</i>	Dailey and Brownell, 1972
<i>Anasakis pacificus</i>	Heptner et al. 1976
<b><u>Trematoda</u></b>	
<i>Campula sp.</i>	Gibson et al. 1998
<i>Fasciola skrjabini</i>	Dailey and Brownell, 1972
<i>Leucasiella subtilla</i>	Heptner et al. 1976
<i>Oschmarinella albamarina</i>	Gibson and Bray, 1997
<b><u>Protozoa</u></b>	
<i>Kyaroikeus cetarius</i>	Sneizek et al, 1995; Schulman and Lipscomb, 1999

### INTERNET RESOURCES:

#### Clinical Medicine:

International Association for Aquatic Animal Medicine: <http://www.iaaam.org>

#### Natural History:

U.S. National Oceanic and Atmospheric Administration:

<http://www.nwr.noaa.gov/mmammals/whales/index.html>

The Marine Mammal Center, Sausalito, California: <http://www.tmmc.org/>

The Whale Museum, Friday Harbor, Washington: <http://WhaleMuseum.org/>

#### Pathology:

The Armed Forces Institute of Pathology: <http://www.afip.org/vetpath/index.html>

#### Regulatory:

U.S. National Oceanic and Atmospheric Administration: <http://www.noaa.gov/>

The Canadian Department of Fisheries and Oceans: <http://www.dfo-mpo.gc.ca/>

U.S. Fish and Wildlife Service: <http://www.fws.gov/>

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### INTRODUCTION:

This protocol is designed to improve knowledge about diseases of free-ranging and captive killer whales (*Orcinus orca*) by providing guidelines for people to complete more comprehensive necropsies and disease testing. It is our hope that once people around the world realize the need to learn more about diseases of killer whales, this protocol also will increase the number of complete postmortem necropsies performed on killer whales. The project was sponsored by the U.S. National Oceanic and Atmospheric Administration (NOAA Fisheries) in response to the relatively sparse amount of information known about diseases of free-ranging killer whales and how disease might impact the recovery of small declining killer whale populations such as the southern resident killer whales. This population has declined 20% between 1997 and 2001 (Gaydos et al., 2004). It is comprised of individuals from three pods (J, K, and L) and is found frequently within the inland waters of Washington (USA) and British Columbia (Canada).

A recent survey found that on average between seven to eight stranded killer whales are found around the world annually, making each killer whale stranding an important opportunity to learn more about the biology and diseases of these animals (Gaydos et al., unpub. data). We hope killer whale researchers and responders to killer whale strandings from around the world will recognize the need to learn more about diseases of free-ranging killer whales and will use this necropsy and disease sampling protocol to help them conduct complete post mortem examinations on all dead stranded animals.

The objectives of this standardized necropsy and disease testing protocol are to:

- Facilitate more comprehensive killer whale post mortem examination and prioritize tissue sampling when complete necropsies are not feasible
- Over time, establish baseline patterns as to the cause of death of stranded killer whales
- Use data derived from increased surveillance and more complete necropsies to compare disease processes between different geographic regions
- Ascertain the possible contribution of contaminants or human interactions as contributory factors to increased morbidity in stranded killer whales
- Provide insights into the natural history and biology of wild killer whales

**A more expanded necropsy and sampling protocol is presented in the following text and relates specifically to the Pacific Northeast. If resources are available, it is recommended that this protocol be followed with all stranded killer whale necropsies. If your facility has appropriate tissue fixatives (formalin), a freezer and access to a microbiology lab, most of the listed tests should be readily accomplished.**

**Alternatively, sample prioritization and a more basic approach to tissue collection is available in Appendix I. At a minimum, grossly noted lesions should be recorded and high priority tissue samples collected.**

**NOTE: if tissues are not collected at the time of necropsy, the opportunity to appropriately sample the animal is lost.**

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### EQUIPMENT CHECKLIST:

Note: This equipment checklist represents an ideal situation and post mortem exams can be completed with less equipment.

1. Standard necropsy instruments. Multiple scalpel handles, scalpel blades, scissors, forceps, knives, knife sharpener, if possible in secure pack
1. Flensing knives and hooks with appropriate sharpening tools, chain saw, axe, or reciprocating saw to cut through the cranium, chest or vertebrae. Hammers, chisels and handsaws.
1. Retractors of various sizes and shapes. Self-retaining retractors with one or two movable arms mounted on a slide bar are most useful.
1. Sterile instruments for culture collection.
1. Flashlights with extra batteries and light bulbs
1. 10% neutral buffered formalin.
1. 4% buffered glutaraldehyde
1. 20% DMSO/saturated saline solution for genetic analysis
1. Isopropol alcohol, for contaminant sampling
1. Containers (from vials to garbage cans) for sample collection, including ice chest, dry ice and if possible liquid nitrogen.
1. Culture swabs, sterile urine cups, glass slides.
1. Serum tubes for blood and urine collection and gas burner to sear organ surfaces and sterilize scalpel blades.
1. Aluminum foil and plastic bags for freezing tissues.
1. Labels and waterproof marking pens.
14. Tape measure (metric), at least 20 meters long
15. Hoist/crane, scales to record organ weights.
16. Gas generator and flood lights with extra bulbs and gasoline
17. Coveralls, aprons, boots, gloves, caps, masks, protective eye and head gear.
18. Accessible water supply with hose.
19. Camera and film, extra batteries, video camera with additional memory cards
20. First aid kit.
21. Multiple plastic tarps, 10 meters.
22. Rope, at least 20 meters.
23. Plastic tape and pylons to cordon off necropsy site.
24. Garbage bags, dish soap, disinfectant, paper towels for clean-up
25. Signs: WARNING—PUBLIC HEALTH HAZARD—DO NOT ENTER
26. Tissue disposition request list and necropsy protocol

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### **LOGISTICS AND NECROPSY RECOMMENDATIONS:**

From a logistical perspective, advanced development of contingency plans will greatly facilitate identification, communication, recovery and necropsy of stranded animals. Key individuals for a killer whale stranding response should be identified and contact information provided to responsible government agencies, regional stranding coordinator, local aquarium facilities, whale watching representatives and stranding networks

Safety of the public and individuals involved with the post mortem examination is a prime consideration. Should a killer whale strand in an inaccessible or remote site, or is identified floating in offshore areas, efforts to recover the animal and relocate by boat to a more accessible site are strongly recommended. If the animal can be re-floated, this move may be accomplished by a large rope or chain secured around the peduncle or immediately behind the pectoral flippers and towed by a suitable vessel. To limit drag, the two front flippers should be tied together and maintained out of the water. To facilitate the post mortem examination, the animal should be positioned in lateral recumbency and secured ashore at high tide with exposure of the carcass attained with ebb tide. As tidal changes may limit the duration of the examination, use of heavy equipment (hoists) and flat bed trucks to transport the animal to a more secure facility or a diagnostic laboratory may be considered. These animals may weigh up to 4000-6000 kg and an appropriate vehicle should be employed. If the carcass is moved by truck, the vehicle should be weighed at a commercial weigh scale before and after transport to obtain the body mass of the carcass. With any field necropsy, there is a risk of human exposure to potential zoonotic pathogens as well as interference with inappropriate public involvement. Use of face masks, protective eyewear and gloves is recommended. In areas with high public exposure, access should be restricted by pylons, tape or rope and use of law enforcement or fisheries officials may be warranted.

Should the animal require euthanasia, consultation with the regional stranding coordinator and an appropriately trained marine mammal veterinary specialist is required. Antemortem blood samples should be collected and appropriately stored for later clinical pathology (hematology and clinical chemistry), serology, archiving, immune function and ancillary diagnostic and research investigations (Appendix V). With a fresh dead animal, post mortem blood may be collected from the pectoral fin or heart. According to the coding scheme employed by Rowles et al, 2001, live stranded marine mammals are graded: code 1, fresh dead: code 2, moderate decomposition: code 3, advanced decomposition: code 4 and severe decomposition: code 5. Even in animals with advanced states of decomposition, efforts to harvest tissues for contaminants, genetics, parasitology, and molecular studies may be undertaken.

To facilitate the flow of the post mortem examination, team members should be identified and assigned to specific tasks before the necropsy is initiated. A lead pathologist or prosector should be designated and individuals appointed to complete data entry, process research samples (Appendix I), label and record diagnostic material (Appendix II), document lesions and observations with photographs, liaise with the media or undertake additional tasks as necessary. Appropriate measurements (Appendix III) should be recorded by designated team members and photographs of the dorsal fin and saddle patch, eye patches, and any other potential identifying features obtained before the necropsy is initiated. A video and digital still camera should be used to record details of the post mortem examination.

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The post mortem approach will be determined to some extent on the animal's position, accessibility, lesions and other factors. Although cosmetic necropsy may be requested, this procedure should not compromise or impede appropriate tissue collection.

With the animal in lateral recumbency, a curvilinear full blubber thickness incision may be made from the caudal limit of the anus, along the dorsolateral aspect of the abdominal and thoracic cavities, terminating at the level of the rostral limit of the mandibular ramus. The lateral skin and blubber can then be reflected with metal retractors from the underlying musculature *en masse*, or divided into suitable 0.5-1.0 m portions and removed. Excised tissues should be placed on a plastic tarp to facilitate clean up and limit environmental contamination.

The abdominal musculature may be incised along the costochondral arch and dorsal limit of the abdominal cavity then reflected laterally to expose the abdominal viscera. The diaphragm should be assessed and if intact, incised and deflated to exclude pneumothorax. If a cosmetic post is requested, the ribs may be detached at the costosternal junction and reflected, or alternatively a chain or reciprocating saw may be employed to remove the thoracic wall. It is important that protective eye wear or face shields be employed by the operator. The tongue may be exteriorized by incision of the blubber and skeletal musculature along the entire length of the medial aspect of the mandibles and then reflected ventrally. If feasible, the lung, heart, trachea and esophagus (pluck) should be removed to a tarp for a more thorough evaluation. With larger animals, dissection of thoracic viscera *in situ* may be warranted. The head may be detached by dissection of the atlanto-occipital articulation and the skin overlying the dorsolateral aspect of the nape and cranium removed. This exposure will facilitate removal of the dorsal aspect of the skull by either chain or reciprocating saw and exposure of the brain. It is important to evaluate the entire length of the vertebral column to assess possible vertebral fractures or subluxations associated with boat strikes and other trauma and representative portion of spinal cord should be recovered from the cervical, mid thoracic, thoracolumbar and lumbar regions.

Due to the importance of the reproductive organs in disease screening and assessment of fecundity (reproductive status), designated individuals should recover and completely excise the reproductive tract for evaluation.

The mesenteric stalk should then be identified and transected to facilitate removal and evaluation of the abdominal viscera. The viscera should be placed on a separate tarp to that of the thoracic contents to limit cross contamination. The entire length of bowel should be detached from the mesenteric attachment and opened for visual inspection. The stomach should then be incised along the greater curvature and the gastric contents recovered and appropriately packaged and labeled. The remaining internal viscera should be evaluated by routine or conventional diagnostic protocols and appropriate research and diagnostic samples harvested and labeled.

With suspect sonar related strandings, arrangements should be made for CT scan of the entire head or ears and close evaluation of the larynx should be undertaken for evidence of submucosal hemorrhage. Samples of peribullar adipose tissue should be collected for histopathology.

An equipment list is attached and diagnostic, as well as research tissues listed in Appendices I and V. Formulation of contact lists should be undertaken by responsible agencies within specific regions and individuals notified in the event of a stranding. A list of contacts and programs identified worldwide is

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available (Gulland et al, 2001), as are additional necropsy and sampling protocols (Geraci et al, 1999). With forensic cases, chain of custody forms should be appropriately completed and forwarded with tissue samples. When tissues samples are forwarded to a reference lab or contact individual outside the country of origin, appropriate authorization and permits from the lead agency, such as CITES and MMPA are required.

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**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**KILLER WHALE NECROPSY PROTOCOL  
SUBMISSION FORM**

**Contributor** \_\_\_\_\_  
**Submitter** \_\_\_\_\_  
**Institution** \_\_\_\_\_  
**Address** \_\_\_\_\_  
**Telephone number** \_\_\_\_\_  
**Facsimile number** \_\_\_\_\_  
**Electronic mail** \_\_\_\_\_

**Animal Information**

**Pod, ID name or number (if known)** \_\_\_\_\_  
**Birth date/Age** \_\_\_\_\_ **Gender** \_\_\_\_\_ **Estimated weight(Kg)** \_\_\_\_\_  
**Morphometric measurements (remit completed checklist on page 42)** \_\_\_\_\_  
**Date of initial observation** \_\_\_\_\_  
**Death/euthanasia date** \_\_\_\_\_  
**Death Location** \_\_\_\_\_  
**GPS coordinates** \_\_\_\_\_  
**Geographic location** \_\_\_\_\_  
**Body of water** \_\_\_\_\_  
**Necropsy date** \_\_\_\_\_  
**Necropsy location** \_\_\_\_\_  
**Post mortem Interval** \_\_\_\_\_

History (initial contact person, circumstances of death, clinical signs)

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**Note: please fax, mail, or e-mail this completed form (pages 16–22) and the histologic findings submission (Appendix X) to: Stephanie Wong, D.V.M., M.P.H., U.S. Navy Marine Mammal Program, Biosciences Division, Code 2351, Space and Naval Warfare Systems Center - San Diego, 53560 Hull Street, San Diego, CA 92152, Tel: 619.767.4335, Fax: 619.553.5068, stephanie.wong@navy.mil.**

**Information submitted will be used to help better understand diseases of killer whales. No data will be published or cited without the consent of the person providing the data.**



**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**3) Body Cavities** (abdominal and thoracic)

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**4) Urinary System** (kidneys, ureters, bladder, urethra)

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**5) Reproductive System** (testes, epididymis, spermatic cord, prostate gland, penis, ovaries, oviduct, uterus, cervix, vagina, urogenital slit, placenta, fetus)

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**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**6) Respiratory System** (blowhole, sinuses, pharynx, larynx, trachea, bronchi, lungs, regional lymph nodes)

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**7) Hemolymphatic System** (spleen, bone marrow, lymph nodes)

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**8) Endocrine System** (thyroids, parathyroids, adrenals, pituitary)

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**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**9) Central Nervous System** (brain, meninges, spinal cord)

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**10) Sensory Organs** (eyes, retrobulbar areas; ears, retrobullar regions)

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**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**11) Cardiovascular System** (heart, pericardial sac, great vessels, myocardium, valves, chambers)

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**12) Digestive System** (mouth, teeth, tongue, esophagus, stomach, small intestine, cecum, large intestine, rectum, liver, pancreas, mesenteric lymph nodes)

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**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**Additional Comments or Observations:**

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Primary Pathologist/Prosector: \_\_\_\_\_ Date: \_\_\_\_\_

Lab \_\_\_\_\_

Address \_\_\_\_\_

Phone \_\_\_\_\_

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**Summarize Preliminary Diagnoses (and please complete and submit Appendix X pathology summary:**

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## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

### COMPREHENSIVE KILLER WHALE TISSUE SAMPLE CHECKLIST

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>	<b>*</b>
Skin, multiple sites	Histopathology	1x2 cm	Formalin	
Skin	Genetics	1x1 cm	DMSO or freeze	
Blubber	Toxicology Contaminant	3x3 cm	Aluminum foil and freeze	
Blubber	Lipid analysis	10x10 cm	Aluminum foil and freeze	
Oral mucosa	Histopathology	1x2 cm	Formalin	
Oropharynx/tonsil	Histopathology	1x2 cm	Formalin	
Oropharynx/tonsil	Bacteriology	Swab	Culture swab	
Oropharynx/tonsil	Molecular studies	Dry swab	Place in plastic bag and chill	
Blowhole	Histopathology	1x2 cm	Formalin	
Blowhole	Bacteriology	Swab	Transport media	
Blowhole	Mycology	Swab	Transport media	
Blowhole	Molecular studies	Dry swab	Place in plastic bag and chill	
Blowhole	<i>Mycoplasma</i> culture	Swab	Culture swab	
Blowhole	Parasitology	Swab	Preserve in Bouin's	
Blowhole	Cytology	Scraping	Air dry and stain	
Tonsil	Histopathology	1x2 cm	Formalin	
Tonsil	Virus Isolation	5 gm	Plastic bag and chill or freeze	
Tonsil	Molecular studies	1x2 cm	Plastic bag and freeze	
Dorsal fin	Anatomy	Excise intact	Seal in plastic and freeze	
Conjunctiva	Histopathology	1x2 cm	Formalin	
Conjunctiva	Bacteriology/ Mycology	Swab	Culture swab	
Conjunctiva	Molecular studies	Swab	Place in whirlpak bag and chill	
Conjunctiva	Electron microscopy	Dry swab	Place in whirlpak bag and chill	
Umbilicus	Histopathology	1x2 cm	Formalin	
Umbilicus	Bacteriology	Swab	Culture swab	
Mammary gland	Histopathology	1x2 cm	Formalin	
Mammary gland	Bacteriology	Swab	Culture swab	
Mammary gland	Cytology	Aspirate	Plastic bag and chill	
Milk	Archive	Aspirate	Plastic bag and freeze	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>	<b>*</b>
Mandible	Morphometric study	Intact	Plastic bag and freeze	
Mandible	Sonar related injury	Internal mandibular fat	Histopathology	
Teeth	Aging	1-2 intact	Plastic bag	
Tongue	Histopathology	1x2 cm	Formalin	
Tongue	Parasitology	3x3 cm	Plastic bag and freeze	
Head	Anatomy	Intact	Plastic bag and freeze	
Eye	Histopathology	Intact, inject with preservative	Formalin	
Eye	Clinical chemistry	Aspirate 3-5 ml of vitreous	Red top tube and chill	
Genital slit	Histopathology	1x2 cm	Formalin	
Genital slit	Bacteriology	Swab	Culture swab	
Genital slit	Mycology	Swab	Culture swab	
Genital slit	Molecular studies	Dry swab	Place in plastic bag and chill	
Vagina	Histopathology	1x2 cm	Formalin	
Vagina	Bacteriology	Swab	Culture swab	
Vagina	Mycology	Swab	Culture swab	
Vagina	Molecular studies	Dry swab	Place in plastic bag and chill	
Uterus	Histopathology	1x2 cm	Formalin	
Uterus	Bacteriology	Swab	Culture swab	
Uterus	Mycology	Swab	Culture swab	
Uterus	Molecular studies	Dry swab	Place in plastic bag and chill	
Uterus	Archive	Tissue samples	Plastic bag and freeze	
Ovary	Histopathology	1x2 cm	Formalin	
Ovary	Bacteriology	Swab	Culture swab	
Ovary	Molecular studies	Dry swab or 1-2 cm tissue sample	Place in plastic bag and chill	
Ovary	Archive	If possible, retain corpora intact	Histopathology	
Oviduct	Histopathology	1x2 cm	Formalin	
Oviduct	Archive	Tissue samples	Plastic bag and freeze	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>	<b>*</b>
Penis/testes	Histopathology	1x2 cm	Formalin	
Penis/testes	Bacteriology	Swab	Culture swab	
Penis/testes	Mycology	Swab	Culture swab	
Penis/testes	Molecular studies	Dry swab	Place in plastic bag and chill	
Accessory sex glands	Histopathology	1x2 cm	Formalin	
Lung	Histopathology	1x2 cm	Formalin	
Lung	Bacteriology	Swab	Culture swab	
Lung	Mycology	Swab	Culture swab	
Lung	Virus Isolation	5 gm	Plastic bag and chill or freeze	
Lung	<i>Mycoplasma</i> culture	Swab	Culture swab	
Lung	Molecular studies	1x1 cm tissue	Place in plastic bag and chill	
Trachea	Histopathology	1x2 cm	Formalin	
Trachea	Bacteriology	Swab	Culture swab	
Trachea	Archive	Tissue samples	Plastic bag and freeze	
Lymph node, multiple sites	Histopathology	1x2 cm	Formalin	
Lymph node, multiple sites	Bacteriology	Swab	Culture swab	
Lymph node, multiple sites	Mycology	Swab	Culture swab	
Lymph node, multiple sites	Virus Isolation	5 gm	Plastic bag and chill or freeze	
Lymph node, multiple sites	Molecular studies	Dry swab	Place in plastic bag and chill	
Lymph node, multiple sites	Archive	Whole or partial nodes	Plastic bag and freeze	
Thymus	Histopathology	1x2 cm	Formalin	
Thymus	Bacteriology	1-2 cm tissue sample	Plastic bag and freeze	
Thymus	Molecular studies	1-2 cm tissue sample	Place in plastic bag and chill	
Thymus	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>	<b>*</b>
Spleen	Histopathology	1x2 cm	Formalin	
Spleen	Bacteriology	1-2 cm tissue sample	Plastic bag and freeze	
Spleen	Virus Isolation	5 gm	Plastic bag and chill or freeze	
Spleen	Molecular studies	1-2 cm tissue sample	Place in plastic bag and chill	
Spleen	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Thyroid gland	Histopathology	1x2 cm	Formalin	
Thyroid gland	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Thyroid gland	Weight	Intact gland	Fresh	
Parathyroid gland	Histopathology	1x2 cm	Formalin	
Parathyroid gland	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Brain - cerebrum	Histopathology	1x2 cm	Formalin	
Brain - cerebrum	Virus Isolation	5 gm	Plastic bag and chill or freeze	
Brain - cerebrum	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Brain - cerebellum	Histopathology	1x2 cm	Formalin	
Brain - cerebellum	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Brain – brainstem, pons, medulla, colliculus	Histopathology	1x2 cm	Formalin	
Brain - brainstem	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Ears	Histopathology	Peribullar fat	Formalin	
Ears	CT scan	Intact	Plastic bag and freeze	
Pituitary gland	Weight	Intact	Fresh	
Pituitary gland	Histopathology	Half	Formalin	
Pituitary gland	Archive	Half	Plastic bag and freeze	
Spinal cord (thoracic)	Histopathology	1x2 cm	Formalin	
Spinal cord (thoracic)	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Brachial plexus	Histopathology	1-2 cm	Formalin	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>	<b>*</b>
Heart, interventricular septa, ventricles, atria, papillary muscle and valve	Histopathology	1x2 cm	Formalin	
Heart	Bacteriology	5 ml post mortem heart blood	Red topped tube or plastic bag and chill	
Heart	Archive	Tissue samples	Plastic bag and freeze	
Aorta and vena cava, multiple levels	Histopathology	Aorta and vena cava, multiple levels	Histopathology	
Pericardium	Tissue culture	Pericardium	Tissue culture	
Pericardial fluid	Cytology and serology	10 ml	Red top tube or plastic bag and freeze	
Liver	Histopathology	1x2 cm	Formalin	
Liver	Toxicology Contaminant	3x3 cm	Aluminum foil and freeze	
Liver	Trace mineral and vitamin analysis	5x5 cm	Plastic bag and freeze	
Liver	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Bile	Archive	5-10 ml	Plastic bag and freeze	
Pancreas	Histopathology	1x2 cm	Formalin	
Pancreas	Archive	5-10 gm	Plastic bag - freeze	
Stomach	Histopathology	1x2 cm	Formalin	
Stomach	Bacteriology	Stomach	Culture swab	
Stomach	Biotoxin assay, prey selection	Entire or portion of ingesta	Place in plastic bag and freeze	
Stomach	Parasitology	Ingesta	Plastic bag and chill	
Small intestine, ileum and jejunum	Histopathology	1x2 cm	Formalin	
Small intestine, ileum and jejunum	Bacteriology	Swab	Culture swab	
Small intestine	Mycology	Swab	Culture swab	
Small intestine, ileum and jejunum	Contents	Ligated bowel	Place in plastic bag and chill	
Small intestine, multiple levels	Archive	Ligated bowel	Plastic bag and freeze	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>	<b>*</b>
Colon	Histopathology	1x2 cm	Formalin	
Colon	Bacteriology	Swab	Culture swab	
Colon	Salmonella culture	Swab	Culture swab	
Colon	Mycology	Swab	Culture swab	
Colon	Archive	Ligated bowel	Plastic bag and freeze	
Kidney	Histopathology	1x2 cm	Formalin	
Kidney	Virus Isolation	5 gm	Plastic bag and chill or freeze	
Kidney	Toxicology Contaminant	3x3 cm	Aluminum foil and freeze	
Kidney	Trace mineral analysis	5x5 cm	Plastic bag and freeze	
Kidney	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Kidney	Weights	Intact	Fresh	
Adrenals	Histopathology	1x2 cm	Formalin	
Adrenals	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Ureter	Histopathology	1x2 cm	Formalin	
Ureter	Archive	Multiple, 1-2 cm portions	Plastic bag and freeze	
Urinary bladder	Histopathology	1x2 cm	Formalin	
Urinary bladder	Archive	5-10 ml urine and bladder wall	Plastic bag and freeze	
Urinary bladder	Urinalysis	5-10 ml	Red top tube	
Urinary bladder	Biotoxin assay, cytology/culture	5-10 ml	Sterile plastic bag and freeze	
Skeletal muscle	Histopathology	1x2 cm	Formalin	
Skeletal muscle	Archive	5x5 cm	Plastic bag and freeze	
Joint fluid	Cytology	5 ml	Red top tube	
Joint fluid	Bacteriology	Swab	Culture swab	
Rib/Bone marrow	Histopathology	1x2 cm	Formalin	
Rib/Bone marrow	Cytology	Smear	Air fix/alcohol	
Rib/Bone marrow	Archive	1-2 ml	Plastic bag and freeze	
Peripheral nerve	Histopathology	1-2 cm	Formalin	
Diaphragm	Histopathology	1-2 cm	Formalin	
Diaphragm	Archive	2x2 cm	Plastic bag and freeze	
Water sample	Molecular studies	10 ml	Plastic bag and freeze	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX I  
SAMPLE PRIORITY AND FIELD TISSUE CHECK**

Circumstances associated with killer whale stranding and resources available at the time of post mortem examination will vary considerably and some flexibility and discretion must be afforded to the necropsy team. In those situations where autolysis, location, equipment, personnel or other factors may restrict access or limit the ability to expedite a thorough necropsy, the tissue sampling and ranking below should facilitate prioritization of sample collection for diagnostic evaluation. Within reason, every effort should be made to collect the high priority samples with each stranding. The ultimate disposition of tissues will be the responsibility of the lead government agency or regional marine mammal coordinator within the respective area.

**1) HIGH PRIORITY SAMPLES: For all killer whales that strand, attempt tissue collection of the samples listed below regardless of post mortem condition of carcass.**

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>
As many representative tissues as possible	Histopathology	1x2 cm	Formalin
Blubber/Liver/Kidney	Toxicology Contaminants	3x3 cm	Aluminum foil and freeze
Skin	Genetics	1x1 cm	DMSO or freeze
Oropharynx/tonsil/blowhole	Molecular studies and culture	Dry swabs and with transport media	Place in plastic bag and chill
Mammary gland	Bacteriology/cytology	3x3 cm tissue	Place in plastic bag and chill
Eye	Clinical chemistry	Aspirate 3-5 ml of vitreous	Red top tube and chill
Genital slit/Urogenital canal	Molecular studies and culture	Dry swabs and with transport media	Place in plastic bag and chill
Ovary	Reproductive and molecular studies	Dry swab and intact ovary	Place in plastic bag and chill
Morphometrics and photographs	Identification	Digital or slide film	Disc
Lung/regional lymph nodes/spleen	Molecular studies and bacteriology	2x2 cm tissue	Place in plastic bag and chill
Post mortem blood sample	Serology and bacterial culture	10-20 ml	Collect in red top tubes and chill
Stomach and small intestine	Biotoxin assay, stomach content analysis	Ligated	Place in plastic bag and freeze
Urinary bladder/bile	Biotoxin assay, cytology/culture/urinalysis	5-10 ml	Sterile plastic bag and freeze

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**INTERMEDIATE PRIORITY SAMPLES:** Collect tissues below if sufficient time and carcass is reasonably fresh (code 1-3: see page 10).

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>
Small intestine	Parasitology	Ingesta	Plastic bag and chill
Tonsil	Histopathology	1x2 cm	Formalin
Tonsil	Molecular studies	5 gm	Plastic bag and chill or freeze
Mandible	Sonar related injury	Internal mandibular fat	Histopathology
Head	Anatomy	Intact	Plastic bag and freeze
Uterus	Molecular studies	Dry swab	Place in plastic bag and chill
Brain, liver, kidney, spleen, lymph nodes and lung	Virus Isolation	5 gm	Individual plastic bags and chill or freeze
Ears	Histopathology	Peribullar fat	Formalin
Ears	CT scan	Intact	Plastic bag and freeze
Liver	Trace mineral and vitamin analysis	5x5 cm	Plastic bag and freeze

**LOW PRIORITY SAMPLES:** If there is sufficient time and resources available, the following samples and morphometrics should be collected.

<b>Tissue</b>	<b>Test</b>	<b>Sample</b>	<b>Preservation</b>
Dorsal fin	Anatomy	Excise intact	Seal in plastic and freeze
Teeth	Aging	1-2 intact	Plastic bag
Organs	Weight	Intact	Fresh
Mandible	Morphometric study	Intact	Plastic bag and freeze
Blubber measurement	Morphometric study	Collect and document	Record information

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

At the time of necropsy, the attached tissue sampling checklist (Appendix 1) and request list should be consulted and tissues from all major organs and lesions collected for histopathology and representative samples frozen for ancillary studies.

Preserve all lesions and as many of the tissues listed below as possible in 10% buffered formalin. Tissue samples should be between 3-5 cm in size and up to 0.5 to 1.0 cm in width and immersed in a ratio of 1 part tissue to 10 parts formalin. If electron microscopy (EM) fixative such as gluteraldehyde is available, preserve minced (1x1 mm) pieces of kidney, liver, spleen and lung.

Representative 3-5 cm blocks of tissue from lesions and major organs (e.g., lung, liver, kidney, spleen) should be placed in individually labeled small (preferably whirlpak) plastic bags and placed on dry or wet ice for initial storage and transportation. Also, collect post mortem serum (from heart blood), urine, eye fluid, bile, ingesta, and any abnormal fluid accumulations. Heart blood should be spun as soon as possible to limit the degree of hemolysis. Upon arrival to a diagnostic or reference laboratory, samples should be frozen at -70 degrees Celsius. If this is unavailable, temporary storage in conventional freezer without automatic defrost cycle is acceptable. A 1-2 cm block of skin, muscle or flipper for genetic analysis should be excised and placed in DMSO/saline solution or foil-wrapped and frozen.

For each lesion, up to 2-3 swabs may be obtained and samples should be chilled for transport to a diagnostic facility. In addition to routine TSA and blood agar cultures, special media for isolation of halophilic bacteria should also be inoculated.

The tissue checklist is designed to follow the sequential post mortem examination of the whale. As organs are excised and appropriate tissues collected and preserved, please mark off the right hand column with an "x".

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX I  
ABRIDGED TISSUE CHECK LIST FOR FIELD USE**

<b>Tissue</b>	<b>Histopathology</b>	<b>Microbiology</b>	<b>Molecular</b>	<b>Contaminants</b>	<b>Archive</b>	
Skin, multiple sites	1x2 cm					
Skin	1x2 cm				DMSO	
Blubber	1x2 cm			Aluminum foil		
Oral mucosa	1x2 cm	Culture swab	Dry swab			
Oropharynx	1x2 cm	Culture swab				
Tonsil	1x2 cm	Fresh tissue				
Blowhole	1x2 cm		Dry swab			
Dorsal fin					Intact	
Conjunctiva	1x2 cm	Culture swab	Dry swab		EM	
Umbilicus	1x2 cm					
Mammary gland	1x2 cm	Culture swab	Dry swab		100 gm	
Milk	1x2 cm	Culture swab	Dry swab	Aluminum foil	50 ml	
Mandible					Intact	
Teeth (1-2)					Intact	
Tongue	1x2 cm				50 gm	
Head					Intact	
Eye	1 globe				5-10 ml	
Genital slit	1x2 cm	Culture swab	Dry swab			
Urogenital canal	1x2 cm	Culture swab	Dry swab			
Uterus	1x2 cm	Culture swab	Dry swab		50 cm	
Ovary	1x2 cm				Retain intact	
Oviduct	1x2 cm				2-4 cm	
Penis	1x2 cm	Culture swab	Dry swab			
Accessory sex glands	1x2 cm					
Lung	1x2 cm	Culture swab fresh tissue	Dry swab		50 cm	
Trachea	1x2 cm	Culture swab	Dry swab		5 cm	
Bronchus	1x2 cm	Culture swab	Dry swab		5 cm	
Lymph node, multiple sites	1x2 cm	Culture swab Fresh tissue	Dry swab		50gm	
Thymus	1x2 cm				50 gm	
Spleen	1x2 cm	Culture swab Fresh tissue				
Thyroid gland	1x2 cm					
Parathyroid gland	1x2 cm					

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

<b>Tissue</b>	<b>Histopathology</b>	<b>Microbiology</b>	<b>Molecular</b>	<b>Contaminants</b>	<b>Archive</b>	
Brain cerebrum	1x2 cm	Culture swab Fresh tissue	Dry swab		50 gm	
Brain cerebellum	1x2 cm	Culture swab Fresh tissue	Dry swab		50 gm	
Brain brainstem	1x2 cm				50 gm	
Ears					Intact	
Ear, fat	1x2 cm					
Pituitary gland	1 cm				10 gm	
Spinal cord (thoracic, lumbar)	1x2 cm				50 gm	
Brachial plexus	1x2 cm					
Heart	1x2 cm				50 gm	
Heart blood		Culture swab			50 ml	
Aorta and vena cava	1x2 cm					
Pericardium	1x2 cm				50 gm	
Pericardial fluid	1x2 cm				10 ml	
Liver	1x2 cm			Aluminum foil	100 gm	
Bile	1x2 cm				20 ml	
Pancreas	1x2 cm				50 gm	
Stomach	1x2 cm				Ingesta	
Small intestine	1x2 cm	Culture swab			Ingesta	
Colon	1x2 cm	Culture swab			Ingesta	
Kidney	1x2 cm	Fresh tissue		Aluminum foil	100 gm	
Adrenals	1x1 cm				50 gm	
Ureter	1x2 cm					
Urinary bladder	1x2 cm					
Urine					50 ml	
Ureter	1x2 cm					
Skeletal muscle	1x2 cm				100 gm	
Joint fluid					10 ml	
Rib/Bone marrow	1x2 cm				100 gm	
Peripheral nerve	1x2 cm					
Diaphragm	1x2 cm				100 gm	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX IIa  
TISSUE DISPOSITION AND SPECIFIC RESEARCH REQUESTS**

**TISSUE DISPOSITION**

<b>Test</b>	<b>Sample</b>	<b>Investigator</b>	<b>Contact information</b>
Algal toxin	Ingesta (stomach)	Dr Wekell/Raverty	206-860-3388/604-556-3003
Algal toxin	Liver/Bile	Dr Wekell/Raverty	206-860-3388/604-556-3003
Anatomy	Mandible	Dr Barrett-Lennard	604-659-3428
Anatomy	Head	Dr Barrett-Lennard	604-659-3428
Anatomy	Dorsal fin	Dr Hanson	206-860-3220
Bacteriology	Multiple tissues	Dr Raverty	604-556-3003
Brucella culture	Representative organs and lymph nodes	Darla Ewalt	515-663-7347
CAT scan	Ears/Head	Dr Hanson	206-860-3220
Cell culture	Representative tissues	Dr Wise	207-228-8050
Clinical chemistry	Serum sample	Dr St Leger	619-225-4259
Fatty acid analysis	Blubber and skin	Dr Ylitalo	206-860-3325
Genetics	Skin biopsy	Dr Barrett-Lennard Dr Barb Taylor	604-659-3428 858-546-5620
Hematology	Blood sample	Dr St Leger	619-225-4259
Histopathology	Formalin fixed tissues	AFIP/Dr St Leger/ Dr Raverty	202-782-2600/619-225-4259 604-556-3003
Molecular studies/ PCR	Multiple tissues	Dr Raverty	604-556-3003
<i>Mycoplasma</i> culture	Respiratory tract and genital slit	Dr T Van Dreumel	519-824-4120, ext 4502
Parasitology	Ingesta	Dr Dailey	415-289-7346
Prey analysis	Stomach contents	Dr Hanson	206-860-3220
Prey analysis	Stomach contents	Dr Ford	250-756-7245
Reproductive	Formalin, intact	Dr B Hanson	206-860-3220
Serology	Heart blood	Dr Saliki/O Nielsen	405-744-6623/204-983-5126
Toxicology	Blubber, liver and kidney	Dr Ylitalo	206-860-3325
Toxicology	Blubber, liver and kidney	Dr Ross	250-363-6806
Trace mineral and vitamin A analysis	Liver and kidney	Dr Raverty	604-556-3003
Virology	Multiple tissues: EDTA blood	Dr Saliki/Dr Raverty	405-744-6623/604-556-3003

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX IIB**

**SPECIFIC RESEARCH REQUESTS**

Dr. Lance Barrett-Lennard  
Vancouver Aquarium Marine Science Centre  
845 Avison Way  
Vancouver,  
British Columbia  
V6G 3E2  
Phone: 604-659-3428  
Email: [Lance Barrett-Lennard \[barrett@zoology.ubc.ca\]](mailto:lance.barrett@zoology.ubc.ca)

**Intact skull or lower jaw (mandible) from stranded killer whales for morphometric studies.** Please contact Dr Barrett-Lennard before conducting the post mortem.

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Dr. Lance Barrett-Lennard  
Vancouver Aquarium Marine Science Centre  
845 Avison Way  
Vancouver,  
British Columbia  
V6G 3E2  
Work: 604-659-3428  
Email: [Lance Barrett-Lennard \[barrett@zoology.ubc.ca\]](mailto:lance.barrett@zoology.ubc.ca)

**Skin samples.** Punch biopsy or excised skin, including epidermis and hypodermis. Placed in either DMSO/saline solution and refrigerate or wrap in aluminum foil and freeze.

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Dr. Paul R Becker  
NIST  
219 Fort Johnson Road  
Charleston, South Carolina  
29412  
Work: 843-762-8503  
Email: [paul.becker@nist.gov](mailto:paul.becker@nist.gov)

**Fresh dead tissue samples for ongoing efforts to collect and appropriately archive harvested tissue samples from multiple indicator species.** Please call before conducting necropsy for additional details.

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Dr. Murray Dailey  
Marine Mammal Center  
Marin Headlands  
1065 Fort Cronkhite

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

Sausalito, CA  
94965-2609, USA  
Phone: office: 415-289-7346, after hours: 415-332-1744  
Email: [daileym@tmmc.org](mailto:daileym@tmmc.org)

**Preservation of parasite samples for ongoing speciation studies.** Samples of stomach worms, frozen in whirlpak bags at -70, alternatively, freeze in standard freezer, ship overnight on dry ice. All other parasites, preserve in 90% ethanol in whirlpak bags. If possible, let flatworms relax in tap water in cooler overnight before fixation.

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Dr. Maureen K. Davidson  
Research Scientist  
Department of Veterinary Pathobiology  
School of Veterinary Medicine  
Purdue University  
725 Harrison Street  
Room VPRB B13  
West Lafayette, IN 47907-2027  
Phone: 765-496-6753  
Email: [mkdavids@purdue.edu](mailto:mkdavids@purdue.edu)

**Tissue samples from live or dead killer whales for special *Mycoplasma* spp culture.**

Please place swabs samples, exudate or fluids directly into blood culture bottles or transport media. For post mortem tissue samples, dry cotton swabs or tissues samples may be placed directly into a sterile transport media. Please contact Dr Davidson prior to shipment.

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Darla R. Ewalt, M.S., Microbiologist  
Mycobacteria and Brucella Section  
Diagnostic Bacteriology Laboratory  
National Veterinary Services Laboratories  
1800 Dayton Road  
Ames, IA 50010  
Phone: 515-663-7347  
Fax: 515-663-7904  
Email: [darla.ewalt@aphis.usda.gov](mailto:darla.ewalt@aphis.usda.gov)

**Frozen tissue samples for *Brucella* culture.**

Dr. Bradley Hanson  
NOAA/NMFS/Northwest Fisheries Science Center  
2725 Montlake Blvd. E  
Seattle, WA 98112  
Work: 206-860-3220  
Fax: 206-860-3475  
Cell Phone 206-300-0282

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

Email: [Brad.Hanson@noaa.gov](mailto:Brad.Hanson@noaa.gov)

**Stomach content, head, ovary and fin for anatomic analysis.** Please either ligate the esophagus and duodenum or remove all the stomach contents, then freeze for analysis. Dorsal fin, remove 10 cm below insertion, place in plastic bag and freeze. Head may be disarticulated, then placed in plastic bag and frozen. Ovaries should be preserved intact in formalin.

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Dr. Michelle Fleetwood  
Armed Forces Institute of Pathology  
6825 16th Street NW  
Washington, DC  
20306-6000  
Phone: 202-782-2442  
Email: [afipvet@afip.osd.mil](mailto:afipvet@afip.osd.mil)

**Fixed tissue samples for ongoing investigation into mortality of stranded killer whales.** A submission form is available on the AFIP website: <http://www.afip.org/vetpath/ddpdf/dd2834.pdf> As regular mail is irradiated at all government facilities, case material should be couriered to the AFIP.

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Dr. John Ford or Graeme Ellis  
Department of Fisheries and Oceans  
Pacific Biologic Station  
3225 Stephenson Point Road,  
Nanaimo, BC, Canada  
V9T 1K3  
Work: 250-756-7245 or 729-8375  
Fax: 250-756-7053  
Email: [FordJo@pac.dfo-mpo.gc.ca](mailto:FordJo@pac.dfo-mpo.gc.ca) or [EllisG@pac.dfo-mpo.gc.ca](mailto:EllisG@pac.dfo-mpo.gc.ca)

**Stomach contents.** Please either ligate the esophagus and duodenum or remove all the stomach contents, then freeze for analysis.

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Dr. Judy St Leger  
Sea World San Diego  
500 Sea World Dr  
San Diego, CA  
92109  
Phone: 619-225-4259  
Email: [judy.st.leger@seaworld.com](mailto:judy.st.leger@seaworld.com)

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

**Fixed tissue samples for ongoing investigation into mortality of stranded killer whales.** Please call before conducting necropsy for additional details.

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Dr. Hendrick Nollens  
Marine Mammal Health Program  
Small Animal Clinical Sciences  
College of Veterinary Medicine  
PO Box 100126  
Gainesville, FL  
32610-0126  
Phone: 352-2392-4777, ext 5286  
Email: [NollensH@mail.vetmed.ufl.edu](mailto:NollensH@mail.vetmed.ufl.edu)

**Fresh and fixed samples of skin lesions suggestive of pox-virus infection.** Tissues for ongoing molecular characterization of poxvirus infections in wild and captive killer whales. Please call before conducting necropsy for additional details.

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Dr. Dawn Noren  
Marine Mammal Group  
NOAA NMFS Northwest Fisheries Science Center  
2725 Montlake Blvd. East  
Seattle, Washington 98112  
206-302-2439  
[dawn.noren@noaa.gov](mailto:dawn.noren@noaa.gov)

**Blubber Volume Determined (Appendix IX):** Validate the method of determining blubber volume in live cetaceans using the truncated cone method.

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Dr. Bets Rasmussen  
Department of Environmental and Biomolecular Systems  
OGI School of Science  
20,000 NW Walker Road  
Beaverton, OR 97006  
Phone: (530) 748-1263

**Post mortem air samples obtained from incised trachea or bronchi for metabolic profiles.** Canisters available upon request. Please contact before conducting necropsy examination for additional details.

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## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

Dr. Stephen Raverty  
Animal Health Center  
1767 Angus Campbell Road  
Abbotsford, BC, Canada  
V3M 2G3  
Phone, work: 604-556-3003  
Phone, work: 800-661-9903  
Email: [Stephen.Raverty@gems3.gov.bc.ca](mailto:Stephen.Raverty@gems3.gov.bc.ca)

### **Fresh and fixed tissue samples for ongoing investigation into mortality of stranded killer whales.**

Please call before conducting necropsy for additional details.

Please also send frozen samples of tongue and masseter muscle as well as diaphragm for *Trichinella* testing, frozen stomach contents and bile for algal toxin testing, multiple frozen tissues for bacteriology, and frozen samples of kidney and liver (wrapped in foil) for trace mineral analysis.

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Dr. Peter Ross  
Institute of Ocean Sciences  
Department of Fisheries and Oceans  
PO Box 6000  
9860 West Saanich Road  
Sidney, BC, Canada  
V8L 4B2  
Work: 250-363-6806  
Email: [RossPe@pac.dfo-mpo.gc.ca](mailto:RossPe@pac.dfo-mpo.gc.ca)

**Samples of liver, kidney and blubber (toxicologic investigation) from stranded killer whales.** Please record species, age, location and date. Wrap 30-50 gm of tissue in aluminum foil then freeze at -20C, ship on dry ice.

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Dr. Teresa Rowles  
NOAA/NMFS/PR2  
1315 East-West Highway  
Silver Spring, Maryland  
20910  
Work: 301-713-2322, Ext: 178  
Email: [teri.rowles@noaa.gov](mailto:teri.rowles@noaa.gov)

**Fresh dead tissue samples for ongoing efforts to collect and appropriately archive harvested tissue samples from multiple indicator species.** Please call before conducting necropsy for additional details.

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## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

Dr. Jeremiah Saliki  
Oklahoma Animal Disease Diagnostic Laboratory  
107 OADDL  
Oklahoma State University  
Stillwater, OK 74078-2007  
Phone: 405-744-6623  
Fax: 405-744-8612  
E-mail: [jsaliki@okstate.edu](mailto:jsaliki@okstate.edu)

**Post mortem heart blood and frozen tissue samples.** Serology, molecular studies and attempted virus isolation on marine mammal specific cell lines. Remove serum from blood sample and freeze at -80C. Various tissues (tonsil, spleen, lymph nodes, kidney, lung, kidney) for virus isolation. If possible, ship chilled same day for overnight delivery; if not, store frozen until shipped.

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Dr. J.L. Stott  
Veterinary Medicine PMI  
1126 Haring Hall  
1 Shields Avenue  
University of California  
Davis, CA 95616  
Phone: 530-752-2543  
Cell: 530-902-3971  
E-mail: [jlstott@ucdavis.edu](mailto:jlstott@ucdavis.edu)

**Blood samples for immune function testing of live animals.** The appropriate vacutainers and instructions for use are listed in Appendix VIII. Prior arrangements **MUST** be made. Leave messages on **both** lab and cell phones and also send e-mail.

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Dr. Barb Taylor  
NOAA/NMFS/Southwest Fisheries Science Center  
Population Identification Program  
8604 La Jolla Shores Drive  
La Jolla, CA 92037-1508  
Phone: 858-546-5620  
E-mail: [Barbara.Taylor@noaa.gov](mailto:Barbara.Taylor@noaa.gov)

**Skin samples.** Punch biopsy or excised skin, including epidermis and hypodermis. Placed in either DMSO/saline solution and refrigerate or wrap in aluminum foil and freeze.

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## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

Dr. Bud Tennant  
C2 009 Veterinary Medical Center  
Cornell University  
Ithaca, New York, 14853  
Phone: 607-253-3280  
Email: [Bct3@cornell.edu](mailto:Bct3@cornell.edu)

**Liver samples:** To screen for viral hepatitis, samples should include liver preserved in 10% buffered formalin, a frozen 10 gm sample of liver and 5 ml of frozen serum. Liver histology sections may also be prepared and forwarded for evaluation.

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Dr. Tony Van Dreumel  
Animal Health Laboratory  
University of Guelph  
Building 49, Macintosh Lane  
Guelph, ON, Canada  
N1G 2W1  
Phone: 519-824-4120, ext 4502  
Email: [Tvandreu@lsd.uoguelph.ca](mailto:Tvandreu@lsd.uoguelph.ca)

**Tissue samples for attempted *Mycoplasma* spp isolation.** Swabs or fresh tissue should be aseptically collected from representative levels of the respiratory system, including the nares, larynx, trachea, tracheal bifurcation, lungs, mediastinal lymph nodes, and urogenital tract. Swabs should be chilled and forwarded by courier.

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Dr. John C. Wekell  
National Oceanic & Atmospheric Agency  
NOAA–Fisheries  
Northwest Fisheries Science Center  
Environmental Conservation Division  
2725 Montlake Blvd. East  
Seattle, WA 98112  
Phone: 206-860-3388  
Fax: 206-860-3335

**Submit frozen sample of representative stomach contents for algal toxin testing.**

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John Pierce Wise, Sr., Ph.D.  
Director, Center for Integrated and Applied Environmental Toxicology  
Associate Professor of Toxicology and Molecular Epidemiology  
Bioscience Research Institute

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

University of Southern Maine  
178 Science Building  
96 Falmouth Street  
Portland, ME 04103  
Phone: 207-228-8047  
Email: WiseLab@usm.maine.edu

**Fresh and frozen tissue samples to be cultured and stabilized (“immortalized”) for subsequent toxicological studies and for placement in the marine mammal cell repository for other permitted researchers.** Please collect skin (w/dermis), kidney, liver, bronchus, testes/ovaries, brain from all young animals and call for storage and shipping recommendations.

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Dr. Stephanie Wong  
US Navy Marine Mammal Program  
Biosciences Division, Code 2351  
Space and Naval Warfare Systems Center — San Diego  
53560 Hull Street  
San Diego, CA 92152  
Phone: 619-767-4335  
Email: stephani.wong@navy.mil

**Collection and analysis of global killer whale stranding and necropsy data.** Please mail or email completed killer whale necropsy submission form (pages 16–22) and completed Appendix X.

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Dr. Gina Ylitalo and Peggy Krahn  
NOAA Fisheries / Northwest Science Center  
2725 Montlake Boulevard East  
Seattle, WA 98112  
Phone: 206-860-3325  
Dr Gina Ylitalo: [Gina.Ylitalo@noaa.gov](mailto:Gina.Ylitalo@noaa.gov)  
Peggy Krahn: [Peggy.Krahn@noaa.gov](mailto:Peggy.Krahn@noaa.gov)

**Samples of liver, kidney blubber, and skeletal muscle (toxicologic investigation) from stranded killer whales.** Please record species, age, location and date. Wrap 30-50 gm of tissue in aluminum foil then freeze at -20C, ship on dry ice (Appendix VI).

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**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX IIC  
REQUEST FOR MARINE MAMMAL POST MORTEM SAMPLES**

**Name** \_\_\_\_\_  
**Date of request** \_\_\_\_\_  
**Affiliation** \_\_\_\_\_  
**Address** \_\_\_\_\_  
**Work phone** (\_\_\_\_) \_\_\_\_\_  
**Home phone** (\_\_\_\_) \_\_\_\_\_  
**Fax** (\_\_\_\_) \_\_\_\_\_  
**Email** \_\_\_\_\_  
**Sample(s) requested** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Purpose of study** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Duration of study** \_\_\_\_\_  
\_\_\_\_\_

**Instructions for sample preparation** \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**Shipping instructions (Permits? Dry ice? Overnight? Will you pay for shipping?)**  
\_\_\_\_\_  
\_\_\_\_\_

**Special instructions**  
\_\_\_\_\_  
\_\_\_\_\_

Attach any additional information. Send to:

- Dr Brad Hanson, NOAA/NMFS/Northwest Fisheries Science Center, 2725 Montlake Blvd. E, Seattle, WA 98112, Office phone: 206-860-3220, Fax: 206-860-3475, Cell Phone 206-300-0282, Email: Brad.Hanson@noaa.gov
- Dr John Ford, Department of Fisheries and Oceans, Pacific Biologic Station, 3225 Stephenson Point Road, Nanaimo, BC, V9T 1K3, Office phone: (250) 729-8375, Fax: (250) 756-7053, Email: FordJ@pac.dfo-mpo.gc.ca

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX III: MORPHOMETRIC ANALYSIS OF STRANDED KILLER WHALES  
(Leger, 2004)**

**Observer** \_\_\_\_\_ **Date** \_\_\_\_\_  
**Identification number** \_\_\_\_\_ **Gender** \_\_\_\_\_ **Weight** \_\_\_\_\_

MEASUREMENTS, BODY (specify units of measure used \_\_\_\_\_)

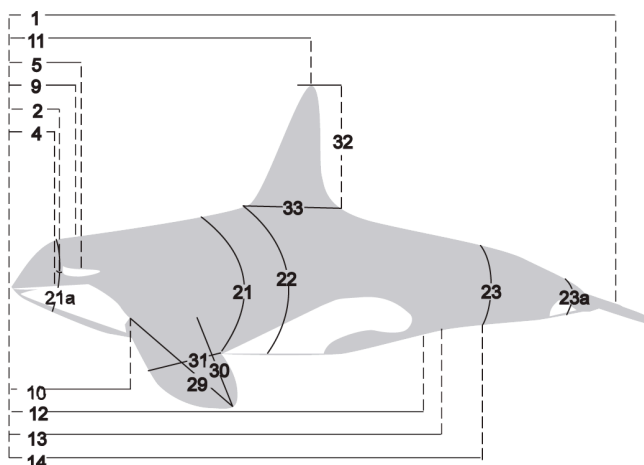
2	Snout to center of eye (left).....		16	Snout to end of ventral groove..	
3	Snout to apex of melon.....		13	Snout to genital slit.....	
15	Projection of the lower jaw.....		14	Snout to anus.....	
4	Length of gape (left).....		1	Total length, snout to notch....	
5	Snout to ear (left).....		17	Blubber thickness, mid dorsal...	
6	Center of eye to ear (left).....		18	Blubber thickness, mid lateral..	
7	Center of eye to angle of mouth...		19	Blubber thickness, mid ventral.	
8	Eye to blowhole (center)(left).....		21	Girth at axilla.....	
9	Snout to center of blowhole.....		21a	Girth at eye.....	
20	Length of throat grooves.....		22	Girth at leading edge of dorsal..	
10	Snout to flipper (left).....		23	Girth at anus.....	
11	Snout to tip of dorsal fin.....		23a	Girth _____ cm before notch.....	
12	Snout to center of umbilicus.....				

MEASUREMENTS, APERTURES (specify units of measurement \_\_\_\_\_)

24	Eye:.....length		27	Blowhole: length _____ width _____	
25	Length of mammary slit..... r _____	l _____			
26	Length genital slit _____ anal slit _____				

MEASUREMENTS, APPENDAGES (specify units of measurement \_\_\_\_\_)

29	Flipper length (ant) (left).....		33	Length of dorsal fin base.....	
30	Flipper length (post) (left).....		34	Width of flukes.....	
31	Maximum width of flipper (left)..		35	Length of flukes (left).....	
32	Height of dorsal fin.....		36	Depth of fluke notch.....	



## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

### APPENDIX IV

#### PATHOGEN AND TISSUE SAMPLE LIST FOR POLYMERASE CHAIN REACTION STUDIES

Current list of Pathogens which may be screened by polymerase chain reaction. These tests may be conducted on tissues harvested from animals recovered in code 1 and 2 and in select cases, code 3.

Pathogen	Tissues
<i>Brucella</i> spp, marine mammal variant and consensus primers	Lymph node, spleen, lung, brain
Canine distemper virus	Lymph node, spleen, lung, brain
Dolphin morbillivirus	Lymph node, spleen, lung, brain
<i>Chlamydophila psittaci</i> - Avian	Lymph node, spleen, lung, brain
<i>Chlamydiophila abortus</i> –Ovine	Lymph node, spleen, lung, brain
<i>Clostridium</i> genotyping (toxin)	Small and large intestine, bacterial isolate
<i>Clostridium piliforme</i> - Tyzzer's disease	Intestine or liver
<i>Coxiella burnetii</i>	Lymph node, spleen, lung, brain
Equine Influenza virus	Lymph node, spleen, lung, brain
<i>Erysipelothrix rhusiopathiae</i>	Lymph node, spleen, lung, brain
<i>Escherichia coli</i> genotyping - Bovine/Porcine	Bacterial isolate
Fungus - Universal	Fungal isolate
Herpesvirus – Universal	Lymph node, spleen, lung, brain
Influenza Virus – Universal	Lymph node, spleen, lung, brain
<i>Leptospira</i> (multivalent)	Liver, kidney
<i>Listeria monocytogenes</i>	Brain, lymph node, spleen, lung
Morbillivirus - Universal	Lymph node, spleen, lung, brain
<i>Mycobacterium</i> - Universal	Lymph node, spleen, lung, brain
<i>Mycobacterium avium</i>	Intestine, mesenteric lymph nodes, feces
<i>Mycobacterium paratuberculosis</i>	Intestine, mesenteric lymph nodes, feces
<i>Mycoplasma (Mollicutes)</i> - Universal	Lymph node, spleen, lung, nares
<i>Neospora caninum</i>	Lymph node, spleen, lung, brain
<i>Nocardia</i> - Universal	Skin, lung, lymph node, spleen
<i>Salmonella</i>	Intestines, feces, isolate
<i>Streptococcus</i>	Isolate
<i>Toxoplasma gondii</i>	Lymph node, spleen, lung, brain
West Nile virus	Brain, lung, lymph node, spleen
Western Equine Encephalomyelitis virus	Brain, lymph node, spleen, lung,

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX V  
LIVE ANIMAL STRANDING DIAGNOSTIC AND RESEARCH TESTS**

Clinical chemistry, hematology and ancillary diagnostic tests on blood samples obtained from live animals (McBain, 2002)

Test	Test Category	Sample Type	Volume
HEMATOLOGY	H	EDTA blood	2ml
Hemoglobin	"	" "	"
Hematocrit/PCV	"	" "	"
RBC	"	" "	"
MCV	"	" "	"
MCH	"	" "	"
MCHC	"	" "	"
RDW	"	" "	"
Platelets	"	" "	"
MPV	"	" "	"
N-RBC	"	" "	"
Reticulocyte	"	" "	"
RBC Morph	"	" "	"
WBC	"	" "	"
Bands	"	" "	"
Neutrophils	"	" "	"
Lymphocytes	"	" "	"
Monocytes	"	" "	"
Eosinophils	"	" "	"
Basophils	"	" "	"
ESR 10	"	" "	"
ESR 20	"	" "	"
ESR 30	"	" "	"
ESR 60	"	" "	"
Fibrinogen	H	citrate plasma	0.25 ml
Total Solids		plasma	0.1 ml

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

Test			0.5-0.75 ml
CHEMISTRY	H	serum	
Glucose	"	"	
BUN	"	"	
Creatinine	"	"	
Bilirubin	"	"	
Cholesterol	"	"	
Triglycerides	"	"	
Total Protein	"	"	
Albumin	"	"	
Globulin	"	"	
Alk Phos	"	"	
ALT	"	"	
AST	"	"	
gGT	"	"	
CK	"	"	
	Test		
Test	Category	Sample Type	Volume
Calcium	"	"	
Phosphorus	"	"	
LHD	"	"	
Sodium	"	"	
Potassium	"	"	
Chloride	"	"	
Bicarbonate	"	"	
Iron	"	"	
Uric Acid	"	"	
Protein Electrophoresis	H	serum	0.1
SEROLOGY			
Morbillivirus	H	serum	1 ml
Brucella	M	serum	1 ml
Leptospira		serum	1 ml
Herpesvirus			
Calicivirus			
Hepatitis B and A			
Influenzavirus	L		
<i>Erysipelas rhusiopathiae</i>			
Equine Encephalitis	L		
Deep Mycotic Panel	M	serum	

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

Test			
TOXICOLOGY			
Heavy metals	M	whole Blood	1 ml
Organochlorines		whole Blood	0.5 ml
Blubber biopsy			
CULTURE SAMPLES		cyto/bact/fung	
Blow ID & sensitivity	M	swab/plate	
Fecal ID & sensitivity	M	tube sample	
Skin ID & sensitivity	M	scrap/punch	
Blood ID & sensitivity	M		
EDTA Blood	H	Virus culture	
MISCELLANEOUS			
Cortisol	M	serum	0.5 ml
Aldosterone	M	serum	0.5 ml
Estrogen	L	serum	0.5 ml
Progesterone	L	serum	0.5 ml
Trace minerals	L	serum	0.5 ml
Thyroid Panel	M	serum	1 ml
Insulin	H/L	serum	?
Vitamin B and A	L	serum	?
Auto-antibodies	M/L	serum	?
Metabolic testing	H	urine (frozen)	30ml
Metabolic testing	H	blood??	??
Blubber biopsy	L		
Test	Category	Sample Type	Volume
IMMUNE PANEL	H	multiple	23 ml
Test	Category	Sample Type	Volume
IL-6	"	serum (frozen)	0.5 ml
Lymphocyte function assays	"	CPT	2x8ml
Leukocyte phenotyping	"	ACD	6ml

TEST CATAGORIES/PRIORITIES

- Immediate health monitoring - H
- Longterm health - M
- Interesting assays - L

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX VI  
MARINE MAMMAL BLUBBER SAMPLING PROTOCOL**

Tissue Sampling for Chemical Contaminant Analyses

*Supplies for sampling will include:*

- 12" x 13" solvent-rinsed Teflon sheets, solvent-rinsed 17-mL Teflon screw top vials for blood and 4-mL amber vials for bile.
- 18 oz. Whirl-pak<sup>®</sup> bags (4.5" x 8.5") or Zip-Lock bags
- ballpoint and marking pens

*Sampling Protocol*

- Priority for collection of samples is: full-thickness blubber with skin, liver, muscle, blood (when possible), bile, (see attached Marine Mammal Sampling Sheet/Data Record for description of how and from where to collect tissue samples).
- See attached Marine Mammal Tissue Sampling Protocol Addendum for additional information on sampling procedure.
- For tissue collection, use a stainless steel knife and rinse the knife between necropsies of each animal.
- Wrap each tissue sample in a pre-rinsed Teflon sheet or in a pre-rinsed vial and then place sample into a labeled Whirl-pak<sup>®</sup> or a Zip-Lock bag. Label each bag with:

Animal ID Number \_\_\_\_\_  
Species \_\_\_\_\_  
Tissue Type \_\_\_\_\_  
Date Collected \_\_\_\_\_

- Remove as much of the air as possible from the bag before it is sealed. Place samples on ice. As soon as possible, freeze at lowest temperature available.
- Please provide copy of full necropsy report.

*Shipment of Samples*

- Ship frozen samples on blue ice or ~5 lbs dry ice, early in the week via FedEx overnight to: Gina Ylitalo/Jon Buzitis, NWFSC, ECD, 2725 Montlake Blvd. E., Seattle, WA 98112-2097. Call Gina (206-860-3325) or Jon (206-860-3309) the day the samples are shipped with the invoice number for tracking, if necessary: **Marine Mammal Tissue Contaminant Analyses**

**Environmental Conservation Division, Northwest Fisheries Science Center  
National Marine Fisheries Service, National Oceanic and Atmospheric Administration  
2725 Montlake Blvd. East, Seattle, WA 98112-2097  
Phone: (206) 860-3325, FAX (206) 860-3335**

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

### MARINE MAMMAL BLUBBER SAMPLING PROTOCOL ADDENDUM

*Blubber collection procedures:* It is important to use standardized sampling procedures so that, even when there are low levels of contaminants present, the differences may be attributed to biological processes and contaminant exposure and not to variation in the collection process. The following procedures are essential to prevent cross-contamination of tissues within an animal and ensure uniformity of samples among animals.

- 1. Collect full-thickness blubber with skin attached, if possible.** This reduces variation caused by possible composition differences within tissues of the same animal. It also provides us with uniform samples and information from all participating organizations which can be directly compared based on the demographics of the animals. Sample size: blubber 10 – 20 g. **NOTE: please collect full thickness blubber from the dorsal region (behind the dorsal fin).**
- 2. If possible, rinse all instruments with isopropyl alcohol before each blubber sample is sampled.** This will minimize cross-contamination of tissues.
- 3. Keep samples as cold as possible after collection.** Some of the organic contaminants are volatile or are degraded by compounds released during cell death. In addition, lipids may be lost (e.g., leaching may occur) if the samples are not kept as cold as possible. To decrease changes in contaminant levels and lipid due to these processes, keep the samples on ice following the necropsy and freeze as soon as possible in a  $-20^{\circ}\text{C}$  freezer or colder freezer (e.g.,  $-80^{\circ}\text{C}$  freezer). For fatty acid analyses, the samples should be stored in a  $-80^{\circ}\text{C}$  freezer.

**KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL**

**APPENDIX VII  
TISSUE SAMPLING FOR ATTEMPTED MYCOPLASMA CULTURE**

Dr. M. Davidson, IACUC D4087/30/04 (rev.)

**Marine Mammal Microbiology Samples**

**University of Florida contact person for collection supplies and shipping of specimens to Dr. Davidson:**

Linda Thomas  
Dept Pathobiology  
(% Dr. Carlos Romero Laboratory)  
College of Veterinary Medicine  
2015 S.W. 16th Ave  
VAB V232  
Gainesville, FL 32611  
Work:352-392-4700-ext 5872  
Home:352-335-7144  
Fax: 352-352-392-1910  
Mobile:352-870-4123

**Shipping of Specimens to Dr. Davidson**

All specimens must be shipped overnight via FedEx or other overnight courier in appropriate packaging. Please call or email to let Dr. Davidson know the specimens are being shipped, and include the airbill number in case we have to track the package.

Ship all specimens to:

Maureen K. Davidson, PhD  
Department of Veterinary Pathobiology  
School of Veterinary Medicine  
Purdue University  
725 Harrison Street  
Room VPRB B13  
West Lafayette, IN 47907-2027  
Phone: 765-496-6753  
Fax: 765-496- 7989  
Email: mkdavids@purdue.edu

Use FedEx account number 1714-0802-4. In the "Internal Reference" part of the form, put 014-1243-1000-73088

**NOTES:** It is ok for the blood bottles, Vacutainer<sup>®</sup> tubes, and the bacterial and mycoplasma cultures to sit at room temperature or below until returned to the laboratory. The samples should not be left to sit in the sun.

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

As long as the temperature of the samples does not exceed 37°C (98°F), the samples will be OK.

Please provide as much history as possible for the animal...i.e., unique identifier for the animal, date samples collected, where caught (TECO, Belize, etc), sex, age (adult, juvenile, calf, etc), general health (ill, healthy, emaciated, etc), other obvious facts (i.e., “this animal is the mother of calf TC 165”, “this animal has fresh propeller cuts”, etc)

### Short Protocol for Blood Culture Collections

*These should be collected from every case, if possible.*

1. Label each tube and culture bottle with the animal’s unique identifier (identification number, whatever label we can use to tell which manatee is which). Use a marker or pen that will not run if wet with alcohol or water. **IF IN DOUBT, USE A #2 PENCIL.**
2. Perform a surgical-type scrub of the venipuncture area.
3. Use a BD Vacutainer blood collection set for the venipuncture.
4. If other tubes of blood are collected for other purposes (hematology, clinical chemistry, etc), they should be collected BEFORE the blood culture specimens.
5. Swab or spray each blood collection tube or blood culture bottle injection port with alcohol prior to collection.
6. Collect the aerobic, anaerobic, and mycobacteria blood cultures directly from the animal. The blood culture bottles should draw the blood into the bottle automatically.

Collect 2 brown cap blood bottles (3-5 ml blood in each bottle)

Collect 1 yellow cap blood bottle (AFB bottle) at least 1 ml blood)

Collect at least 1 green top (heparin) vacutainer of blood from each animal. Collect 2 tubes green top tubes, if possible. Remember to mix the vacutainers well by inverting the tubes repeatedly immediately after collection.

7. Transport all of the blood bottles and vacutainers back to the laboratory. Ship them to Dr. Davidson as soon as possible. It is OK if the samples have to wait a day or two to be shipped.

## KILLER WHALE NECROPSY AND DISEASE TESTING PROTOCOL

### Short Protocol for Collection of Swabs for Mycoplasma Cultures

NOTE: Sampling of these sites are not intended to be “invasive” or traumatic to the animal, so collection is left to the discretion of the person in charge of the live animal capture. It is quite possible that all sites will be easily collected from some animals, while none of the samples can be collected from other animals. Whatever swabs from the sites that can be sampled easily should be collected to provide a good survey of the mycoplasmas present in the different populations of manatees.

1. Sterile, **cotton** tipped, wooden shaft, swabs and 15 ml screw cap, centrifuge tubes containing medium are provided for collection of swab samples for mycoplasma cultures. (These tubes can also be used for small pieces of tissue, exudates, body fluids, etc. if these samples need to be collected from animals that are ill, etc).
2. Label each tube with the animal’s unique identifier (identification number, whatever label we can use to tell which manatee is which). Use a marker or pen that will not run if wet with alcohol or water. **IF IN DOUBT, USE A #2 PENCIL**
3. Use a sterile, **cotton** tipped, wooden shaft, swab for all collections.
4. Sites to be sampled, as appropriate for the situation: conjunctiva, nasal passages, throat, mouth, genital tract, urinary tract, skin after surgical scrub for collection of blood.
5. After swabbing the site, place the swab in the tube containing the medium and break it off in the tube. It is OK if only the cotton part of the swab remains in the tube. Try not to put the part of the shaft that has been contaminated by fingers, etc into the tube.
6. Transport the tubes containing the swabs back to the laboratory. Ship them to Dr. Davidson as soon as possible. It is OK if the samples have to wait a day or two to be shipped.

**NOTE: Mycoplasma transport medium may be used for transport of other specimens if no antibiotics are added to the medium.**

## **Stranded or Beached Marine Mammals: Short Protocol for Collection of Swabs for Bacterial Cultures**

**Live Animals:** Sampling of these suggested sites from live animals are not intended to be “invasive” or traumatic to the animal, so collection is left to the discretion of the person in charge of the live animal.

**Necropsy Specimens:** Collection of specimens at the time of necropsy is left to the discretion of the persons performing the necropsy. Cultures from animals that are in an advanced state of decay should be limited to obvious gross lesions as these are the only specimens that could possibly give any pertinent information. Even then, the results should be viewed with caution as bacteria move and multiply within minutes to all sites once death has occurred.

***This protocol will suffice for most samples. Fluids or exudates also can be injected into blood culture bottles (best) or added directly to the transport medium. Bits of tissues can be placed in the transport medium.***

1. Sterile, **cotton** tipped, wooden shaft, swabs and 15 ml screw cap, centrifuge tubes containing medium are provided for collection of swab samples for cultures. (These tubes can also be used for small pieces of tissue, exudates, body fluids, etc. if these samples need to be collected from animals that are ill, etc).
2. Label each tube with the animal’s unique identifier (identification number, whatever label we can use to tell which manatee is which). Use a marker or pen that will not run if wet with alcohol or water. **IF IN DOUBT, USE A #2 PENCIL**
3. Use a sterile, **cotton** tipped, wooden shaft, swab for all collections.
4. Sites to be sampled, as appropriate for the situation: conjunctiva, nasal passages, throat, mouth, genital tract, urinary tract, skin after surgical scrub for collection of blood.
5. After swabbing the site, place the swab in the tube containing the medium and break it off in the tube. It is OK if only the cotton part of the swab remains in the tube. Try not to put the part of the shaft that has been contaminated by fingers, etc into the tube.
6. Transport the tubes containing the swabs back to the laboratory. Ship them to Dr. Davidson as soon as possible. It is OK if the samples have to wait a day or two to be shipped.

**NOTE: Mycoplasma medium without antibiotics is an excellent transport medium for bacteria as this medium is very rich and allows the growth of fastidious organisms. This is the usual transport medium supplied, but blood culture bottles may also be used. Simply remove the cap and place the specimen in the bottle and replace the cap.**

**APPENDIX VIII  
BLOOD SAMPLING FOR IMMUNE FUNCTION TESTS**

1. **One EDTA vacutainer (purple top)(3 ml draw):** This is for a CBC and is best conducted ASAP at a local lab on the same day; if sent to us overnight the quality of the differential leukocyte count will suffer considerably. Total white count and differential should be faxed to our laboratory immediately upon completion as this information is needed for us to process the blood received in Davis the following morning.
2. **One sodium citrate vacutainer (light blue-top)(3 ml draw):** This is for quantification of fibrinogen in the plasma. Plasma should be removed and frozen ASAP and shipped on Dry Ice to our lab at your convenience.
3. **One serum collection vacutainer (red top)(8 ml draw):** This is for both serum chemistry and serology. This sample should be split with one sample being sent to a lab of your choice for clinical chemistries and one sample sent to our lab for possible future serology.
4. **One ACD vacutainer (yellow top)(8 ml draw):** This is for analytical flow cytometry and analysis of leukocyte subpopulations. This tube must be stored cool (do not let blood tube come in direct contact with frozen ice packs as the blood sample will hemolyze. Must be shipped overnight for AM arrival at our lab. **Samples more than 24 hours old cannot be analyzed.**
5. **Three CPT cell preparation tube vacutainers (blue/black camo top)(8 ml draw each):** Tubes MUST be filled to the blue band near top and rocked EXTENSIVELY. This sample is for lymphocyte function. As many killer whales have low lymphocyte counts, especially if they are ill, 3 to 4 tubes of blood is desirable. These samples must be handled and shipped as described in #3 above. **Samples more than 24 hours old cannot be analyzed.**
6. **Two PAXgene vacutainers (red top containing fluid)(each tube requires 2.5 ml of blood):** These RNA blood tubes are for preservation of leukocyte mRNA. They should be shipped as described above in #3. This is for quantitation of inflammatory mediators and immune response messengers.

Ship to: Dr. J.L. Stott  
Vet Med PMI  
1126 Haring Hall  
1 Shields Avenue  
University of California  
Davis, CA 95616  
Lab: 530-752-2543  
Cell: 530-902-3971  
e-mail: [jlstott@ucdavis.edu](mailto:jlstott@ucdavis.edu)

**APPENDIX IX  
BLUBBER VOLUME DETERMINATION**

***Purpose of Measurements:***

Validate the method of determining blubber volume in live cetaceans using the truncated cone method.

Dawn Noren

***Protocol:***

Animal condition must be excellent – body fully intact and fresh so that blubber has not deteriorated or been lost through decay.

Measurements to be made: total body length, total body weight, girths at multiple sites along the body, lengths between the girth measurements, blubber thickness at each of the girth sites measured by a ruler inserted into small slits cut into the dorsal, ventral, and lateral surface at each site, blubber thicknesses measured by ultrasound at the dorsal, ventral, and lateral surface at each site, weight of all blubber and skin.

Killer Whale Necropsy and Disease Testing Protocol

Date: \_\_\_\_\_

Animal I.D.: \_\_\_\_\_

Species: \_\_\_\_\_

Age: \_\_\_\_\_

Sex: \_\_\_\_\_

Condition: \_\_\_\_\_

\_\_\_\_\_

Stranding Location: \_\_\_\_\_

Possible Cause of

Death: \_\_\_\_\_

Total Body Length (Tip of rostrum to fluke notch) – Straight line above

body: \_\_\_\_\_

Total Body Length (Tip of rostrum to fluke notch) \_\_\_\_\_

Curvilinear along body: \_\_\_\_\_

Total Body Mass: \_\_\_\_\_

Total Blubber and Skin Mass: \_\_\_\_\_

Date: \_\_\_\_\_

Animal I.D.: \_\_\_\_\_

Killer Whale Necropsy and Disease Testing Protocol

Measurement Site	Length from tip of Rostrum to Measurement Site	Length Between Measurement Site and Previous Site	Girth at Measurement Site	Dorsal Blubber Thickness (ruler) n=3	Lateral Blubber Thickness (ruler) n=3	Ventral Blubber Thickness (ruler) n=3	Dorsal Blubber Thickness (ultrasound) n=3	Lateral Blubber Thickness (ultrasound) n=3	Ventral Blubber Thickness (ultrasound) n=3
Ear or directly posterior to end of melon									
Anterior pectoral fin insertion									
Posterior pectoral fin insertion									
Anterior dorsal fin insertion									
Posterior dorsal fin insertion									
Mid-way between posterior dorsal fin insertion and anus									
Mid-way between anus and fluke notch									
Fluke Notch			-----	-----	-----	-----	-----	-----	-----

**APPENDIX X**  
**Standardized Killer Whale Histopathology Tracking Form – Page 1 of 5**

Tissue	Gross Obs	Histo Obs	Dist	Duration	Severity	Other Descriptors	Inflam	Primary WBC	Infect	Microbe	Microbial Relevance	Overall Relevance
STANDARD VARIABLE OPTIONS:	Normal Body condition score Abnormal List gross diagnoses	Normal Abnormal	Focal Multifocal Segmental Diffuse Focally extensive	Peracute Acute Subacute Chronically active Chronic	Mild Moderate Severe	Congested (C) Hemorrhagic (H) Necrotic (N)	Yes No	Mononuclears Basophilic Eosinophilic Lymphocyteic Hemorrhagic Necrotizing Fibrinous Hisstiocytic Supporative Lymphohistiocytic Granulomatous Pyogranulomatous Granulation tissue Abscessation Fibrosing Giant cell formation Syncytia	Yes No	Bacterial Mycotic Parasitic Viral	Pathogen Contaminant  Confirmed How: Culture Serology Electron microscopy Special stains PCR IFA IHC  Follow up serology of live animals  Suspected	Primary Secondary Insignificant Unknown
Skin												
Dermis												
Blubber												
Conjunctiva												
Umbilicus												
Thoracic cavity												
Abdominal cavity												

**Standardized Killer Whale Histopathology Tracking Form – Page 2 of 5**

Tissue	Gross Obs	Histo Obs	Dist	Duration	Severity	Other Descriptors	Inflam	Primary WBC	Infect	Microbe	Microbial Relevance	Overall Relevance
Teeth												
Oral mucosa												
Oropharynx												
Tonsil												
Larynx												
Esophagus												
Stomach, glandular												
Stomach, nonglandular												
Pancreas												
Liver												
Bile												
Small intestine												
Colon												
Mesentery												
Omentum												
Adipose tissue												

**Standardized Killer Whale Histopathology Tracking Form – Page 3 of 5**

Tissue	Gross Obs	Histo Obs	Dist	Duration	Severity	Other Descriptors	Inflam	Primary WBC	Infect	Microbe	Microbial Relevance	Overall Relevance
Ovary												
Oviduct												
Uterus												
Vagina												
Urogenital slit												
Mammary gland												
Penis												
Accessory sex glands												
Testes												
Blowhole												
Trachea												
Bronchus												
Lung												
Lymph node, multiple sites												
Thymus												
Spleen												
Thyroid gland												

**Standardized Killer Whale Histopathology Tracking Form – Page 4 of 5**

Tissue	Gross Obs	Histo Obs	Dist	Duration	Severity	Other Descriptors	Inflam	Primary WBC	Infect	Microbe	Microbial Relevance	Overall Relevance
Parathyroid gland												
Adrenal gland												
Brain cerebrum												
Brain cerebellum												
Brain brainstem												
Meninges												
Pituitary gland												
Spinal cord (thoracic, lumbar)												
Brachial plexus												
Heart												
Aorta and vena cava												
Pericardium												
Pericardial fluid												
Peripheral vasculature												
Kidney												
Ureter												

**Standardized Killer Whale Histopathology Tracking Form – Page 5 of 5**

<b>Tissue</b>	<b>Gross Obs</b>	<b>Histo Obs</b>	<b>Dist</b>	<b>Duration</b>	<b>Severity</b>	<b>Other Descriptors</b>	<b>Inflam</b>	<b>Primary WBC</b>	<b>Infect</b>	<b>Microbe</b>	<b>Microbial Relevance</b>	<b>Overall Relevance</b>
Urinary Bladder												
Urethra												
Urogenital canal												
Skeletal muscle												
Rib/Bone marrow												
Diaphragm												

**Note: please fax, mail, or e-mail this completed 5-page histologic findings form as well as the submission form (page 16) to:**

**Stephanie Wong, D.V.M., M.P.H.**  
**U.S. Navy Marine Mammal Program, Biosciences Division, Code 2351**  
**Space and Naval Warfare Systems Center - San Diego**  
**53560 Hull Street**  
**San Diego, CA 92152**  
**Tel: 619.767.4335, Fax: 619.553.5068**  
**stephanie.wong@navy.mil**