

NIST Special Publication 1211

**Risk Assessment of Brucellosis for
Tissues Processed and Archived
at the Marine Environmental
Specimen Bank**

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NIST
**National Institute of
Standards and Technology**
U.S. Department of Commerce

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March 2017



U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
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**National Institute of Standards and Technology Special Publication 1211
Natl. Inst. Stand. Technol. Spec. Publ. 1211, 25 pages (March 2017)**

**This publication is available free of charge from:
<https://doi.org/10.6028/NIST.SP.1211>**

Table of Contents

1	NIST Marine Environmental Specimen Bank (Marine ESB)	1
1.1	Background	1
1.2	Marine ESB Facility.....	1
1.3	Collections.....	1
1.3.1	Northern Gulf of Mexico Cetacean Unusual Mortality Event.....	1
1.3.2	National Marine Mammal Tissue Bank.....	2
1.3.3	Other Collections.....	3
2	Risk Assessment	3
2.1	Known Agent and Strain <i>Brucella</i>	3
2.2	<i>Brucella</i> Testing Results.....	5
2.3	Activities with Risk of Exposure.....	5
2.3.1	Receiving and Shipping Samples	5
2.3.2	Collection and Processing of Samples for NMMTB.....	9
2.3.3	Cryohomogenization of Archived Tissues	10
2.4	Personnel Training and Expertise.....	13
2.4.1	Required trainings and certifications.....	13
2.4.2	Responsible Parties and Authorized Personnel	13
2.5	Evaluate and Prioritize Risk	14
2.6	Develop or evaluate controls to mitigate exposure	14
3	Emergency Procedures	16
4	References	16
	Appendix A	18

1 NIST Marine Environmental Specimen Bank (Marine ESB)

1.1 Background

The National Institute of Standards and Technology (NIST) has been involved in environmental specimen banking since 1979 through collaborations with various research and monitoring programs. Samples from on-going marine related projects are collected and archived at ultra-cold and cryogenic temperatures (-80 C° and -150° C) in NIST's Marine Environmental Specimen Bank (Marine ESB), Hollings Marine Laboratory (HML), Charleston, SC. These samples include: sediments, mussels, oysters, marine mammal tissues and fluids, bird egg contents and feathers, sea turtle tissues and fluids, coral tissue, coralline algae, and fish tissues.

1.2 Marine ESB Facility

The Marine ESB was designed and operates under ISO Certified Class 5, 6 and 7 clean room conditions. Stored samples are brought into the bank facility through an ISO Class 6 anteroom and are stored in liquid nitrogen vapor-phase and ultra-cold electric freezers in the ISO Class 7 Freezer Room, Room D104. These clean air laboratories control the concentration of airborne particles to specified limits and are essential in minimizing contamination of samples during processing. In addition, the Marine ESB is a secure area with key-card access restricted to trained Marine ESB personnel or other trained researchers who are escorted by Marine ESB personnel. Personal protective equipment (PPE) is required at all times when entering and working in the Marine ESB. PPE includes disposable Tyvek coverall (full body), open-faced hood and mask or eyes-only hood, boot covers, safety glasses, and gloves (general laboratory vinyl gloves and/or cryogenic gloves). The work activities and required PPE for the Marine ESB are listed in the Job Hazard Analysis Forms which are approved and signed by all Marine ESB personnel, the NIST Supervisor, and the Area Safety Representative for the HML as well as the NIST Hazard Review System which are approved by the Group Leaders, Division Chief, and Lab Directors, when necessary. Environmental conditions in the laboratory (humidity, temperature, and oxygen levels) and freezer temperatures are monitored 24/7 using a monitoring system (1).

1.3 Collections

1.3.1 Northern Gulf of Mexico Cetacean Unusual Mortality Event

NIST has been indirectly involved in the response to the 2010 Deep Water Horizon Gulf of Mexico Oil Spill incident. In support of the National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Office of Protected Resources (NOAA/NMFS/OPR), NIST has become the lead repository for frozen marine mammal (primarily bottlenose dolphin, *Tursiops truncatus*) samples collected in response to this event. Sources of these samples are: stranding events (including the ongoing Northern Gulf of Mexico Cetacean Unusual Mortality Event), live capture health assessments, and dart biopsy collections

conducted throughout the Gulf of Mexico region. Since May 2010, NIST has received thousands of tissue samples (no microbial cultures) that were collected in the field and shipped frozen to the Marine ESB for storage following strict Chain of Custody guidelines. The majority of the frozen samples received have come from strandings as a part of the Northern Gulf of Mexico Cetacean Unusual Mortality Event. These samples were collected and shipped from marine mammal stranding network participants, including the Institute for Marine Mammal Studies (IMMS), Gulf World Marine Park, the Emerald Coast Wildlife Refuge (ECWR), NOAA Southeast Fisheries Science Center, Dauphin Island Sea Lab (DISL), and the Audubon Aquatic Institute.

Samples maintained at the Marine ESB are requested by NMFS/OPR to be sent to various laboratories for testing and analyses, including testing for zoonotic pathogens, such as *Brucella* and Morbillivirus and analyses for polycyclic aromatic hydrocarbons (PAH's) and biotoxins.

In October 2011, NIST Marine ESB personnel were informed by NMFS/OPR that samples shipped out for testing in July, August, and October of 2011 tested positive for marine *Brucella*, the causal agent of brucellosis. A total of 33 frozen tissue samples, including lung, spleen, kidney, umbilicus, cerebrospinal fluid, placenta and lung lymph node, were shipped from the Marine ESB to Michael Kinsel at the University of Illinois, Zoological Pathology Program, in Maywood, IL for *Brucella* testing. Since 2011 more samples have tested positive for marine *Brucella*. Additional samples maintained at the Marine ESB are currently being requested to be shipped for marine *Brucella* testing as well as more testing in the future. According to a recent study the prevalence of *Brucella* and Morbillivirus infections, which were investigated as potential alternative causes for increased dolphin deaths, was low in UME dolphins after the oil spill and was no different compared to reference dolphin populations (2).

1.3.2 National Marine Mammal Tissue Bank

The National Marine Mammal Tissue Bank (NMMTB) consists of two ongoing environmental monitoring projects, the Alaska Marine Mammal Tissue Archival Project (AMMTAP), established in 1987 and the Marine Mammal Health and Stranding Response Program (MMHSRP), established in 1990. These projects provide a large percentage of samples banked at the Marine ESB. The NMMTB was formally established by the Marine Mammal Health and Stranding Response Act (Public Law 102-587) and expanded to become a larger program that resulted in several components; the Marine Mammal Stranding Networks, the NMMTB, and a Monitoring and Quality Assurance Program. The MMHSRP is focused on animal health assessment, real-time contaminant monitoring, specimen banking, response to strandings and mass mortalities, quality assurance/quality control of analytical results, and the management of a nationwide database on the health of marine mammal populations. NIST currently maintains the NMMTB and the Monitoring and Quality Assurance Program. The NMMTB houses liver, kidney,

blubber/adipose, muscle, and brain tissues from stranded animals which include cetaceans, pinnipeds, and polar bears (3).

1.3.3 Other Collections

Tissue collections from other projects are obtained from healthy animals or animals that are not suspected to be infectious. These include sea bird egg contents, marine mammal tissues taken from Alaskan subsistence harvested animals, marine mammal tissues taken from incidental takes, marine mammal fluids taken from human managed animals, bivalves, sport fish tissue, sea turtle tissues and fluids, sea turtle egg contents, and specimens from live captured cetaceans. These samples pose little zoonotic risk when handled with required PPE.

2 Risk Assessment

2.1 Known Agent and Strain *Brucella*

Marine mammal isolates of the genus *Brucella* (*Brucella pinnipedialis* and *Brucella ceti*, sometimes referred to as *Brucella maris*) are potentially zoonotic, causing the disease Brucellosis. However, the frequency of transmission to humans is unknown. There have been a total of four confirmed human infections of marine *Brucella*. Three of these infections are naturally occurring thought to have been caused by the consumption of raw fish (4), (5) since none of the patients had any contact with marine mammals. The fourth, a laboratory acquired infection, was mild and presented no complications (6). Marine mammal isolates of *Brucella* are not considered select agents by the Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture/Animal and Plant Health Inspection Service (APHIS) (7).

Brucella is gram negative coccobacilli. *Brucella* species are thought to be stable in the environment in carcasses and organs up to 135 days, and up to 180 days in blood stored at 4°C. With regards to terrestrial *Brucella*, laboratory infections typically occur when the bacteria is grown in large cultures. Routes of exposure can include ingestion, direct contact with skin abrasions and mucous membranes, as well as inhalation of the aerosolized agent. Reservoirs of infectious agent include infected tissues, blood, urine, vaginal discharge, and aborted fetuses (8, 9). Routes of exposure are poorly understood in marine *Brucella*, with little evidence to support any route of infection. Infectious doses of terrestrial *Brucella* species are as little as 10 to 100 organisms (10, 11) and the incubation period for terrestrial *Brucella* can run from 1 to 15 weeks. The infectious dose and incubation period of marine *Brucella* is not known. Infections can be symptomatic or asymptomatic. When symptoms are present the disease is extremely variable and clinical signs may appear abruptly or insidiously. The disease typically begins with nonspecific flu-like signs such as fever, headache, back pain, malaise and generalized aches. Some patients will recover spontaneously while others develop persistent symptoms that wax and wane. Occasionally additional complications arise including, arthritis, spondylitis, chronic fatigue, anemia, internal abscesses, nephritis, endocarditis and dermatitis.

2.5 Evaluate and Prioritize Risk

Each task or activity in which specimen bank personnel can come into contact with a potential source of *Brucella* is evaluated to determine the hazard and the likelihood the hazard will occur. Table 1 outlines the hazard associated with each task by project.

Table 1. Exposure risk based on tasks or activities conducted with animal tissues in the Marine Environmental Specimen Bank.

Task or Activity	Exposure Risk			
	Hazard	Hazard Description	Likelihood	Risk
Receiving and shipping samples				
Northern Gulf of Mexico UME	Direct contact	Open containers	Moderate	Low
	Sharps	Broken glass	Moderate	High
NMMTB	Direct Contact	Open containers	Unlikely	Low
Other collections	Direct Contact	Open containers	Unlikely	Low
Collection and processing				
Collection from necropsy	Aerosols/Splash	Manipulating the carcass and tissues	Low	Low
	Direct Contact	Spill through open container	Unlikely	Low
Processing for NMMTB archive	Sharps	Cuts and knicks via knives	High	High
	Direct Contact	Through sleeves, paperwork, surface contact	High	Moderate
	Splash	Cleaning tissues, splatters during processing	High	Moderate
	Sharps	Cuts while cleaning knives	Moderate	Moderate
Cryohomogenization of tissues				
NMMTB	Splatter/Splash	Breaking jar and dislodging tissue	Moderate	Moderate
	Aerosols	Leaking during milling, manipulating frozen powder with scoops and spatula.	Moderate	Moderate
	Direct Contact	Through surface contact	Moderate	Moderate
	Splash	Cleaning mills in sink	Moderate	Moderate
Other projects	Splatter/Splash	Breaking jar and dislodging tissue	Moderate	Low
	Aerosols	Leaking during milling, manipulating frozen powder with scoops and spatula.	Moderate	Low
	Direct Contact	Through surface contact	Moderate	Low
	Splash	Cleaning mills in sink	Moderate	Low

2.6 Develop or evaluate controls to mitigate exposure

Engineering controls and workplace practices provide the primary barrier to injury or exposure while PPE provides secondary protection. Table 2 outlines the engineering and workplace controls in place to mitigate hazards related to potential *Brucella* exposure for tasks and activities in the Marine ESB.

Table 2. Hazards and recommended controls to reduce laboratory worker exposure to *Brucella*.

Task or Activity	Hazards and Recommended Controls		
	Hazard	Engineering Controls or Workplace Practices	PPE
Receiving and shipping samples			
Northern Gulf of Mexico UME	Direct contact	Absorbent paper on benchtop and disinfection of all work surfaces using 10 % bleach solution.	Standard PPE* plus N-95 respirator
	Sharps	Place broken glass in available sharps container and dispose as hazardous biological waste.	
NMMTB	Direct Contact	None	Standard PPE*
Other collections	Direct Contact	None	Standard PPE*
Collection and processing			
Collection from necropsy site	Aerosols/Splash	Personnel will not participate in active removal of tissues. Tissues are placed in clean FEP bag and sealed using a zip tie with a label then placed in a cooler on ice for transport.	Disposable field frock, washable shoes, Gloves, and N-95 respirator.
	Direct Contact		
Processing for NMMTB archive	Direct Contact	Processor will not handle paperwork. Suits are discarded after processing. Excess tissue is discarded.	Standard PPE*, N-95 respirator unless subsistence hunted animal, cut-resistant glove on non-cutting hand.
	Splash	Cleaning takes place in high sided bin, users are careful to minimize splashing/splatters.	
	Sharps	When taken apart the metal knife should be placed in a plastic container and washed with a brush. Do not use fingers to loosen screws.	
Cryohomogenization of tissues			
NMMTB	Splatter/Splash	FEP bags are placed over the open end of the jar before attempting to crack open. Dislodging is done inside a freezer typically arm's length away from the face.	Standard PPE*, N-95 respirator unless subsistence animal
	Aerosols	Mill is tightly closed using the shaker's lever; a lid is used to minimize risk from moving parts and possibly aerosols. Wait at least 5 seconds before opening the lid to allow for particles to "settle."	
	Direct Contact	None	
Other projects	Splash	Water will not be turned on directly on top of mill to minimize splashing.	Standard PPE*
	Splatter/Splash	FEP bags are placed over the open end of the jar before attempting to crack open. Dislodging is done inside a freezer typically arm's length away from the face.	
	Aerosols	Mill is tightly closed using the shaker's lever; a lid is used to minimize risk from moving parts and possible aerosols.	
	Direct Contact	None	
	Splash	Water will not be turned on directly on top of mill to minimize splashing.	

* Standard PPE (disposable Tyvek coverall [full body], open-faced hood and mask or eyes-only hood, boot covers, safety glasses, and gloves [general laboratory vinyl gloves and/or cryogenic gloves]).

3 Emergency Procedures

In the event of an injury or accident

- Call 9-911 (if needed), treat the injured, and prevent further injury/damages
- Collect information on the incident including who, what, when, where, why, and how, and if applicable, gather information from witnesses and others involved in the incident.
- Notify your supervisor or the lab lead (or if unreachable, any supervisor) and the NOAA HML Safety office as soon as possible.

In the event of a spill

- Alert people in the area.
- Cover an area twice the size of the spill with disinfectant soaked-paper towels.
- Wipe down any contaminated stationary equipment or furniture with disinfectant.
- Use forceps, tongs, or broom to remove broken glass and other items; place in sharps container or red bag.
- Remove towels and re-clean area with disinfectant solution.
- Disposable items used are to be put in the biohazard bag for incineration
- Reusable clean-up items and other reusable equipment are to be decontaminated using chemical treatment.

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Appendix A.

Animals with tissues archived in the Marine ESB that have tested positive for *Brucella* from the Deep Water Horizon (DWH) Gulf of Mexico UME. Testing was conducted using polymerase chain reaction (PCR) based assays and confirmed with culture assays. Tissues archived, may or may not include the tissue tested for *Brucella*. Tissues archived presently or in the past and their quantities are listed along with the earliest receipt date for any tissue from that animal including the present or past freezer locations. Lung LN (Lung associated lymph node), CSF (cerebrospinal fluid)

DWH Gulf of Mexico UME		Test Result	Tissues in Marine ESB	Earliest Date Received
Field Number	Tissue Tested	PCR / Culture	Tissues Archived (quantity)	
01IMMS011313	Lung Lung LN	+ / + -	Skin/Blubber (3)	17 Jul 2013
07DISL030413	Lung Lung LN	+ / + + / +	Brain, Lung LN (2), Lung, Spleen, Kidney, Liver, Skin/Blubber	17 Jul 2013
09DISL030612	Lung	+ / +	Skin/Blubber, Liver, Lung, Spleen, Kidney, Skin	17 Jul 2013
09IMMS022812	Lung	+ / +	(Lung, Spleen, Kidney, Liver, Adrenal Gland, Cerebrum, Cerebellum, Brain Stem, Thoracic Fluid, Mesenteric LN) (3) Skin/Blubber, Liver in glass, Lung in glass, Urine in glass	18 Sept 2012
23IMMS031713	Lung	+ / +	Skin/Blubber (6)	17 Jul 2013
29IMMS033113	Lung	+ / +	Skin/Blubber (3), (Lung, Spleen, Kidney, Liver) (3)	17 Jul 2013
36IMMS042213	Lung	+ / +	Skin/Blubber (3)	17 Jul 2013
39IMMS042713	Lung LN Lung Spinal Cord Brain	+ / + - - -	Skin/Blubber (3), Brain, Lung, (Viral: Lung, Spleen, Kidney, Liver, Lung LN, Brain, Adrenal Gland) (3), Skin, Brucella – PCR: CSF, Lungworms, Testicle, Pulmonary LN, Mesenteric LN	17 Jul 2013
58IMMS031211	Lung	+ / +	Swab (4), Skin (5), Brain (12), Lung (3)	28 Apr 2011
CDD-20110916-LA002	Lung LN Lung Brain	+ / + + / + -	Brain (2), Lung (2), Lung LN (2), Spleen, Adrenal Gland, Kidney, Liver, Dry Tissue	17 Jul 2013
DCA-20130619-LA001	Lung LN Lung Brain	+ / + + / + -	Skin/Blubber (2), Brain (3), Lung (2), Spleen (2), Adrenal Gland (2), Kidney (2), Liver(2)	9 Dec 2014
DCA-20130924-LA001	Spinal Cord Lung Lung LN Brain	+ / + - - -	Skin/Blubber (2), Brain (3), Lung LN (2), Lung (2), Spleen (2), Adrenal Gland (2), Kidney (2), Liver (2), CSF (2), Amniotic Fluid (2), Placenta (2), Umbilical Cord (2), Uterine Horn (2), Lungworm, Mesenteric LN	9 Dec 2014

LFH-20120131- LA001/Y12	Brain Stem/ Spinal Cord Lung Lung LN Lung Abscess Lung	+ / + - - - -	Cerebellum (2), Brain Ventricle (2)	18 Sept 2012
01DISL012413	Lung	+	Lung (3), Brain (2), Skin/Blubber	17 Jul 2013
02IMMS010912	Brainstem caseous material Whole Blood Blowhole Swab Feces Brainstem Ventral Brain Lung Lung LN	- / + - - - - - - -	(Viral: Lung, Spleen, Kidney, Liver, Adrenal Gland, Cerebrum, Cerebellum, Brain Stem, Prescapular LN (2)) (3), Serum (4), Skin, Skin/Blubber, Liver, Lung (2), Urine (2), Bone Marrow, Genital LN, Abdominal Fluid, Testicle	18 Sept 2012
05DISL022413	Lung	+	Brain (3), Lung, Spleen, Adrenal Gland, Kidney, Liver, Skin/Blubber, Spinal Cord (2)	17 Jul 2013
05IMMS012712	Lung	+	Skin/Blubber	17 Jul 2013
06IMMS030214	Amniotic Fluid Brain Fetal Tissue Uterus	+ - - -	Brain (4), Lung LN(3), Lung (4), Spleen (3), Adrenal Gland (3), Kidney (3), Liver (3), Skin/Blubber (3), Uterus, Pulmonary LN, Mesenteric LN, Amniotic Fluid (2), Umbilical Cord, Brainstem/Spinal Cord, Placenta (2)	9 Dec 2014
08IMMS012913	Lung	+	Skin/Blubber (3)	17 Jul 2013
08IMMS030210	Lung	+	Skin, Lung, Stomach, Spleen, Intestines, Heart, Blubber (2), Muscle, Kidney	28 Apr 2011
12IMMS020813	Lung	+	Skin/Blubber (3)	17 Jul 2013
13IMMS021013	Lung	+	Skin/Blubber (3)	17 Jul 2013
14IMMS031012	Lung	+	Skin/Blubber	17 Jul 2013
20IMMS031613	Lung Lung LN	+ +	Skin/Blubber (3)	17 Jul 2013
24IMMS031912	Lung	+	Skin/Blubber	17 Jul 2013
32IMMS022311	Lung	+	Blubber (2), Lung (2), Liver (2), Mixed Frozen Tissues, RNA	28 Apr 2011
35IMMS041813	Testicle CSF Brain	+ + + / +	(Viral: Brain, Lung LN, Lung, Spleen, Adrenal Gland, Kidney, Liver) (3), Lungworms, Brainstem/spinal Cord, Pulmonary LN, Mesenteric LN	17 Jul 2013

	Lung	-		
38IMMS022611	Lung	+	Swab (3), Blubber (4), Liver (5), Lung (4), RNA Extract	28 Apr 2011
40IMMS102612	Genital LN	+	Cerebrum, Cerebellum, Brain Stem, Lung (2), Spleen,	17 Jul 2013
	Amniotic Fluid	+	Adrenal Gland, Kidney, Liver, Genital LN, Amniotic Fluid,	
	Umbilicus	+	Placenta, Uterus, Skin/Blubber, Brain,	
	Fetus	-		
	Lung	-		
	Brain	-		
	Spinal Cord	-		
	Bone Marrow	-		
40IMMS042713	Uterus	+	Skin/Blubber (3), (Viral: Lung, Spleen, Kidney, Liver, Lung	17 Jul 2013
	CSF	+	LN, Brain, Adrenal Gland) (3), Brain (2), Lung, Lungworms,	
	Lung	+	Brainstem, Pulmonary LN, Mesenteric LN	
51IMMS030711	Lung	+	Guaze Swab, Skin (4), Blubber (5), Lung (5), Frozen Tissues,	28 Apr 2011
			Skull, RNA Extract	
62IMMS031511	Lung	+	Mixed Tissue Samples, Swabs (3), Meconium, Skin, Adrenal	28 Apr 2011
			Gland (4), Lung (4), Brain (12)	
65IMMS031711	Lung	+	Mixed Frozen Samples, Whole Stomach, Blubber (4), Lung	28 Apr 2011
	Lung LN	+	(5), Kidney (3), Liver (5), Aqueous Humor (2) Swab (3),	
			Feces (2), Spleen, Lung LN (2), RNA Extracts	
66IMMS031811	Lung	+	Swabs (4), Skin (3), Skin Lesion (6), Pulmonary LN (5),	28 Apr 2011
			Liver (10), Adrenal Gland (5), Spleen (5), Thymus (4), Lung	
			(4), Kidney (4), Blubber (5), Feces (5), Small Intestine, Brain,	
			Cerebrum, Cerebellum, Midbrain	
70IMMS032111	Lung	+	Swab (2), Skin (3), Brain (4), Adrenal Gland (4), Liver (5),	28 Apr 2011
			Spleen (4), Lung (4), Meconium	
77IMMS032811	Lung	+	Brain (12), Spleen (4), Adrenal Gland (4) Liver (5), Lung (4),	22 Jul 2011
			Skin, Brain Swab, Meconium	
BP-2010-LA-168	Spinal Cord	+	Gauze Swab (2), Skin, Plasma (3), Serum (40), Blood (2),	16 Nov 2010
	Lung	-	Liver (2), Lung (2), Blubber (2), Urine (2), RNA Extract,	
			Spinal Cord	
CCC-20120117-LA001	Lung	+	Spleen (2), Kidney (2), Brain (2), Adrenal Gland (2), Lung	18 Sept 2012
	Lung LN	+	LN (2), Lung (3), Liver (3), Skin/Blubber, Urine in glass	
	Brain	-		
CES-20110218-LA001	Lung	+		22 Mar 2011

	Skin	-	Skin, Stomach, Kidney (2), Liver (2), Lung (2), Spleen (2), Trachea, Brain (3), Mesenteric LN, Lung LN (2), Blubber (2), Muscle, Feces, Umbilicus, RNA Extracts (4)	
JSH-20130318-LA002	Lung Brain Stem	+ +	Skin/Blubber (2), Lung (2), Brain, Adrenal Gland, Kidney, Liver	17 Jul 2013
JSH-20130404-LA001	Lung	+	Skin/Blubber, Lung (2), Adrenal Gland, Spleen, Kidney, Brain (5), Liver	17 Jul 2013
JSH-20130417-LA001	Spinal Cord Testicle Lung	+ - -	Skin/Blubber (2), Brain (4), Kidney, Liver (2), Lung LN, Lung (3), Spleen, Adrenal Gland, Lungworms, Skin Lesion (3), Spinal Cord, Pulmonary LN, Spinal Cord, Skin Lesions (3), Testes	17 Jul 2013
JSH-20130429-LA001	Lung	+	Skin/Blubber, Lung, Skin, Blubber (2)	23 Sept 2014
MCT-20101203-LA001	Lung	+	Mixed Frozen Tissues, Culturette of Lung, Liver, Spleen, Kidney, Feces	18 Mar 2011
MCT-20110228-LA001	Pulmonary LN CSF Lung	+ + -	Kidney, Lung (2), Liver, Leeches, Blubber (2), Muscle, CSF, Urine, Blood, Bile, Stomach, Stomach Contents, Peritoneal Fluid, Mid Peduncle Granuloma, Spleen, Trachea, Mesenteric LN, Pulmonary LN (2), Cervical LN, Skin, Otoliths, RNA Extract (4)	11 Mar 2011
MCT-20110321-LA001	Lung	+	Blubber	18 Dec 2014
MRB-20110315-LA001	Lung Lung LN	+ / + +	Mixed Tissue Samples, Whole Stomach, Skin, Skin/Blubber, RNA Extract (3)	18 Mar 2011
MSB-20110501-LA001	Lung Lung LN Brain	+ - -	Brain (2), Lung LN (2), Lung (2), Spleen, Adrenal Gland, Kidney, Liver, Skin/Blubber	17 Jul 2013
RIB-20110111- LA002/LA441	Lung	+	Swabs (3), Liver, Kidney, Muscle, Stomach Contents, Blubber, Lung (3), Feces, Urine, Blood, Intestine, Mixed Frozen Samples, RNA Extract	18 Mar 2011