

NIST Internal Report
NIST IR 8555

The National Marine Mammal Tissue Bank

Updated Collection, Processing, and Archive Protocols

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Abstract

The National Marine Mammal Tissue Bank (NMMTB) was initiated in 1987 by the National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) to collect select marine mammal specimens for a long-term cryogenic archive. In 1992, the NMMTB was formally established by Federal Legislation (Public Law 102-587) and a portion of specimens collected by NMFS's Marine Mammal Health and Stranding Response Program (MMHSRP) are archived in the NMMTB. The MMHSRP obtains specimens from marine mammal strandings and from animals taken incidentally during commercial fishing operations and emphasizes contaminant monitoring and information management. Specimens from Alaska are archived in the NMMTB through the Alaska Marine Mammal Tissue Archival Project (AMMTAP). AMMTAP was initiated in 1987 with sponsorship from the U.S. Department of the Interior, former Minerals Management Service (MMS). The goal of the AMMTAP is to establish a representative collection of marine mammal specimens collected during Alaska Native subsistence activities for future contaminant analyses and documentation of long-term trends in environmental quality. NIST manages and maintains specimens for both AMMTAP and MMHSRP, and although they are identified as separate projects, the specimen processing and archiving protocols are identical. This report will describe the updated protocols for specimen collection and processing for the NMMTB, as well as specimen accessioning once received at the NIST Biorepository to be archived in the NMMTB. The cryogenic banking facilities for the NMMTB are operated by the NIST Chemical Sciences Division, Biospecimen Science Group, at the NIST Biorepository in Charleston, South Carolina, at the Hollings Marine Laboratory.

Keywords

Archive; Biorepository; Biospecimen Science; Marine Mammal; Protocol

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1. THE ROLE OF NIST IN ENVIRONMENTAL SPECIMEN BANKING

The National Institute of Standards and Technology (NIST) has been involved in long-term biological and environmental specimen banking for over 45 years. In 1979, NIST established the National Biomonitoring Specimen Bank (NBSB) in Gaithersburg, Maryland, as a direct result of a pilot environmental specimen bank program that was sponsored by the Environmental Protection Agency (EPA) (Wise and Zeisler, 1985). As part of this collaboration with the EPA, NIST developed standardized protocols for the collection, processing, and storage of specimens (Wise and Zeisler, 1984). NIST expanded specimen banking efforts beyond the EPA program of banking human livers to include specimens from the marine environment, including sediment, oysters, fish, and other marine animal tissue and fluids in collaboration with the National Oceanic and Atmospheric Administration (NOAA), other government agencies, and organizations. NIST continues many of these collaborations, particularly as they relate to marine animal research by improving the quality of exogenous contaminant measurements through analytical method development and application, quality assurance activities, expansion of environmental specimen banking activities, and the promotion of beneficial collaborative interdisciplinary research among other partners.

In 2002, NIST was established as a partner at the Hollings Marine Laboratory in Charleston, South Carolina, with NOAA, the South Carolina Department of Natural Resources, the Medical University of South Carolina, and the College of Charleston. NIST developed a satellite specimen bank facility, the Marine Environmental Specimen Bank (Marine ESB) to increase the storage capacity of the NBSB with a focus on banking specimens from the marine and coastal environment. NIST operated both the NBSB and Marine ESB as a collaborative facility overseeing the legacy and active biobanking projects for many years. NIST personnel from both facilities collaborated in specimen bank project planning, development of standardized banking protocols, and day-to-day operations. In 2010, the NBSB permanently closed and over several years, all specimens were transferred to the Marine ESB. To reflect the variety of specimens archived, which included non-marine related specimens, NIST formally changed the facility name to the NIST Biorepository in 2019. The management of the NIST Biorepository is led by the Biospecimen Science Group of the Chemical Sciences Division.

1.1. Alaska Marine Mammal Tissue Archival Project (AMMTAP)

In 1987, NIST began a project in collaboration with NOAA's Outer Continental Shelf Environmental Assessment Program to collect and bank marine mammal specimens from Alaskan species, particularly in areas of potential coastal development by the oil and gas industry. This project, the Alaska Marine Mammal Tissue Archival Project (AMMTAP), was supported by the U.S. Department of the Interior, former Minerals Management Service (Becker *et al.*, 1988). The project was conducted as a collaboration with NOAA's National Marine Fisheries Service (NOAA/NMFS), the U.S. Geological Survey's Biological Resources Division (USGS/BRD) and NIST. The original goal of AMMTAP was to establish a representative collection of marine mammal specimens for future contaminant analyses and to document long-term trends in environmental quality (Zeisler *et al.*, 1992). Initial specimens were collected

during Alaska Native subsistence harvests, but today the AMMTAP also includes specimens collected by the marine mammal stranding and research communities.

1.2. Marine Mammal Health and Stranding Response Program (MMHSRP)

In 1990, the NOAA/NMFS initiated the MMHSRP to coordinate the response to stranded marine mammals, which consists of four components: 1) Specimen Bank, 2) Stranding Network, 3) Monitoring, and 4) Quality Assurance (Becker, et al., 1994). In 1992, the National Marine Mammal Tissue Bank (NMMTB) was formally established by Federal Legislation (Public Law 102-587). This legislation was the direct result of the mass mortality of bottlenose dolphins that occurred between 1987-1988 on the U.S. Atlantic coast (Scott et al., 1988). Although an algal toxin was implicated as a causative factor, high levels of polychlorinated biphenyl ethers (PCBs) and chlorinated pesticides were also found in these dolphins. It was not known if these levels were normal for these animals, or what role these chemicals might have had in their deaths (Geraci 1989). A baseline of contaminant levels was needed for marine mammals, but no specimens were available for analysis. It was assumed that new contaminants would likely be identified in the future and understanding bioaccumulation and biotransfer of these compounds would be needed to understand trends over time and health risks to marine mammals. Thus, a specimen banking and chemical monitoring program was established, and a portion of specimen was collected by NMFS's MMHSRP, as well as the specimens collected by AMMTAP, were archived as a part of the NMMTB.

NIST manages and maintains specimens for both AMMTAP and MMHSRP, and although they are identified as separate projects, the specimen processing and archiving protocols are identical. This report will describe the protocols for NMMTB specimen collection and processing, as well as accessioning and archiving once specimens are received at the NIST Biorepository.

1.3. The National Marine Mammal Tissue Bank (NMMTB)

The NMMTB was formally established by Federal Legislation (Public Law 102-587) in 1992 and is the longest collaborative project with NOAA that remains active today (Lillestolen et al., 1993). Under Title IV (4), Section 407 of the Marine Mammal Protection Act (MMPA), (16 USC 1421f), these tissues/fluids will be archived in the NMMTB. The majority of specimens are located in the NIST Biorepository in Charleston, SC. Following biobanking best practices, select duplicates of marine mammal specimens were transferred to the NIST Office of Reference Materials Standard Reference Material (SRM) Warehouse in Gaithersburg, MD, for permanent archive, serving as a secondary storage location.

In 1992, the NMMTB program was formally established by the Marine Mammal Health and Stranding Response Act (Public Law 102-587) and was expanded and combined with the Marine Mammal Stranding Network to become a larger program that resulted in several components: Stranding Networks, the NMMTB, and Monitoring and Quality Assurance. This expansion is now known as the Marine Mammal Health and Stranding Response Program (MMHSRP) and is coordinated by the NMFS in cooperation with the USFWS. 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. The NMMTB and the quality assurance program are administered by NIST. In 1995, the quality assurance program was formalized and became the National Marine Analytical Quality Assurance Program (NMAQAP).

2. SPECIMEN COLLECTION PROTOCOL

The updated collection protocols described below for the NMMTB are modifications of those previously developed by NIST, including the EPA human liver pilot program (Harrison et al., 1981; Zeisler et al., 1983a), NOAA's National Status and Trends program for the collection of fish, bivalves, and sediment specimens (Lauenstein, 1986), the AMMTAP (Becker et al., 1988; 1991), and the original protocols for the NMMTB (Lillestolen, et al., 1993 and Becker et al., 1999). The intent of the collection protocol is to ensure the highest quality specimen is archived. This is accomplished by obtaining fresh specimens uncontaminated by external sources, and packaging and transporting as quickly as possible under conditions that eliminate or minimize degradation prior to storage. When the NMMTB protocol was developed, liver, kidney, blubber, muscle, and brain were collected and archived. Today only liver, kidney, and blubber are collected, and specimens must meet specific criteria to be processed and archived as part of the NMMTB, as described in Becker et al., 1999. Animals designated as necropsy Code 2 from Marine Mammals Ashore (Geraci and Lounsbury, 2005) are acceptable for the NMMTB.

From 1987 to 2024, ~300 g of specimen was collected for the NMMTB and divided into two, 150 g portions: specimen "A" and specimen "B." Due to advanced analytical techniques and smaller size requests, in 2025 total specimen size was reduced from 300 g to between 200 and 150 g for each specimen type; liver, kidney, and blubber. After collection, the specimens are then divided into two, ≤ 75 g portions: an "A" specimen and "B" specimen. Specimen "A" is collected for long-term storage (i.e., decades) and specimen "B" is designated to be processed into homogenous aliquots that can be requested. Although Appendix D, NMMTB Specimen Access Policy, indicates that 150 g for specimens are collected for "A" and "B," smaller sizes are acceptable for the NMMTB.

Specimen storage and inventory procedures are in accordance with those routinely performed at the NIST Biorepository, including storage under liquid nitrogen (LN₂) vapor-phase temperatures (≤ -150 °C). NIST and NOAA work together to identify NOAA researchers, state and local government institutions, non-profit organizations, and academic institutions authorized/permitted to collect specimens for the AMMTAP and MMHSRP, and willing to collect and process specimens for the NMMTB. To ensure conformance to the standardized protocol for quality control, the NIST Biorepository staff provide formal training, documentation outlining the processing steps, and all collection and processing supplies.

Specimen collection is completed in three stages: specimen removal, specimen processing to obtain representative portion, and packaging/shipping. The division of the protocol into stages is used as an aid in organizing and simplifying NMMTB collection procedures.

2.1. Required Supplies and Materials

Supplies and materials required for processing NMMTB specimens are provided by the NIST Biorepository. It is imperative for quality assurance purposes that only these supplies and materials are used for processing.

Basic supply list:

- Adhesive, chemical resistant polytetrafluoroethylene (PTFE) cover
- Cutting board
- Disposable field frock(s)
- Perfluoroalkoxy (PFA) jars with recessed lid; 180 mL, 90, mL, or 60 mL
- Specimen jar side labels
- Clear, cryogenic lab tape for securing jar side labels
- Lid label tabs
- Non-powdered vinyl co-polymer gloves
- Cut resistant glove(s)
- Fluorinated ethylene propylene (FEP) sheets
- FEP bags for specimen collection and transport from the field to the processing facility
- Label tags & zip ties
- Lint free cloths
- Carboy, or bottle, of 18.2 resistivity (M Ω) ultra-pure water
- Titanium blade/ Polytetrafluoroethylene (PTFE) handled knife(s)
- 1 PFA squirt bottle for ultra-pure water
- 1 PFA squirt bottle for high grade ethanol
- Shipping labels and supplies
- Biological dry shipper(s) (LN₂ cooled) with outer protective shipping container
- NMMTB datasheet(s)
- NMMTB training poster

Supplies available upon request include:

- Insulated gloves for handling frozen specimens
- Safety glasses
- Balance for weighing

Additional supplies to be shipped directly from the manufacture:

- 200 proof, ACS grade ethanol
- Isopropyl alcohol cleaning spray

As supplies are depleted, contact the NIST Biorepository for replenishment and/or replacement.

2.2. Stage I. Specimen Removal

The removal of specimens from marine mammals may occur under different environmental conditions: in the field, in a necropsy laboratory, etc. Necropsy and specimen sampling procedures may vary across institutions and organizations depending upon the species, but the same NMMTB protocol should be implemented by all organizations when sampling individuals of the same species. This protocol will only describe the steps associated with the removal of blubber, liver, and kidney specimens for submission to the NMMTB.

Basic Considerations:

- No animal will be considered a candidate for sampling if specimens cannot be collected within the specified time after death and/or if handling of the animal has not followed protocol criteria or cannot be documented. Animals designated as NOAA Condition Code 2 (fresh dead, or no rigor) are acceptable for the NMMTB.
- The anatomical location of specimen removal is specified to maintain consistency and comparability between the same specimen types of the species.
- The size of the specimen removed from an animal should be sufficient to provide two (2) specimens of approximately 75 g each for archival; approximately 150 to 200 g total. This quantity is required to provide sufficient material for long-term storage as well as aliquots for periodic analysis. If there is not enough specimen to collect 150 to 200 g, smaller sizes are acceptable.
- Clean, non-powdered vinyl co-polymer gloves are used by all personnel during specimen removal and handling. Caution must be taken throughout the procedures to reduce the risk of chemical contamination. Contamination may originate from the individual performing the work, the atmosphere, the skin of the animal, the instruments used in the dissection, and any chemicals in the area where the work is being performed. While in the field, best efforts should be made to wear field frocks to prevent contamination.

2.2.1. Procedures for Specimen Removal

The following procedure has been adapted from Fay, *et al.*, 1979. As cetaceans are the most commonly sampled taxon within the NMMTB, the following procedures are written for cetacean specimen removal, though some notes on other taxa are included. The required NMMTB datasheet is attached (Appendix A) and instructions for completing the datasheet, including definitions and explanations, can be found in Appendix B.

- Record locality details, time taken, and other pertinent data from the individual animal on the NMMTB datasheet.
- Record all animal measurements required on the NMMTB datasheet.
- Confirm and record the animal's sex.

- Ideally, cuts should be made using high-quality stainless-steel dissection tools, or NIST provided titanium knives which are cleaned following NIST protocols, or rinsed in the NIST provided ultra-pure water and ethanol in specific cases discussed below.
- For Blubber thickness measurements, use a stainless-steel instrument to make an incision through the skin and blubber on the dorsal side, anterior to the dorsal fin or mid-line and posterior to the blowhole, to measure the blubber thickness (mm).
- Remove the specimens as soon as possible after opening the body cavity.

2.2.2 Blubber Removal - The anatomical site of blubber removal will depend on the distribution of fat layers on the animal; therefore, it will be rather species specific. For cetaceans, make an incision with a titanium knife or stainless-steel blade, anterior to the dorsal fin, or midline, and posterior to the blowhole. Make two more cuts perpendicular to the first incision near the fin, or midline, and blowhole respectively on the left side of the animal (i.e., a three-sided flap of skin is cut).

- For larger animals during necropsy, if the left side is unavailable, the right side is suitable for sampling and should be noted on the NMMTB datasheet. For all other marine mammal species (pinnipeds, fissipeds) consult the respective organization's necropsy protocols and procedures for removing specimens. Marine Mammals Ashore (Geraci and Lounsbury, 2005) is a recommended reference tool.
- When possible, use the titanium knife to remove a section of blubber from the muscle layer. If a stainless-steel scalpel is used to remove the blubber section, the layer of muscle and blubber sampled that may have been touched by the scalpel blade will be removed during processing. Place the blubber in a clean and labeled FEP bag for immediate transport to the processing area. If a scalpel was used instead of a titanium knife, remove all edges and layers where the stainless-steel blade touched the specimen during processing using the titanium knife provided. After the blubber is separated from the muscle, separate the blubber from the skin in the same manner. Note the general appearance of the blubber before including any unusual coloration, texture, parasites, etc., and record observation on the NMMTB datasheet. A total of 150 to 200 g of blubber is ideal for "A" (≤ 75 g) and "B" (≤ 75 g) specimens.

2.2.3 Liver Removal - Note the general appearance of the liver before removal including any unusual coloration, texture, shape, etc., and record observation on the NMMTB datasheet. Using a stainless-steel instrument or titanium knife, remove the entire liver and place it in a clean and labeled FEP bag. If the entire liver cannot be removed, use a titanium knife to remove a 150 g to 200 g section from both sides of the medial indentation at the distal end of the liver and place in a clean FEP bag for immediate transport to the processing area. If a stainless-steel instrument was used instead of a titanium knife to remove the specimen, remove all edges and layers where the stainless-steel blade touched the specimen using the provided titanium knife. A total of 150 to 200 g of liver is ideal for "A" (≤ 75 g) and "B" (≤ 75 g) specimens.

2.2.4 Kidney Removal - Note the general appearance of the kidney before removal, including any unusual coloration, texture, shape, etc., and record observation on the NMMTB datasheet. Using a stainless-steel instrument or titanium knife, remove kidney(s) and place in a clean and labeled FEP bag. If a stainless-steel instrument was used instead of a titanium knife to remove the specimen, remove all edges and layers where the stainless-steel blade touched the specimen using the provided titanium knife. A total of 150 to 200 g of kidney is ideal for “A” (≤ 75 g) and “B” (≤ 75 g) specimens.

- For smaller species (e.g., seals and sea otters, pups and calves), remove both kidneys from the animal and place in a clean FEP bag for immediate transport to the processing area. Both kidneys are required to provide a total specimen size of 150 g. Note the left and the right kidney when labeling the specimens. Attachments of the kidney may be cut using surgical scissors that have been previously rinsed with ethanol from the PFA bottle.
- For larger species (e.g., sea lions and cetaceans), remove the left kidney from the animal and place in a clean FEP bag for immediate transport to the processing area. Attachments of the kidney may be cut using surgical scissors that have been previously rinsed ethanol from the PFA bottle.
- If the entire kidney is too large to be transported back to the processing area, remove a 150 to 200 g section from the posterior end of the left kidney using the titanium knife and place in a clean FEP bag for transport.

Following the removal of the NMMTB specimens, any additional specimens collected for other researchers and measurements taken should be included as requested on the NMMTB datasheet.

Special Consideration - If any deviations occur during specimen removal, they should be noted on the NMMTB datasheet. Examples include:

- Removal of specimen using anything other than stainless-steel blades (scalpel)
- Removal of specimen from alternate site; right side vs. left side sampling
- Adverse weather conditions
- Potential external sources of contamination
- Gloves worn other than NIST provided gloves
- Pre-freezing and thawing prior to processing

In some cases, specimens collected for the NMMTB are not able to be processed immediately by the collector and, if arranged ahead of time with a NIST researcher, may be sent to the NIST Biorepository for processing. Specimens should be clearly labeled with identification numbers, species or common name, and collection date. Unprocessed specimens should be shipped

frozen to the NIST Biorepository in FEP bags secured with zip ties and labels. Appropriate necropsy forms should also accompany the unprocessed specimens.

2.3. Stage II. Specimen Processing

Specimen processing should occur under laboratory conditions when possible: cleanroom, HEPA filtered hood, shipboard laboratories, etc. (Pugh et al., 2007). It is acceptable to process specimens for the NMMTB in an outdoor setting, but conditions should be noted on the NMMTB datasheet. At a minimum, the specimen processing should take place in a covered and enclosed area free of obvious sources of contamination such as cigarette smoke, fuel oil fumes and smoke, laboratory formaldehyde, etc. The processing area should be cleaned to remove dust, and the working surfaces covered with PTFE surface sheeting. See Figure 1. In addition, only titanium knives provided by NIST are to be used to cut specimens during Stage II. Titanium knife cleaning procedures can be conducted in the field if multiple specimens are processed, and details are presented in the section below.

Ideally, the NMMTB processing should be completed with at least two trained personnel. This allows for minimal and/or no additional contamination of the specimen(s) and quicker processing times of specimen(s).

2.3.1. Specimen Processing Set-Up

- Don the provided field frock while taking care to keep the garment as clean as possible.
- While wearing clean, non-powdered vinyl co-polymer gloves, cover the cutting board with the PTFE adhesive, chemical resistant sheeting. If needed, two layers of sheeting may be used. Once covering is complete, spray isopropyl alcohol, or ethanol, onto the cutting surface and wipe with a lint free cloth.
- Place a lint free cloth on one of the top corners of the cutting board, then place a clean titanium knife onto the cloth. If needed, cover the titanium knife with another lint free cloth until processing begins.
- Gather PFA jars, side and lid labels, lab tape, scale, NMMTB datasheet, and indelible pen or fine-tipped marker.
- While taking care to not contaminate the straw inside the PFA squirt bottle, fill the PFA squirt bottles with their respective liquids; one for ethanol and the other for NIST provided ultra-pure water. Be sure to label the bottles with the appropriate liquid.
- While wearing a clean pair of non-powdered vinyl co-polymer gloves, place an FEP sheet onto the clean, covered cutting board surface.



Figure 1. NMMTB Specimen Processing Set-Up

2.3.2. Blubber Processing

- Fold the edges of the FEP bag away from the blubber specimen. While wearing clean, non-powdered vinyl co-polymer gloves, remove the blubber specimen from the FEP bag. Hold the blubber specimen over a sink or bin and rinse the surface with ultra-pure water. Pour, or squirt, enough ultra-pure water from the bottle onto the blubber specimen to wash off blood, other fluid, or debris. Rub the blubber specimen with a gloved hand if necessary to remove blood, etc. Allow the blubber specimen to drain for several seconds to prevent dripping on surfaces. Place the flat, rinsed blubber specimen on the clean FEP sheet on the cutting board and discard the used FEP bag.
- As a safety precaution, a cut resistant glove is provided. The specimen processor should put on a pair of non-powdered vinyl co-polymer gloves, then the cut resistant glove on the non-dominant hand, and cover it with one, or two, additional non-powdered vinyl co-polymer gloves to prevent contamination from the cut resistant glove.
- Use the titanium knife to remove any remaining portions of muscle or skin attached to the blubber specimen and trim the edges that were cut with the stainless-steel implements. Also, any specimen that came in contact with a contaminant (e.g., animal hair, hemostats, etc.), should be excised and discarded. Cut the remaining specimen into smaller pieces for distribution into the storage jars.
- Tare the mass of one pre-cleaned PFA jar. Place approximately 75 g of blubber into the jar and secure the lid tightly. Label appropriately using the side label, securing it with the roll of tape provided and ensuring the tape is wrapped around the jar and on itself tightly at least three times. Clearly indicate the Animal ID, Tissue Type, weight in grams, and "A" on the lid label. Ensure the lid label is placed in the recessed slot on the top of

the lid. Record the mass of the specimen collected on the NMMTB datasheet. This is “A” specimen.

- Repeat the procedure above to obtain the “B” specimen indicating “B” on the side and lid labels.
- Freeze immediately at -70 °C or lower, preferably LN₂ vapor-phase temperature ($\leq$ -150 °C).
- Upon completion of the blubber processing, rinse the titanium knife with ultra-pure water and remove all blood and fluids from the knife, using a lint-free cloth, if necessary, before it has time to dry.
- If additional specimens are to be processed from the same animal, rinse the titanium knife with ethanol, allowing the ethanol to run down the blade of the knife. Place the knife on a lint free cloth, cover it with an additional lint free cloth, and allow it to air dry. Use a clean, new knife if specimens from a different animal are to be processed. Should another knife not be available, clean the titanium knife as well as possible before proceeding noting the modification on the NMMTB datasheet.
- While wearing clean, non-powdered vinyl co-polymer gloves, squirt ultra-pure water onto the cutting surface and wipe clean. Using a lint-free cloth, wipe away any fluids and discard unused specimen appropriately. Repeat wiping until the cutting surface is clean. As a final step, spray isopropyl alcohol onto the cutting surface and wipe clean.



Figure 2. NMMTB Blubber Processing

2.3.3. Liver Processing

- Start with a clean cutting surface. Fold the edges of the FEP bag away from the liver specimen. While wearing clean, non-powdered vinyl co-polymer gloves, remove the liver from the FEP bag, being cautious not to touch the liver to the outside of the bag. Hold the liver specimen over a sink, or bin, and rinse the surface with ultra-pure water

from the PFA bottle. Pour, or squirt, enough ultra-pure water from the bottle onto the liver specimen to wash off blood, or debris. Rub the liver specimen with a gloved hand if necessary to remove blood and/or debris. Allow the liver specimen to drain for several seconds to prevent dripping. Place the rinsed liver specimen on the clean FEP sheet on the cutting board and discard the used FEP bag.

- As a safety precaution, a cut resistant glove is provided. The specimen processor should put on a pair of non-powdered vinyl co-polymer gloves, then the cut resistant glove on the non-dominant hand, and cover it with one, or two, additional non-powdered vinyl co-polymer gloves to prevent contamination from the cut resistant glove.
- If the whole liver was transported to the processing facility, remove approximately 150 g to 200 g from the distal end of both sides of the medial indentation using a titanium knife. Trim the edges that came in contact with other materials (e.g. scalpel blade) using the titanium knife provided and dissect into two equal parts; one section for specimen "A" and the other for specimen "B". Cut the liver into smaller pieces for distribution into the storage jars.
- Tare the mass of one pre-cleaned PFA jar. Place approximately 75 g of liver into the jar and secure the lid tightly. Label appropriately using the side label, securing it with the roll of tape provided and ensuring the tape is wrapped around the jar and on itself at least three times. Clearly indicate the Animal ID, Tissue Type, weight in grams, and "A" on the lid label. Ensure the lid label is placed in the recessed slot on the top of the lid. Record the mass of the specimen collected on the NMMTB datasheet. This is specimen "A."
- Repeat the procedure above to obtain specimen "B" indicating "B" on the side and lid labels.
- Freeze the jars containing the specimens immediately at -70 °C or lower, preferably LN₂ vapor-phase temperature ($\leq$ -150 °C).
- Upon completion of the liver processing, rinse the titanium knife with ultra-pure water and remove all blood and fluids from the knife, using a lint-free cloth if necessary, before it has time to dry.
- If additional specimens are to be processed from the same animal, rinse the titanium knife with ethanol, allowing the ethanol to run down the blade of the knife. Place the knife on a lint free cloth, cover it with an additional lint free cloth, and allow it to air dry. Use a clean, new knife if specimens from a different animal are to be processed. Should another knife not be available, clean the titanium knife as well as possible before proceeding noting the modification on the NMMTB datasheet.
- While wearing clean, non-powdered vinyl co-polymer gloves, squirt ultra-pure water onto the cutting surface and wipe clean. Using a lint-free cloth, wipe away any fluids or extra specimen and discard. Repeat wiping until the cutting surface is clean. As a final step, spray isopropyl alcohol onto the cutting surface and wipe clean.



Figure 3. NMMTB Liver Processing

2.3.4. Kidney Processing

- Start with a clean cutting surface. Fold the edges of the FEP bag away from the kidney specimen. While wearing clean, non-powdered vinyl co-polymer gloves, remove the kidney specimen from the FEP bag. Hold the kidney specimen over a sink, or bin, and rinse the surface with ultra-pure water. Pour, or squirt, enough ultra-pure water from the bottle onto the kidney specimen to wash off blood, other fluid, or debris. Rub the kidney specimen with a gloved hand if necessary to remove blood, etc. Allow the kidney specimen to drain for several seconds to prevent dripping on surfaces. Place the rinsed kidney specimen on the clean FEP sheet on the cutting board and discard the used FEP bag.
- As a safety precaution, a cut resistant glove is provided. The tissue processor should put on a pair of non-powdered vinyl co-polymer gloves, then the cut resistant glove on the non-dominant hand, and cover it with one, or two, additional non-powdered vinyl co-polymer gloves to prevent contamination from the cut resistant glove.
- For smaller species where the left and right kidneys are collected, each kidney should approach the weight recommendation for each specimen, approximately 75 g, and no additional subsampling will be necessary. The right kidney will provide specimen “A” and the left kidney will provide specimen “B.” If additional cutting is required, or if cutting of the specimen is necessary to fit the specimen into the jar, use the titanium knife to remove a section from the kidney.
- For larger species where the left kidney was collected, trim the edges of the kidney that came in contact with other materials using the titanium knife provided.
- Tare the mass of one pre-cleaned PFA jar. Place approximately 75 g of kidney into the jar and secure the lid tightly. Label appropriately using the side label, securing it with the

roll of tape provided and ensuring the tape is wrapped around the jar and on itself tightly at least three times. Use only the NIST provided tape to ensure it stays adhered to the jar once stored at cryogenic temperatures. Clearly indicate the Animal ID, Tissue Type, weight in grams, and specimen "A" or specimen "B" on the lid label, Figure 4. Ensure the lid label is placed in the recessed slot on the top of the lid. Record the mass of the specimen collected on the NMMTB datasheet. This is specimen "A."

- Repeat the procedure above to obtain specimen "B."



Figure 4. NMMTB Lid Label

- Freeze each specimen immediately at -70 °C or lower, preferably LN₂ vapor-phase temperature (≤ -150 °C).
- Upon completion of the kidney processing, rinse the titanium knife with ultra-pure water and remove all blood and fluids from the knife, using a lint-free cloth if necessary, before it has time to dry.
- If additional specimens are to be processed from the same animal, rinse the titanium knife with ethanol, allowing the ethanol to run down the blade of the knife. Place the knife on a lint free cloth, cover it with an additional lint free cloth, and allow it to air dry. Use a clean, new knife if specimens from a different animal are to be processed. Should another knife not be available, clean the titanium knife as well as possible before proceeding noting the modification on the NMMTB datasheet.
- While wearing clean, non-powdered vinyl co-polymer gloves, squirt ultra-pure water onto the cutting surface and wipe clean. Using a lint-free cloth, wipe away any fluids and discard unused specimen appropriately. Repeat wiping until the cutting surface is clean. As a final step, spray ethanol onto the cutting surface and wipe clean.



Figure 5. NMMTB Kidney Processing

2.3.5. Clean-up and waste disposal

Upon completion of NMMTB processing, discard disposable soiled items (i.e., lint-free wipes, non-powdered vinyl co-polymer gloves, FEP sheets & bags, and field frock(s)). Remove the protective covering from the cutting board and discard it. Clean the cutting board with mild soap and water, if necessary, and allow to air dry. Used titanium knives should be shipped back to the NIST Biorepository for cleaning and sharpening. In the event the cut-resistant glove is soiled, rinse the glove with water and scrub with a mild soap. Allow the cut resistant glove to air dry for reuse. Follow the appropriate waste disposal procedures for biological and/or hazardous waste for the appropriate agency/institution.

The process of packing and shipping should be relatively standard and not vary between species or specimen, unless previously discussed with the NIST Biorepository staff. Specimens ready to be archived in the NMMTB are generally shipped using LN₂ vapor-phase ($\leq -150\text{ }^{\circ}\text{C}$) dry shippers. Shipments must be coordinated in advance with the NIST Biorepository to ensure specimens are received in a timely manner.

- Check the NMMTB datasheet for completeness and accuracy. Any deviations from or modifications to the protocol must be noted on the NMMTB datasheet.
- Ensure jar label and lid label are secured to each specimen jar prior to adding to shipper.
- Upon request to the NIST Biorepository, a charged LN₂ vapor-phase ($\leq -150\text{ }^{\circ}\text{C}$) dry shipper can be provided for shipping the processed specimens to the NIST Biorepository. Personnel handling LN₂ vapor-phase charged biological shippers are advised to wear closed-toed shoes, cuff-less trousers, non-absorbent aprons, loose insulating gloves, and safety glasses. Alternatively, specimens may also be shipped in a

well vented cooler with adequate dry ice to remain frozen for the length of the shipment.

- Once the LN₂ vapor-phase dry shipper is received, remove the specimens from the freezer and place inside dry shipper (Fig. 5).
- Place the completed NMMTB datasheet(s), Level A datasheets and necropsy notes (if applicable) and appropriate marine mammal collection permit(s) inside the outer protective shipping container that houses the dry shipper.
- Immediately make arrangements with the appropriate shipping courier to return the shipper to the NIST Biorepository using the provided shipping label.



Figure 6. Liquid Nitrogen Vapor-Phase Dry Shipper and Outer Protective Shipping Container

- Used titanium knives can be carefully wrapped and included in the outer shipping container for cleaning and sharpening at the NIST Biorepository.
- Closure instructions are included with the outer protective container.
- Specimens should be shipped as soon as possible using express package service to:

NIST Biorepository
331 Fort Johnson Road
Charleston, SC 29412
- Avoid scheduling shipments for late in the week (i.e., Thursday or Friday) or before holidays, unless special arrangements have been made with the shipping service and the NIST Biorepository staff.
- Notify a NIST Biorepository staff member as soon as possible after the specimens have shipped.

3. ARCHIVING NMMTB SPECIMENS

The NIST Biorepository uses a specimen management system which curates specimen storage locations and metadata, including aliquot use history for all biobanking projects, including the NMMTB.

After an LN₂ vapor-phase biological dry shipper containing processed NMMTB specimens is received on site, the specimens are inspected to ensure they are frozen and properly labeled. Specimen label information is verified to ensure it corresponds to the information on the completed NMMTB datasheet(s) provided by the field collector. All specimens are temporarily placed inside a stainless-steel basket in an LN₂ vapor-phase freezer until a permanent archival location is determined. Each specimen incorporated into the NMMTB is assigned a NIST storage identification number according to the diagram shown below.

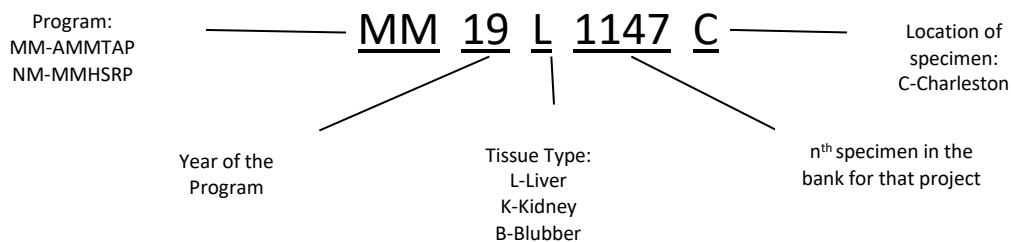


Figure 7. NMMTB Storage ID

Prior to January 1, 2018, NIST storage datasheets were completed to track physical specimen location(s) within a freezer. However, the practice of creating NIST storage datasheets was discontinued in 2018, and specimens are currently tracked using an electronic sample management database system. An example of the archived NIST storage datasheet is available in Appendix C.

After an NMMTB Storage ID has been assigned, a permanent storage location is chosen by determining the next location available in the freezer according to the database. Next, each jar will receive a barcoded label encoded with the “globally unique aliquot ID” affixed to the lid label.

The PFA jars are placed in pre-labeled cardboard tubes according to their designation; “A” or “B.” Specific freezers are designated as an “A” or “B” freezer in accordance with the International Society of Biological and Environmental Repositories (ISBER) best practices (Snapes *et al.*, 2023) to prevent total specimen loss in the event of a temperature failure event to one freezer. Specimen data is entered into the NMMTB project using metadata from the NMMTB datasheet and any additional information from the necropsy form, if available. Permanent storage location information is also assigned at that time.

4. TITANIUM KNIFE CARE & FIELD CLEANING PROCEDURE

In the event where several marine mammals and their respective specimens are to be processed, care should be taken to thoroughly clean the titanium processing knife between each specimen. Typically, a scalpel blade will be used to remove specimens from an animal. The titanium knife should then be used to cut specimen edges where the scalpel blade, or other type of knife, touched the specimen surface. If available, a different titanium knife should be used for processing a different animal's specimens. If multiple titanium knives are not available, thoroughly clean the knives between specimens (see below) and make note of the modification (the same titanium knife was used to process several different animals' specimens) on the NMMTB datasheet(s).

If the knife is to be used again for field sampling, perhaps during a mass stranding, thoroughly rinse the knife with ultra-pure water to remove any adhering blood or tissue, rinse with ethanol, and place the knife in a clean FEP bag for storage and transport to the next sampling site. The knife, or implement, should at no time be touched with ungloved hands during processing.

5. NMMTB Specimen Access Policy

The scientific community, contributors, and principal investigators can request specimens from the NMMTB through a formal specimen access policy established by the NOAA/NMFS Office of Protected Resources. The detailed NMMTB specimen access policy can be found on the NOAA NMFS website (Appendix D).

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Appendix A. NMMTB Datasheet

National Marine Mammal Tissue Bank Form

Animal Information -
Page 2

Field ID: _____

Genus species: _____

Sex: ☐ Female ☐ Male
☐ Unknown

Total length: _____

☐ cm ☐ in

☐ Actual ☐ Estimated

Total weight: _____

☐ kg ☐ lb

☐ Actual ☐ Estimated

Age Class: ☐ Adult ☐ Subadult ☐ Actual
(choose one) ☐ Pup/calf ☐ Yearling ☐ Estimated
☐ Unknown

Age: _____ By whom: _____ Date aged: dd /mm / yy

Method Used: ☐ Teeth (GLG's) ☐ Baleen ☐ Bone ☐ Ear Plugs
(choose one) ☐ Other: _____

Epiphysis: ☐ Open ☐ Closed fused ☐ Fused invis

Disposition of specimen: ☐ Photo ☐ Slide (Please attach copy of photo or slide)

Reproductive condition:

☐ Sexually Mature
☐ Pregnant
☐ Lactating

Testis/Ovaries:
(circle one)

Length: _____ Mid-Width: _____ Mid-depth: _____ Weight: _____

Left: _____

☐ cm

☐ g

Right: _____

☐ in

☐ oz

Fetus length: _____
☐ cm ☐ in

Corpora lutea #: _____ Corpora albicantia #: _____ Corpora hemorrhagicum #: _____

Specify Units of Measurement: ☐ cm ☐ in

Cetaceans:

Snout to ant. ins. of flipper: _____

Girth: _____

Axillary: _____

Snout to center of genital aperture: _____

Max: _____

Snout to center of anus: _____

Anal: _____ (Location)

Flipper length: _____

Blubber thickness: _____

Thoracic: _____

Fluke width: _____

Dorsal: _____

Fluke notch to anus: _____

Lateral: _____

Tooth counts: UL/LL: _____ UR/LR: _____

Ventral: _____

Pinnipeds:

Nose to tail length: _____

Ant. length of hind flipper: _____

Ant. length of foreflipper: _____

Blubber thickness over post. end of sternum: _____

Axillary girth: _____

Other blubber thickness: _____

Baculum length: _____

(Location)

Polar Bears:

Girth of neck of axis: _____

Skull length: _____

Girth of neck at shoulders: _____

Sea Otters:

Snout to angle of mouth: _____

Right forepaw width: _____

Skull length: _____

Skull width: _____

Axillary girth: _____

Tooth Wear: ☐ Heavy ☐ Medium ☐ Light ☐ None

Estimate of body fat stores: _____

None: Little: Average: Excessive:

Subcutaneous: ☐ ☐ ☐ ☐

Groin: _____ cm ☐ ☐ ☐ ☐

Kidneys: ☐ ☐ ☐ ☐

Mesenteric: ☐ ☐ ☐ ☐

National Marine Mammal Tissue Bank

Additional Samples List -
Page 3

Field ID Number: _____

Genus species: _____

Was animal necropsied? ☐ Yes ☐ No

Necropsied by: _____ dd / mm / yy
(Please attach necropsy report) Date

Samples collected:

Histological samples:

Individual/Organization: _____

Final destination: _____

Tissues sampled: ☐ Liver ☐ Kidney ☐ Blubber ☐ Stomach ☐ Heart ☐ Intestine
(Choose all that apply) ☐ Lung ☐ Pancreas ☐ Adrenals ☐ Brain ☐ Muscle ☐ Skin
☐ Trachea ☐ Spleen ☐ Thymus ☐ Colon ☐ Thyroid ☐ Esophagus

Other: _____

(Please list)

Lymph Nodes: ☐ Submandibular ☐ Prescapular ☐ Axillary ☐ Hilar ☐ Mesenteric

Other l.n.: _____

Other samples collected:

Type of storage:

(Z-frozen, F-formalin, DMSO, ETOH)

Where located (Ind./Org.):

Teeth: _____

Genetics (skin): _____

Skull: _____

Reproductive tract: _____

Mammary tissue: _____

Ovaries: _____

Gonads/testes: _____

Parasites:

■ Number of parasites: ☐ 0-20 ☐ 21-100 ☐ 101+

■ List type and location: _____

Stomach: _____

■ List contents if applicable: _____

Other contaminant samples: _____

(List tissue type, storage type and where located)

Additional samples: _____

(List tissue type, purpose of collection, storage type and where located)

National Marine Mammal Tissue Bank

General Notes -
Page 4

Field ID Number: _____

Genus species: _____

Photos taken of animal: ☐ Yes ☐ No

☐ Digital ☐ Film

If yes, how many? _____

Video taken of animal: ☐ Yes ☐ No

(Please send copy with samples for NIST archive)

Disposition: _____

*(primary location
for photos and/or
video)*

General comments: _____

(Field notes)

General appearance of individual: _____

General appearance of organs: _____

NMMTB Protocol: ☐ Standard ☐ Modified

Please note any modifications: _____

Form prepared by: _____

Name (Print)

Affiliation (Print)

**A copy of this form and Level A Data Form
should be shipped with samples to:**

ATTN: Rebecca Pugh or Amanda Moors
NIST Biorepository
Hollings Marine Laboratory
331 Fort Johnson Rd
Charleston, SC 29412
843-460-9864 / 843-460-9814

NMMTB's Chain of Custody

Field ID Number:

Other ID Number:

NMMTB Storage ID Numbers:

1.	Collector's signature	Method of transfer to processing stage	dd / mm / yy Date
2.	Processor's signature	Method of transfer to shipping stage	dd / mm / yy Date
3.	Shipper to NMMTB's signature	Method of transfer to NIST Biorepository	dd / mm / yy Date
4.	Receiver's signature		dd / mm / yy Date

Each person in possession of the tissue must sign and date the form.

PAPERWORK REDUCTION ACT INFORMATION

A Federal agency may not conduct or sponsor, and a person is not required to respond to, nor shall a person be subject to a penalty for failure to comply with an information collection subject to the requirements of the Paperwork Reduction Act of 1995 unless the information collection has a currently valid OMB Control Number. The approved OMB Control Number for this information collection is 0648-0468. Without this approval, we could not conduct this survey/information collection. Public reporting for this information collection is estimated to be approximately 45 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the information collection. All responses to this information collection are mandatory. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden to the National Marine Fisheries Service at: 1315 East West Highway, 13th Floor, Silver Spring, MD 20910, Attn: Sarah Wilkin, Coordinator, Marine Mammal Health and Stranding Response Program, sarah.wilkin@noaa.gov if desired.

OMB Control #: 0648-0468

Expiration Date: 5/31/2027

Appendix B. NMMTB Datasheet Instructions

National Marine Mammal Tissue Bank Datasheet Instructions

Introduction.

The purpose of this document is to clarify protocol for completing the National Marine Mammal Tissue Bank (NMMTB) Form (NOAA Form 0648-0468) for the collection of specimens from marine mammals in the United States. This measure will standardize the field data sent to the National Institute of Standards and Technology (NIST). NIST maintains the NMMTB at the Hollings Marine Laboratory, South Carolina Marine Resources Center in Charleston, South Carolina (SC). The NMMTB provides a resource of samples that have been collected in a systematic and well-documented manner for comparing results over time to identify whether environmental trends exist, provides for future retrospective analyses for new analytes of interest, and allows for future analyses of samples collected today using improved analytical techniques of tomorrow. Many of the fields on this form may have several interpretations. For consistencies in information and accuracy of the database, please use this guide as the common convention in understanding and completing the form.

Background.

In 1989 the National Oceanic and Atmospheric Administration (NOAA) and the National Marine Fisheries Service (NMFS) initiated the development of the National Marine Mammal Tissue Bank (NMMTB) as a result of a massive die-off of bottlenose dolphins (*Tursiops truncatus*) in 1987-88. A large number of animals stranded along the Atlantic coast of the United States and although it was concluded that a naturally occurring toxin, brevetoxin, was likely the cause of death (Geraci, 1989), environmental pollution was suspected due to high levels of contaminants. It was also determined then that baseline data of anthropogenic contaminants was relatively unknown but needed for future reference. The NMMTB was developed to provide this reference data as well as other valuable information by collecting and banking marine mammal tissues for long-term storage for retrospective analyses. The guidelines for the NMMTB were based on the already established goals and protocols of the Alaska Marine Mammal Tissue Archival Project (AMMTAP). This project was established in 1987 through an agreement between NOAA's National Ocean Service (NOS), the National Institute of Standards and Technology (NIST) and the U.S. Department of the Interior, former Minerals Management Service (MMS) to help determine contaminant levels of marine mammals that were taken primarily during native subsistence hunts in Alaska (Becker, *et al.*, 1988; 1991, 1993). Tissues were also collected and banked for long-term archive using standardized protocols. Currently the AMMTAP is conducted by the United States Geological Survey (USGS) Biological Resource Division in cooperation with NOAA's NMFS and NIST.

In 1990, a demonstration phase of the NMMTB began evaluating the collection protocols that were developed in order to obtain tissues from animals taken during incidental catches and strandings from coastal locations throughout the U.S. The New England Aquarium (NEA), Boston, MA., assisted NOAA/NMFS and NIST personnel in the initial collection of two species of marine

mammals, the harbor porpoise (*Phocoena phocoena*), taken during incidental catches, and the pilot whale (*Globicephala melaena*), taken during mass strandings. Protocols were developed that identified the criteria to determine whether an animal was suitable for sampling as well as the logistics and procedures for collecting and processing samples from these events. Efforts have been extended to sampling on the west coast, as well as the southeast Atlantic and Gulf of Mexico regions. Animals are collected through incidental catches, single and mass strandings, as well as subsistence hunts in Alaska through the AMMTAP, and the species list has expanded to include other cetaceans as well as some pinnipeds, and fissipeds.

In 1992, the NMMTB program was formally established by the Marine Mammal Health and Stranding Response Act (Public Law 102-587) and was expanded and combined with the Marine Mammal Stranding Network to become a larger program that resulted in several components; Stranding Networks, the NMMTB, and Monitoring and Quality Assurance. This expansion is now known as the Marine Mammal Health and Stranding Response Program (MMHSRP) and is coordinated by the NMFS in cooperation with the USFWS. 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. The NMMTB and the quality assurance program are administered by NIST. In 1995, the quality assurance program was formalized and became the National Marine Analytical Quality Assurance Program (NMAQAP).

After the demonstration phase, the New England Aquarium continued to collect tissues for the NMMTB. In addition, other federal and non-federal partners have been formally trained by NIST personnel and collect samples for the NMMTB. These current and former partners include:

- Alaska Department of Fish and Game
- Alaska Nanuuq Commission
- Alaska Sea Life Center
- Alaska Veterinary Pathology Services
- Aleut Community of St. Paul Island
- Cape Cod Stranding Network
- Hubbs Sea World Research Institute
- Lowcountry Marine Mammal Network
- Mote Marine Laboratory
- National Marine Fisheries Service (NMFS)
- National Oceanic and Atmospheric Administration (NOAA)/National Ocean Service (NOS) Center for Coastal Environmental Health and Biomedical Research (CCEHBR) Laboratory
- New England Aquarium
- NOAA, Panama City Laboratory
- North Slope Borough, Department of Wildlife Management United States Fish and Wildlife Service (USFWS)
- The Marine Mammal Center
- University of Alaska-Fairbanks

Over 1,000 animals have been collected from 40 species of cetaceans, pinnipeds, and fissipeds. There have been approximately 1,500 marine mammal tissue specimens collected from 27 species outside of Alaska and approximately 1,700 tissue specimens collected from 22 species in Alaska. Blubber or other adipose tissue, liver, and kidney tissues are the primary tissues collected from each animal for the NMMTB. In the past muscle specimens were collected for animals that were collected for subsistence uses.

The goal of the NMMTB was initially established for the purposes of anthropogenic chemical evaluations by:

1. archiving samples for future retrospective analyses for new analytes of interest;
2. archiving samples for future analyses using improved analytical techniques and;
3. providing a resource of samples that have been collected and stored in a systematic and well documented manner for comparing results over time to identify if environmental trends exist.

Initial specimen collection and archival protocols were developed for chemical contaminants (Becker, *et al.*, 1999) and were based on the AMMTAP for Alaska species (Becker *et al.*, 1988; 1991). Although there have been updates made over time, the goals and focus of the NMMTB have remained primarily on chemical contaminants and non-essential elements.

Tissue or fluid specimens are primarily obtained from stranding network personnel, subsistence harvest collaborators, or biologists or veterinarians during live capture release programs who have been trained by NIST personnel to collect and process tissues for the NMMTB. During stranding or subsistence events, animals' tissues and/or fluids selected for collection and archive must meet specific criteria before they can be collected. The following is the criteria that an animal must meet before it is considered for archival in the NMMTB as a representation of a 'normal' animal:

- Animal appears to be 'normal' and 'healthy' as defined by lack of evidence of chronic disease, evidence of recent human interaction (ship strike, entanglement) or incidental to other human activities, or good robust body condition with no known cause of death;
- Animal is Code 2 (fresh dead);
- There is no obvious scavenger damage;
- Body cavity is intact and has not been breached; and
- Elapsed post-mortem time is less than 24 hours or the animal has been kept refrigerated or frozen for 48 hours post-mortem.

NMMTB Tissue Access Policy.

The NMMTB Tissue Access Policy was developed in order to outline the procedure for requesting use of tissues/fluids in the NMMTB, to prioritize the use and release of tissues/fluids from the NMMTB, and to track such tissue/fluid distribution. A NMMTB Tissue Request Form and research proposal must be submitted to the MMHSRP Program Manager, Office of Protected Resources,

NMFS for review of the request. A copy of this access policy as well as the specimen request form can be found at <https://www.fisheries.noaa.gov/national/marine-mammal-protection/national-marine-mammal-tissue-bank>.

After tissues/fluids are collected following the NMMTB protocols, they are shipped frozen to, and maintained by, the NIST at the NIST Biorepository in Charleston, SC, located at the Hollings Marine Laboratory where they are stored in liquid nitrogen vapor phase freezers ($\leq -150^{\circ}\text{C}$). Subsample A is stored for long term archival and Subsample B is available for analysis. The 2 subsamples of each tissue are stored in separate freezers for security purposes so if a freezer breaks down or overfills with liquid nitrogen, the entire sample is not compromised.

Reporting.

One of the goals of the NMMTB is to provide a resource of samples that have been collected and stored in a systematic and well-documented manner for comparing results over time to identify if environmental trends exist. In order to achieve this goal, data associated with the collection, processing and storage of the samples must be reported. Reporting burden for this collection of information is estimated to average 45 minutes per animal collection, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information, including completing the paper form and entering the information into the NMMTB Database. Comments regarding this burden estimate, or any other aspect of this collection of information, including suggestions for reducing this burden, may be sent to the National Marine Fisheries Service, Office of Protected Resources, Marine Mammal and Turtle Conservation Division, 1315 East-West Highway, Silver Spring, MD 20910.

Protocols developed by NIST for collecting and archiving tissues/fluids are designed to: (1) provide sufficient material for multiple analyses, (2) minimize the possibility of sample change and/or loss during storage, (3) minimize inadvertent contamination during sample handling and ensure sample integrity, (4) provide for long-term sample stability through cryogenic techniques, and (5) track and maintain a record of sample history.

Sources of specimen from dead animals include fresh stranded animals, animals taken incidental to fishing activities, and animals taken for subsistence uses.

Indicator species include: harbor seal (*Phoca vitulina*), California sea lion (*Zalophus californianus*), northern fur seal (*Callorhinus ursinus*), ringed seal (*P. hispida*), pilot whale (*Globicephala melas*), harbor porpoise (*Phocoena phocoena*), Atlantic white-sided dolphin (*Lagenorhynchus acutus*), pygmy sperm whale (*Kogia breviceps*), bottlenose dolphin (*Tursiops truncatus*), rough-toothed dolphin (*Steno bredanensis*), common dolphin (*Delphinus delphis*), beluga whale (*Delphinapterus leucas*), bowhead whale (*Balaena mysticetus*), polar bear (*Ursus maritimus*). Additional species have also been included. See Table 1 for a list of all species collected as a part of the NMMTB.

Table 1. Species of Marine Mammals Collected as a Part of the National Marine Mammal Tissue Bank (NMMTB).

Marine Mammal Health and Stranding Response Program (MMHSRP)	Alaska Marine Mammal Tissue Archival Project (AMMTAP)
Harbor Porpoise (<i>Phocoena phocoena</i>)	Ringed Seal (<i>Phoca hispida</i>)
Pilot Whale (<i>Globicephala melas</i>)	Harbor Seal (<i>Phoca vitulina</i>)
Short Finned Pilot Whale (<i>Globicephala macrorhynchus</i>)	Spotted Seal (<i>Phoca largha</i>)
Pygmy Sperm Whale (<i>Kogia breviceps</i>)	Bearded Seal (<i>Erignathus barbatus</i>)
Dwarf Sperm Whale (<i>Kogia simus</i>)	Elephant Seal (<i>Mirounga angustirostris</i>)
North Atlantic Right Whale (<i>Eubalaena glacialis</i>)	Stellar Sea Lion (<i>Eumetopias jubatus</i>)
Blainville's Beaked Whale (<i>Mesoplodon densirostris</i>)	Northern Fur Seal (<i>Callorhinus ursinus</i>)
Gervais Beaked Whale (<i>Mesoplodon europaeus</i>)	Pacific Walrus (<i>Odobenus rosmarus divergens</i>)
Bottlenose Dolphin (<i>Tursiops truncatus</i>)	Beluga Whale (<i>Delphinapterus leucas</i>)
Striped Dolphin (<i>Stenella coeruleoalba</i>)	Bowhead Whale (<i>Balaena mysticetus</i>)
Dolphin (<i>Stenella spp.</i>)	Polar Bear (<i>Ursus maritimus</i>)
Risso Dolphin (<i>Grampus griseus</i>)	Sea Otter (<i>Enhydra lutris</i>)
White Side d Dolphin (<i>Lagenorhynchus acutus</i>)	Harbor Porpoise (<i>Phocoena phocoena</i>)
California Sea Lion (<i>Zalophus californianus</i>)	Gray Whale (<i>Eschrichtius robustus</i>)
Common Dolphin (<i>Delphinus delphis</i>)	Ribbon Seal (<i>Phoca fasciata</i>)
Hooded Seal (<i>Cystophora cristata</i>)	Stejneger's Beaked whale (<i>Mesoplodon stejnegeri</i>)
Harbor Seal (<i>Phoca vitulina</i>)	Humpback Whale (<i>Megaptera novaeangliae</i>)
Ringed Seal (<i>Phoca hispida</i>)	
Harp Seal (<i>Phoca groenlandica</i>)	
Grey Seal (<i>Halichoerus grypus</i>)	
Rough-Toothed Dolphin (<i>Steno bredanensis</i>)	
Fin Whale (<i>Balaenoptera physalus</i>)	
Cuvier's Beaked Whale (<i>Ziphius cavirostris</i>)	
Atlantic Spotted Dolphin (<i>Stenella frontalis</i>)	
Eastern Spinner Dolphin (<i>Stenella longirostris orientalis</i>)	
Pantropical Spotted Dolphin (<i>Stenella attenuata</i>)	
Clymene Dolphin (<i>Stenella clymene</i>)	
Mink Whale (<i>Balaenoptera acutorostrata</i>)	
Beluga Whale (<i>Delphinapterus leucas</i>)	
Melon-headed Whale (<i>Peponocephala electra</i>)	
Bryde's Whale (<i>Balaenoptera edeni</i>)	
Pygmy Killer Whale (<i>Feresa attenuata</i>)	

Under Title IV of the Marine Mammal Protection Act (MMPA), Section 407, the Secretary of Commerce shall make provision for the storage, preparation, examination, and archiving of marine mammal tissues/fluids. These tissues/fluids will be archived in the NMMTB. In addition, the Secretary shall consult with the Marine Mammal Commission, the Secretary of Interior, and individuals with knowledge and experience in marine science, marine mammal science, marine mammal veterinary and husbandry practices, and conservation to issue guidance for analyzing tissue samples as a means to monitor and measure overall health trends in representative species or populations of marine mammals after the public has an opportunity to review and comment.

The MMHSRP working with the federal partners is developing an integrated information management system which will serve to assist the Secretary of Commerce and Interior to meet their goals of the Act for the following:

- Facilitate the collection and dissemination of reference data on the health of marine mammals and health trends of marine mammal populations in the wild;

- Correlate the health of marine mammals and marine mammal populations with available data on physical, chemical, and biological environmental parameters; and
- Coordinate effective responses to unusual mortality events by establishing a process in the Department of Commerce in accordance with Section 404 of the Marine Mammal Protection Act.

Under Section 407 (c) of Title IV, the Secretary of Commerce shall maintain a central database that provides an effective means for tracking and accessing data on marine mammal tissues collected for and maintained in the NMMTB. This database should contain reference data on the health of marine mammals, their populations and species that are subject to unusual mortality events. In addition, the Secretary shall consult with the Secretary of the Interior to establish criteria for access to marine mammal tissues in the tissue bank; analyses conducted; and marine mammal information within the database after the public has an opportunity to review and comment.

NIST SAMPLE PROCESSING.

Field ID: _____ Other ID Number: _____ Common Name: _____ Genus species: _____

Field ID is the unique identifier assigned to the animal from which the tissue(s) or fluids were obtained. The format is open to each agency's requirements; however, please remain consistent within your agency.

Other ID Number is any other identifier(s) related to this animal Examples include: previous field ID numbers if this animal previously stranded; ID numbers assigned by other organizations (including authorized rehabilitation facilities to which the animal is transferred), former identification numbers from scientific research projects, etc.

Common Name is the commonly used name for this species (e.g., harbor porpoise).

Genus, Species are the Latin or scientific names for the species using standard binomial nomenclature.

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Collection Type:	☐ Single Strand	☐ Biopsy	☐ Incidental Take (choose one) (specify): _____	☐ Fisheries or Other	☐ Subsistence (choose one) (specify): _____	☐ Clubbed
	☐ Mass Strand	☐ Repeat Event				☐ Bow/Arrow
	☐ UME	☐ Live Capture Release				☐ Gunshot
	☐ Rescue	☐ Other (specify): _____				☐ Other (specify): _____

Collection Type is the description of the event(s) or circumstances pertaining to the animal from which the samples are taken and the collection method used. Examples include: strandings (single or multiple); unusual mortality event, repeat stranding event, rescue, live capture release, biopsy, etc.

Single Strand: Check this box if one animal stranded and was responded to by your organization or another.

Mass Strand: Check this box if the animal from which the samples were collected was part of a mass stranding. A “*mass stranding*” is more than 2 cetaceans that strand, other than cow-calf pairs. A “*cow/calf pair*” would be two animals stranding where one is the mother and the other is the offspring (a mom/pup pair would also qualify).

UME: Check this box if the animal was part of an official “*unusual mortality event*” (UME). An “*unusual mortality event*” is a die-off that has been officially designated as a UME by the Working Group on Marine Mammal Unusual Mortality Event (WGMMUME).

Rescue: Check this box if the animal was part of a rescue during an oil spill, disentanglement, out of habitat, or other such event.

Biopsy: Check this box if the sample was collected using remote biopsy (e.g., dart) or using a punch pole.

Repeat Event: Check this box if the animal stranded during a morbidity or mortality event that has been designated as a repeat event by the WGMMUME.

Live Capture Release: Check this box if the animal was part of a live-capture release research effort and samples were collected (e.g., blood, surgical biopsy)

Other (specify): Check this box if the animal was part of an event that is not listed in this section and specify what the event is on the line provided next to the check box (e.g. research).

Incidental Take: Check if the animal was killed incidental to any human activities.

Fisheries: Check this box if the animal was obtained incidental to fishery activities.

Other (specify): Check this box if the animal was obtained incidental to other human activities (e.g., incidental to permitted research activities, ship strike) and fill in the circumstances in the comment field.

Subsistence: Check this box if the animal was killed for subsistence use by Native Americans under co-management agreements. List the means by which the legal take occurred:

Clubbed: Check this box if the cause of death was a striking with a cudgel, stick, bat, baton, nightstick, staff, shillelagh, or billy club during a hunt.

Bow/Arrow: The animal cause of death was due to the shooting of a bow and arrow during a hunt.

Ballistics or Gunshot: Check this box if the cause of death was from a projectile fired from a gun. When possible, please enter the caliber of the projectile used.

Other (specify): Check this box if the cause of death was from a weapon other than the one listed on the form such as, a knife, a machete, etc. and specify weapon.

Condition: <i>(choose one)</i> ☒ Alive ☐ Fresh Dead (Code 2) ☐ Euthanized	Was animal in rehabilitation? ☒ Yes ☐ No
If euthanized: With what: _____ How much: _____ Where: _____	If yes: Where: _____ From: dd / mm / yy To: dd / mm / yy <i>(please attach clinical/medical records)</i>

Condition indicates the physical state of the animal (carcass or live animal) on the date when the samples were removed from the animal. The codes used follow the guidelines used in the Level A data sheet. Choose only one. If the animal was alive, but euthanized, choose euthanized.

- Code 1 – alive
- Code 2 – fresh dead
- Code 3 – moderate decomposition
- Code 4 – Advanced decomposition
- Code 5 – Mummified/Skeletal
- Code 6 – Unknown

Alive (Code 1): Check this box if the animal was alive during the removal of the specimen. This option does not apply to samples collected for the MMHSRP or AMMTAP.

Fresh Dead (Code 2): Check this box if the carcass was in good condition (fresh/edible).

Normal appearance, usually with little scavenger damage; fresh smell; minimal drying and wrinkling of skin, eyes and mucous membranes; eyes clear; carcass not bloated, tongue and penis not protruded; blubber firm and white; muscles firm, dark red, well-defined; blood cells intact, able to settle in a sample tube; non-hemolyzed serum; viscera intact and well-defined, gut contains little or no gas; brain firm with no discoloration, surface features distinct, easily removed and intact. If animal is early Code 3, moderate decomposition, note this in the Additional Comments section on Page 1 of the NMMTB Datasheet.

Euthanized: Check this box if the animal was found alive and was euthanized by an authorized entity for humane welfare, or medical reasons.

If euthanized indicates if the animal was euthanized by an authorized entity, please provide the following information about the method used:

With what Chemical: Indicate the drug(s) used to chemically euthanize the animal

How much: Indicate the dosage amount of the drug (total dose in milliliters) and include the concentration (mg/ml) of the drug as well as dose given.

Where: Indicate how and where the drug was administered to the animal by drug (e.g., IM acepromazine along left peduncle).

Was animal in rehabilitation?

Yes: Check this box if the animal was in an authorized rehabilitation facility when the samples were removed.

No: Check this box if the animal was not in an authorized rehabilitation facility.

If the animal was in a rehabilitation facility, please provide the following information about the animal medical history:

Where: Indicate the name of the authorized rehabilitation facility.

From: DD/MM/YYYY **To**: DD/MM/YYYY - Indicate the date that the animal was admitted into the rehabilitation facility and the date that the animal was removed from the rehabilitation facility upon the animal's death following the described date format. Also, attach the animal's medical history. If you are completing the form using the NMMTB Database, you may submit the medical history electronically.

Animal Location:	State: _____	County: _____	City/Island/Community: _____
	Ocean/Bay/Sea: _____		
	Locality Details: _____		
	Latitude: _____ N (dec degrees)	Longitude: _____ W (dec degrees)	

Animal location is the geographic location of animal when the samples were collected, or where the animal stranded prior to rehabilitation if applicable.

State: The standard two letter abbreviation of a state in the United States.

County or Parish: The name of the county or parish in the state, if known.

City/Island/Community: The names for the city, island or community of the location. This should include boroughs, parishes, provinces, commonwealths, and territories.

Ocean/Bay/Sea: The name of the ocean, sea, gulf, bay, inlet or estuary that the animal was found on the shore of a body of water where the tissue was removed.

Locality Details: Using known landmarks, describe the precise locality where the animal was found. Compass bearings and relative distances are useful.

Latitude: These are the GPS coordinates of the geographic location of where the animal was when the tissues were collected. The units of measurements should be decimal degrees.

Longitude: These are the GPS coordinates of the geographic location of where the animal was when the tissues were collected. The units of measurements should be decimal degrees.

Time of death..... (Zulu)	dd / m / yy	hr	Place of Death:	
			Internal body temp. of animal:	☒ C ☐ F Rigor? ☒ Yes ☐ No
If transported before tissue removal:	Vehicle Type: _____ Length of Transport: _____ Ambient weather condition: _____ Remarks: _____			
Time of tissue removal (Zulu)	dd / mm / yy	hr	Place of tissue removal:	
			Internal body temp. of animal just before tissue removal:	☐ C ☒ F
If transported before processing:	Transportation storage: ☐ Dry ice ☐ Wet ice Other: _____ Ambient weather condition: _____ Interim storage of tissue: ☐ Teflon bag ☐ Teflon jar Other: _____ Remarks: _____			
Time of tissue processing.... (Zulu)	dd / mm / yy	hr	Place of tissue processing:	
			Ambient temperature at processing:	
Time of interim freezing..... (Zulu)	dd / mm / yy	hr	Freezer type:	☐ LN2 ☐ -80° C ☐ -30° C Other: _____
Time shipped to NIST..... (Zulu)	dd / mm / yy	hr		
Time received at NIST..... (Zulu)	dd / mm / yy	hr		

If samples were collected from a dead animal:

Time of Death: Indicate the date (DD/MM/YYYY) of the animal's death and time using Zulu which pertains to the hour in military time (i.e. 2 pm equals 1400).

Place of Death: Indicate the place of the animal's death. This may be different from the location of the animal during sample collection.

Internal Body Temp. of Animal: Indicate the animal's internal body temperature at the time of death.

Celsius (C): Check this box if the unit of measurement is Celsius.

Fahrenheit (F): Check this box if the unit of measurement is Fahrenheit.

Rigor?

Yes: Check this box if *rigor mortis*, the stiffening of the body muscles, was observed during the time of tissue collection.

No: Check this box if *rigor mortis* did not occur during the time the carcass was being observed prior to sample collection.

If transported before tissue removal: If the animal was transported before sample removal, please provide the following information describing the method of the animal relocation from initial site.

Vehicle Type: Indicate the type of transportation provided to relocate the animal from the initial site, such as, pick-up truck, open truck, closed truck, van, etc.

Length of Transport: Indicate the length of time the animal was in transport.

Ambient Weather Conditions: Indicate the temperature of the environment during transport. Provide units of measurements as Celsius (C) or Fahrenheit (F).

Remarks: Provide additional comments regarding the animal relocation.

Time of Tissue Removal: Indicate the date (*DD/MM/YYYY*) of the removal of samples from the animal and time using Zulu which pertains to the hour in military time (i.e. 2pm equals 1400).

Place of Tissue Removal: Indicate the location of the animal when the samples were removed. (e.g., necropsy laboratory, beach, landfill, etc.).

Internal Body Temperature Before Tissue Removal: Indicate the animal's internal body temperature at the time of tissue removal. Check the units of measurement as Celsius (C) or Fahrenheit (F).

If transported before processing: Please provide information in this section about the storage of the tissue(s) that are transported prior to processing.

Transportation Storage: Check whether the storage unit used dry ice, wet ice or other to preserve the tissue while being transported before processing to NIST. If select "other", please indicate other storage method used (i.e. portable freezer, refrigerator/freezer, etc.)

Ambient Weather Conditions: Indicate the environmental parameters for the transport event. Include temperature and if in an open area (outside) include precipitation and wind factor. Provide units of measurements as Celsius (C) or Fahrenheit (F).

Interim Storage Container: Check one of the boxes to indicate whether the tissue was in a Teflon bag, Teflon jar or other. If select "other", please describe the interim storage container.

Remarks: Provide additional comments regarding the storage of the tissue.

Time of Tissue Processing: Indicate the date (*DD/MM/YYYY*) and time (using Zulu which pertains to the hour in military time (i.e. 2pm equals 1400) that the sample collected was processed for long term storage.

Place of Tissue Processing: Indicate the place where the tissue was processed.

Ambient Temperatures at Processing: Indicate the temperature of the environment where the samples are being processed and if processing occurs outside include wind and precipitation. Provide units of measurements as Celsius (C) or Fahrenheit (F).

Time of Interim Freezing: Indicate the date (DD/MM/YYYY) and time (using Zulu which pertains to the hour in military time (i.e. 2pm equals 1400) of the initial freezing of the samples collected.

Freezer Type and temperature: Check the freezer type temperature as LN₂, -80 °C, -30 °C or other. If select “other”, please provide the units of measurements for the freezing temperature as Celsius (C) or Fahrenheit (F).

Time Shipped to NIST: Indicate the date (DD/MM/YYYY) and time (using Zulu which pertains to the hour in military time (i.e. 2pm equals 1400) the sample was shipped to NIST from the site using the format provide on the form in this section

Time Received at NIST: Indicate the date (DD/MM/YYYY) and time (using Zulu which pertains to the hour in military time (i.e. 2pm equals 1400) the sample was received at NIST from the site using the format provide on the form in this section.

Additional comments: _____							

Sample weights:	Blubber (g):	Liver (g):	Kidney (g):	Whole Blood (mL):	Plasma (mL):	Serum (mL):	Other:
A	_____	_____	_____	_____	_____	_____	_____
B	_____	_____	_____	_____	_____	_____	_____

Additional Comments: Provide additional remarks regarding the tissues being processed. For instance, if animal is early Code 3, note it here.

Sample Weights: Indicate the sample weight for tissues classified, A or B. The weight should be measured in grams for the following tissues: blubber, liver, kidney. The measure for whole blood, plasma and serum should be in milliliters. If other, specify the tissue and provide the unit of measurement.

ANIMAL INFORMATION

Field ID: _____		Genus species: _____	
Sex:	☐ Female ☐ Male	Total length: _____	☐ cm ☐ in ☐ Actual ☐ Estimated
	☐ Unknown	Total weight: _____	☐ kg ☐ lb ☐ Actual ☐ Estimated

Field ID is the unique identifier assigned to the animal from which the sample(s) were obtained. Format is open to each agency's requirements; however, please remain consistent within your agency. It is carried over from NIST Sample Processing – Page 1.

Genus Species is the Latin name for the animal in standard binomial nomenclature. It is carried over from NIST Sample Processing – Page 1.

Sex is the sexual classification of the animal.

Female: Check this box if the animal is confirmed as female.

Male: Check this box if the animal is confirmed as male.

Unknown: Check this box if the animal's sex cannot be confirmed by examination. It is expected that all unknown will be genetically identified as to 'sex'.

Total Length is the straight length (not contoured) measurement of the animal on the date of the initial examination. Please note in the comment field if the animal was towed or otherwise stretched prior to measurement.

Cm: Check this box if the measurement is taken in centimeters (metric system is preferred).

In: Check this box if the measurement is taken in inches.

Actual: Check this box if this is the actual physical measurement using standard measuring device (e.g., tape measure).

Estimated: Check this box if this is a visual measurement or if the animal's carcass is not intact (e.g. flukes degraded or severed).

Total Weight is the weight of the entire animal on the date of the initial examination.

Kg: Check this box if the weight of the animal is measured in kilograms (metric system is preferred).

Lb: Check this box if the weight of the animal is measured in pounds.

Actual: Check this box if this is the actual physical weight obtained using a scale.

Estimated: Check this box if this is an estimate based on length/growth charts or visual observation using an item of known length because the carcass was not intact or could not be measured. (e.g. flukes degraded or severed or floating).

Age Class: (choose one)	☐ Adult ☐ Subadult ☐ Actual ☐ Pup/calf ☐ Yearling ☐ Estimated ☐ Unknown	Age: _____ By whom: _____ Date aged: dd /mm / yy Method Used: ☐ Teeth (GLG's) ☐ Baleen ☐ Bone ☐ Ear Plugs (choose one) ☐ Other: _____ Disposition of specimen: _____ ☐ Photo ☐ Slide (Please attach copy of photo or slide)
Epiphysis:	☐ Open ☐ Closed fused ☐ Fused invis	

Age Class is the classification of the animal into one of six age classes using morphometric data, aging techniques on specific specimen (e.g., teeth, baleen, claw, bone, etc.) or having known age animals.

Adult: Check this box if the age class for an animal is determined to be physically or sexually mature.

Subadult: Check this box if the age class for an animal is determined to be greater than one year old, but not yet mature physically or sexually.

Pup/calf: Check this box if the age class for an animal is determined to be younger than one year old.

Yearling: Check this box if the age class for an animal is determined to be approximately one year old, using length or time of year or age determination.

Unknown: Check this box if the age class of an animal is unable to be determined.

Actual: – Check this box if the age can be verified.

Estimated: Check this box if the age is estimated.

Epiphysis is an area at the end of a long bone and other bones (e.g., vertebrae) that is the point of bone growth in length. The line of growth is called the physeal line and is made of cartilage in young immature animals. Over time and with physical maturity the physeal line reduces as the cartilage becomes ossified and the bones fuse. The timing of that fusion or ossification is species dependent and bone dependent, therefore for most species only gives approximate age classification not exact age. *Ossification* is the process of bone formation, in which connective tissues, such as cartilage are turned to bone or bone-like tissue. Measures of epiphyseal closure indicate physical maturity (classification as adult).

Open: Check this box if the epiphyses are open with the physeal line of cartilage apparent and in many cases the bone ends may separate from the main portion of the bone.

Closed fused: Check this box if the epiphyses are partially closed (ossified) but the physeal line is still quite visible.

Fused Invis: Check this box if the epiphysis is fused (ossified) to the rest of the bone and the physeal line is invisible.

Age is the approximate number of years determined for the animal based on aging techniques.

By Whom is the name of the qualified individual who determined the age classification of the animal.

Date Aged: The date (DD/MM/YYYY) that the age classification was determined.

Method Used: If the age was determined using a standard aging technique, please indicate the type of technique used to estimate the age of the animal.

Teeth (GLG's): Check this box if the age estimation is determined by counting the growth layer groups (GLGs) or tooth layers of the animal's teeth, e.g. odontocetes' age classification is determined by counting the GLG's of the mid-mandibular teeth; pinnipeds' age classification is determined by counting the GLG's of the canines and first 3-4 post canines (incisors and premolars); walruses' lower canines; and sea otters' lower first premolars.

Baleen: Check this box if the age estimation is determined using baleen plates.

Bone: Check this box if the age estimation is determined by counting the growth plate on the bone end; e.g., remove the tympanic bullae for a count of the layers or rings.

Ear plugs: (also known as "wax plug" in some mysticetes) Check this box if the age estimation is determined by removing the ear plug at the proximal end on the auditory canal and counting the ear plug layers.

Other: Check this box if other method was used, please indicate in the comment field the method used with a brief description of the method.

Disposition of Specimen: Use this line to indicate where the photos or slides of the sample taken to determine the age classification of the animal are housed.

Photo: Check this box if you have pictures of the specimens used to determine the age classification of the animal. If possible, attach copies of the photographs to the NMMTB form.

Slide: Check this box if you have slides of the specimens used to determine the age classification of the animal. If possible, attach copies of the slides to the NMMTB form.

Reproductive condition:		Length:		Mid-Width:	Mid-depth:	Weight:
☐ Sexually Mature	Testis/Ovaries: (circle one)	Left:			☒ cm	☐ g
☐ Pregnant		Right:			☐ in	☐ oz
☐ Lactating						
Fetus length:		Corpora lutea #:	Corpora albicantia #:	Corpora hemorrhagicum #:		
	☒ cm ☐ in					

Reproductive Condition is the state of the animal's reproductive cycle at the time of the sample collection.

Sexually Mature: Check this box if the male or female has characteristics of reproductive maturity such as pregnancy, ovulation, corpora lutea or albicans, presence of milk, size of the uterus, sperm apparent on smear or histological examination.

Pregnant: Check this box if the animal is carrying one or more embryos or fetuses. If pregnant, please indicate fetal length.

Lactating: Check this box if the animal shows evidence of increased mammary development and milk can be expressed from the nipple or on cut surface of the mammary gland.

Fetus Length is the measurement of the length in centimeters of the fetus or embryo as a total straight length (tip of nose to notch in fluke or tip of tail).

cm: Check this box if the measurement is taken in centimeters (the metric system is preferred).

in: Check this box if the measurement is taken in inches.

Testis/Ovaries: Gonadal measurements are used to identify and quantify reproductive maturity and are evaluated for evidence of reproductive status. In this section, please provide the measurements of the gonadal length and weight.

Length: The full-length measurement of the gonads (left and right separately).

Mid-Width: The arc girder length from the center of the gonad to the end.

Mid-Depth: The depth from the center of the gonad.

cm: Check this box if the measurement is taken in centimeters (the metric system is preferred.)

in: Check this box if the measurement is taken in inches.

Weight: The total weight amount of gonad. For testis, weigh without epididymis attached.

g: Check this box if the weight of the animal is measured in grams or kilograms (metric system is preferred).

oz: Check this box if the weight of the animal is measured in ounces.

Corpora lutea #: The Corpora lutea is the mass of tissue that forms when the granulosa cells move into the Graafian follicle after ovulation. The presence of a CL is indicative of recent ovulation and the uterus should be examined closely for the presence of a blastocyst.

Corpora albicantia #: The mass of white scar tissue formed in an ovary replacing the corpus luteum. Because corpora albicantia persist in cetaceans, the number of corpora albicantia can be used to approximate the maximum number of pregnancies.

Follicle # and maximum diameter: The presence of a large follicle indicates imminent ovulation and is a sign of sexual maturity. Maximum follicle diameter is measured as a mechanism to understand seasonality of ovulation and whether an immature female is close to maturity.

Cetaceans:			
Snout to ant. ins. of flipper:	_____	Girth: _____	Axillary: _____
Snout to center of genital aperture:	_____		Max: _____
Snout to center of anus:	_____		Anal: _____ (Location)
Flipper length:	_____	Blubber thickness: _____	Thoracic: _____
Fluke width:	_____		Dorsal: _____
Fluke notch to anus:	_____		Lateral: _____
Total counts: UL/LL: _____ UR/LR: _____			Ventral: _____

Specify units of measurements - indicate using centimeters (cm) (metric system is preferred) or inches (in).

For measurement of all species, use straight length, not contoured. Refer to Geraci, J.R. and V.J. Lounsbury. 2005. *Marine Mammals Ashore: A Field Guide for Strandings*, Second Edition. National Aquarium in Baltimore, Baltimore, MD.

Cetaceans:

Snout to ant. (anterior) ins. (insertion) of flipper: This is measured from snout to anterior insertion of flipper while the animal is lying flat, dorsal side up if possible.

Snout to center of genital aperture: This is measured from snout to center of genital aperture.

Snout to center of anus: This is measured from snout to center of anus.

Flipper length: This is measured from axilla (posterior attachment of the flipper to the body) to the tip of the flipper.

Fluke width: This is measured from tip to tip across the widest part of the fluke while the animal is lying flat, dorsal side up if possible.

Fluke notch to anus: This is measured from fluke notch to center of anus.

Total Counts: These are the total tooth counts of the animal.

UL/LL: Upper left/Lower left.

UR/LR: Upper right/Lower right.

Girth: The measurement around the body of the cetacean.

Axillary: This is the circumference of the animal measured at the caudal insertion of the flippers.

Max: This is the maximum circumference of the animal measured. Specify location next to the measurement.

Anal: This is the circumference of the animal measured at the level of the anus.

Blubber Thickness: For small cetaceans, such as the beluga, this is the sternal blubber thickness. For large cetaceans, such as the bowhead whale, the measurement might have to be made midway along the side depending on how the animal is lying.

Thoracic: This is the measurement of blubber thickness along the body over the thorax with three levels being measured.

Dorsal: This is the measurement of the blubber thickness along the dorsal surface (midline) over the thorax.

Lateral: This is the measurement of the blubber thickness at the mid-lateral position (halfway between the dorsal midline and the ventral midline) over the thorax.

Ventral: This is the measurement of the blubber thickness at the ventral midline over the thorax.

<u>Pinnipeds:</u>			
Nose to tail length:		Ant. length of hind flipper:	
Ant. length of foreflipper:		Blubber thickness over post. end of sternum:	
Axillary girth:		Other blubber thickness:	(Location)
Baculum length:			

Pinnipeds:

Nose to tail length: This measurement is the total length of the animal, from tip of snout to tip of tail.

Ant.(anterior). length of foreflipper: This is the straight measurement from the base of the foreflipper anteriorly to the tip of the foreflipper.

Axillary girth: This is the circumference of the animal measured just posterior of the front flippers.

Baculum length: The length of the baculum from tip to tip after excision from the penis.

Ant.(anterior) length of hind flipper: This is the measurement of base of the hind flipper anteriorly to the tip of the hind flipper.

Blubber thickness over post. (posterior) end of sternum: This is the thickness of the blubber at the posterior end of the sternum.

Other blubber thickness: If additional blubber thickness measurements are taken, note here the thickness and location.

Polar Bears:

Girth of neck of axis:

Skull length:

Girth of neck at shoulders:

Polar Bears:

Girth of neck of axis: This measurement is the circumference at the top of the neck, just below the head and behind the ears.

Girth of neck at shoulder: This is the circumference at the base of the neck, just above the shoulders.

Skull length: (condylobasal length) Measure using calipers with bear in lateral recumbent position. Using your knee, bend the head to near 90 degrees at the neck to facilitate feeling the back of the skull. Measure distance between upper dental plate and bony process at the base of the skull.

<u>Sea Otters:</u>																													
Snout to angle of mouth:	_____	Right forepaw width:	_____																										
Skull length:	_____	Skull width:	_____																										
Axillary girth:	_____	Tooth Wear:	☒ Heavy ☐ Med. ☐ Light ☐ None																										
Estimate of body fat stores:	<table border="1"> <thead> <tr> <th></th> <th>None:</th> <th>Little:</th> <th>Average:</th> <th>Excessive:</th> </tr> </thead> <tbody> <tr> <td>Subcutaneous:</td> <td>☐</td> <td>☐</td> <td>☐</td> <td>☐</td> </tr> <tr> <td>Groin: _____ cm</td> <td>☐</td> <td>☐</td> <td>☐</td> <td>☐</td> </tr> <tr> <td>Kidneys:</td> <td>☐</td> <td>☐</td> <td>☐</td> <td>☐</td> </tr> <tr> <td>Mesenteric:</td> <td>☐</td> <td>☐</td> <td>☐</td> <td>☐</td> </tr> </tbody> </table>					None:	Little:	Average:	Excessive:	Subcutaneous:	☐	☐	☐	☐	Groin: _____ cm	☐	☐	☐	☐	Kidneys:	☐	☐	☐	☐	Mesenteric:	☐	☐	☐	☐
	None:	Little:	Average:	Excessive:																									
Subcutaneous:	☐	☐	☐	☐																									
Groin: _____ cm	☐	☐	☐	☐																									
Kidneys:	☐	☐	☐	☐																									
Mesenteric:	☐	☐	☐	☐																									

Sea Otters:

Snout to angle of mouth: Open the sea otter's mouth and measure from the tip of the snout to the angle of the mouth.

Skull length: Cut away tissues so that the base of the skull is exposed where the neck vertebrae joins the skull. Place the large calipers at the base of the skull and extend it to the gum line of the upper incisors. Measure the distance between the jaws of the calipers in centimeters (cm) on the plastic ruler or measuring tape to the nearest 0.1 cm.

Axillary girth: On a flat surface, with the animal in ventral position, place the measuring tape beneath it. Wrap the tape around the otter at the xiphoid process (the tape should be snug but not pulled tight). The xiphoid process is located at the point where the last rib connects at the bottom of the sternum (breastbone). Record the circumference to the nearest 0.5 cm.

Right forepaw width: Measure the width of the right forepaw across the widest portion of the foot at the level with the pad using a small, plastic calipers. Record the measurement to the nearest 0.1 cm.

Skull width: Cut away tissues at the zygomatic arch to expose the bone. The zygomatic arch is the "cheek bone" and it is an arch of bone that extends along the side of the skull beneath the eye. Place the large calipers on the outermost point of each zygomatic arch. Measure the distance between the jaws of the calipers in centimeters on the plastic ruler or measuring tape to the nearest 0.1 cm.

Tooth Wear: Measure base of upper right canine tooth with plastic calipers from front to back at gum line. Measure widest part visible without pushing gums down. Record to the nearest 0.1 cm.

Heavy: Check this box if the incisors and molars are worn to or near gumline, tips of canines are rounded, and molars are flattened with heavy pitting.

Med. (Medium): Check this box if the incisors and molars show wear but the wear does not extend as far as the gumline. Moderate pitting in the molars may be observed.

Light: Check this box if there is slight rounding of the cusps of the molars and incisors, little or no pitting in the molars.

None: Check this box if the fresh pointed canines, incisors, and cusps of molars show no flattening or pitting on them. This is usually observed in animals less than or equal to one year of age. Check to see if the molars are in the process of replacement with adult teeth.

Estimate of body fat stores:

Body fat stores in sea otters are evaluated at four levels:

- None: no visible fat on tissue.
- Little: some fat visually detected on tissue.
- Average: fat is easily detected on tissue.
- Abundant: fat is simple and covers much of the surface of the tissue.
- Fat is evaluated at four distinct sites on the animals.

Subcutaneous: Fat that is located between the skin and muscle layers that is readily visible after the animal has been skinned.

Groin: Fat in the area on the inside of the thigh where the leg meets the body. This is measured by slicing the fat layer with the scalpel and inserting a plastic ruler, measuring to the nearest 0.1cm.

Kidneys: Fat located on the top of the kidney and the fat occurring between the reniculi of the kidney itself. Whole right kidneys are being collected to attempt to develop a quantitative measure for this fat store.

Mesenteric: Fat associated with the supportive membranes which supply the intestines with blood.

ADDITIONAL SAMPLES LIST

Field ID Number: _____			Genus species: _____			
Was animal necropsied? ☒ Yes ☐ No						
Necropsied by: _____ (Please attach necropsy report)			dd / mm / yy Date			
Samples collected:						
Histological samples:						
Individual/Organization: _____			Final destination: _____			
Tissues sampled: (Choose all that apply)	☐ Liver	☐ Kidney	☐ Blubber	☐ Stomach	☐ Heart	☐ Intestine
	☐ Lung	☐ Pancreas	☐ Adrenals	☐ Brain	☐ Muscle	☐ Skin
	☐ Trachea	☐ Spleen	☐ Thymus	☐ Colon	☐ Thyroid	☐ Esophagus
Other: (Please list)						

Lymph Nodes: ☐ Submandibular ☐ Prescapular ☐ Axillary ☐ Hilar ☐ Mesenteric						
Other I.n.: _____						

Field ID Number is the unique identifier assigned to the animal from which the tissue(s) were obtained. Format is open to each agency's requirements; however, please remain consistent within your agency. This is automatically populated from Page 1.

Genus species is the Latin name for the animal in standard binomial nomenclature. It is carried over from NIST Sample Processing – Page 1.

Was animal necropsied?

Yes: Check this box if a full necropsy was undertaken to examine the carcass determining the cause of death as well as obtaining tissues samples for submission.

No: Check this box if a necropsy was not completely performed but tissues samples were obtained for submission.

Necropsy by: List the name and contact information of the primary person/facility who conducted the necropsy. In addition, attach a copy of the necropsy report to the tissue bank form.

Necropsy Date: Provide the date (DD/MM/YYYY) when the necropsy was done.

Samples collected: Indicate the tissue samples collected other than the NMMTB samples from the same animal for other research or diagnostic or archival purposes from the checklist provided in this section of the form.

Histological samples: If samples are collected for histopathological examinations, these are recorded here. This indicates that specimens taken from the same animal for histological analysis are used for the direct assessment of the animal's cause of death.

Individual/Organization: List the person or facility that conducted the removal of these tissue samples.

Final Destination: This is the final location of where the histological samples/slides will be sent for analysis.

Tissue Samples:

Tissue List: Check the box next to each tissue collected for histological analysis. These tissues are liver, kidney, blubber, stomach, heart, intestine, lung, pancreas, adrenals, brain, muscle, skin, trachea, spleen, thymus, colon, thyroid, and esophagus (i.e. check the box next to the liver if the liver was collected).

Other: List additional tissues that were collected for histological analysis that were not included in the tissue sample list above.

Lymph Nodes: Check the box next to each lymph node if that lymph node was collected for histological analysis (i.e. check submandibular if the submandibular lymph node was collected...).

Other l.n. (lymph nodes): List additional lymph node samples that were collected for histological analysis that were not included in the lymph node list above.

Other samples collected:	Type of storage: (Z-frozen, F-formalin, DMSO, ETOH)	Where located (Ind./Org.):
Teeth:		
Genetics (skin):		
Skull:		
Reproductive tract:		
Mammary tissue:		
Ovaries:		
Gonads/testes:		
Parasites:		
■ List type and location:		
Stomach:		
■ List contents if applicable:		
Other contaminant samples: <i>(List tissue type, storage type and where located)</i>		
Additional samples: <i>(List tissue type, purpose of collection, storage type and where located)</i>		

Other Samples Collected: Samples collected from the same animal for other research purposes are recorded in this section. This information includes the kind of sample (s): Teeth, Genetics (skin), Skull, Reproductive tract, Mammary tissue, Ovaries and Gonads/testes.

Type of Storage: (Z-frozen, F-Formalin, DMSO, ETOH-ethyl alcohol, other.)

Where located (Ind./Org.): Indicate the name and organization of the person (s) where the tissues are located.

Parasites (List type and location): In addition to type of storage and where located (Ind./Org.), also list the type of parasite (s) collected and where in the cavity the parasite came from and indicate the number of parasites (0-20, 21-100, 100+).

Stomach (List contents if applicable): In addition to type of storage and where located (Ind./Org.), identify food items if known and record some impression of degree of fullness. Note if internal parasites are present as well.

Other contaminants/biotoxin samples: (list the tissue type, storage type and where located). If other tissues are collected for contaminants or biotoxin analysis, list the tissue type sampled, storage type and where the tissue is being stored (Ind./Org.).

Additional samples: (list tissue type, purpose of collection, storage type and where located) If additional samples are collected that were not listed above, list tissue type, purpose of collection, storage type and where the sample is located (Ind./Org.).

Field ID Number:				Genus species:	
Photos taken:	☐ Yes ☐ No	☐ Digital ☐ Film	If yes, how many?		
Video taken:	☐ Yes ☐ No	(send copy with samples for NIST archive)			
Disposition:	(primary location for photos and/or video)				

Field ID Number is the identifier assigned to the animal from which the tissue(s) were obtained. Format is open to each agency's requirements; however, please remain consistent within your agency. It is carried over from NIST Sample Processing – Page 1.

Genus species is the Latin name for the animal in standard binomial nomenclature. It is carried over from NIST Sample Processing – Page 1.

Photos taken:

Yes: Check this box if photographs were taken of the tissue sample or animal.

Digital: Check this box if photographs were taken with a digital camera.

Film: Check this box if photographs were taken with a film camera.

If yes, how many?: Indicate the number of photographs taken of the tissue specimen or animal. In addition, send copy of images with the samples for NIST archive.

No: Check this box if there were no photographs taken.

Video taken:

Yes: Check this box if video was taken of the tissue sample or animal.

No: Check this box if there was no video taken.

Disposition (Primary location of photos and/or video): If photos and/or video were taken, list where the photos and/or video are located and the individual and organization responsible for the photos and/or video.

General comments:	
<i>(Field notes)</i>	
General appearance of individual:	
General appearance of organs:	
NMMTB Protocol: ☒ Standard ☐ Modified	
<i>Please note any modifications:</i>	

General comments (Field notes): Any additional comments which were obtained from the field that were not indicated elsewhere on the data sheet.

General appearance of individual: Any comments, which describe the healthy or unhealthy appearance of the animal, external parasites, evidence of trauma, or body condition of the animal.

General appearance of organs: Any unusual appearances of any of the internal organs, particularly those to be sampled. If they all appear normal, note this also.

NMMTB Protocol:

Standard: Check this box if there were no changes made to the protocol to obtain the tissues for the NMMTB.

Modified: Check this box if changes were made to the protocol to obtain the tissues for the NMMTB and indicate modifications below.

Please note any modifications: List the modifications made to the NMMTB protocol here.

NMMTB's Chain of Custody			
Field ID Number:			
Other ID Number:			
NMMTB Storage ID Numbers:			

1.	Collector's signature	Method of transfer to processing stage	dd / mm / yy Date
2.	Processor's signature	Method of transfer to shipping stage	dd / mm / yy Date
3.	Shipper to NMMTB's signature	Method of transfer to NIST Biorepository	dd / mm / yy Date
4.	Receiver's signature		dd / mm / yy Date

Each person in possession of the tissue must sign and date the form.

Form prepared by: Indicate the Name and Affiliation of the person (s) recording the data for the form.

Field ID: This is the identifier assigned to the animal from which the tissue(s) were obtained. Format is open to each agency's requirements; however, please remain consistent within your agency.

Other ID Number: This is the identifier related to the Field ID or identification of the animal. Examples include: previous field ID numbers if this animal previously stranded; ID numbers assigned by other organizations (including authorized rehabilitation facilities to which the animal is transferred), former identification numbers from scientific research projects, etc.

NMMTB/Reference/Storage ID Numbers: These numbers will be assigned by NIST personnel when the samples are received at the NMMTB in Charleston, SC.

Collector's Signature: This is the signature of the person that removed the tissue (collected) from the animal prior to processing the tissue.

Method of Transfer to Processing Stage: This is the method that was used to transfer the tissues from the collection site to the processing site, if applicable. (i.e. by hand, by truck, by cooler, Federal Express, DHL, UPS, USPS or indicate other if needed).

Date: This is the date (DD/MM/YYYY) that the samples were transferred from the collection site to the processing site.

Processor's Signature: This is the signature of the person that processed the tissue for eventual liquid nitrogen freezing and shipment to the NMMTB in Charleston, SC

Method of Transfer to Shipping Stage: This is the method that was used to transfer the tissues from the processing site to the shipping site, if applicable. (i.e. by hand, by truck, by cooler, Federal Express, DHL, UPS, USPS or indicate other if needed).

Date: This is the date (*DD/MM/YYYY*) that the samples were transferred from the processing site to the shipping site.

Shipper to NMMTB's Signature: This is the signature of the person that shipped the samples to the NMMTB in Charleston, SC.

Method of Transfer to MESB: This is the method that was used to transfer the tissues from the shipping site to the NMMTB in Charleston, SC. (i.e. by hand, by truck, by cooler, Federal Express, DHL, UPS, USPS or indicate other if needed).

Date: This is the date (*DD/MM/YYYY*) that the samples were transferred from the shipping site to the NMMTB in Charleston, SC.

Receiver's Signature: This is the signature of NIST personnel at the NMMTB in Charleston, SC that received the samples for archival.

Date: This is the date (*DD/MM/YYYY*) that the samples were received at the NMMTB in Charleston, SC.

References

- Becker, P.R., S.A. Wise, B.J. Koster, R. Zeisler. 1988. Alaskan Marine Mammal Tissue Archival Project: A Description Including Collection Protocols. U.S. Dep. Commer., National Bureau of Standards, NBSIR 88-3750. Gaithersburg, Maryland.
- Becker, P.R., S.A. Wise, B.J. Koster, R. Zeisler. 1991. Alaska Marine Mammal Tissue Archival Project: Revised Collection Protocol. USDOC, National Institute of Standards and Technology, NISTIR 4529. Gaithersburg, MD.
- Becker, P.R., Porter, B.J., Mackey, E.A., Schantz, M.M., Demiralp, R., and Wise, S.A. 1999. National Marine Mammal Tissue Bank and Quality Assurance Program: Protocols, Inventory, and Analytical Results. U.S. Department of Commerce, National Institute of Standards and Technology, NISTIR 6279. Gaithersburg, MD. 183 pp.
- Doroff, Angela M. and Daniel Mulcahy. 1997. A Field Guide to General Necropsy and Tissue Collection for Sea Otters (*Enhydra lutris*) in Alaska. U.S. Fish and Wildlife Service, Marine Mammals Management Technical Report: MMM 1997-03. 26pp.
- Geraci, J.R. and V.J. Lounsbury. 2005. Marine Mammals Ashore: A Field Guide for Strandings, Second Edition. National Aquarium in Baltimore, Baltimore, MD.

Appendix C. NIST Storage Datasheet

NIST SPECIMEN BANK																	
Sample Storage																	
		Storage ID				Wt (g)	Date In			Date Out			Initials				
		Fz	Rack	Box			Day	Mo	Yr	Day	Mo	Yr					
Comments																	
Subsamples		Storage ID				Wt (g)	Date In			Date Out			Initials				
LN2	Other	Fz	Rack	Box	XY		Day	Mo	Yr	Day	Mo	Yr					
B001																	
B002																	
B003																	
B004																	
B005																	
B006																	
B007																	
B008																	
B009																	
B010																	
B011																	
B012																	
B013																	
B014																	
B015																	
B016																	
B017																	
B018																	
B019																	
B020																	

Archived-NIST Datasheet no longer in use

Appendix D. NMMTB Specimen access policy



NATIONAL MARINE MAMMAL TISSUE BANK SPECIMEN ACCESS POLICY

The goal of the National Marine Mammal Tissue Bank (NMMTB) is to maintain quality controlled marine mammal specimens that will permit retrospective analyses to determine such things as environmental trends of contaminants and other analytes of interest and that will provide the highest quality specimens for analyses using new and innovative techniques. The NMMTB was formally established in 1992 by Public Law 102-587. This legislation gives lead responsibility to the National Oceanic and Atmospheric Administration (NOAA) and designates the Department of the Interior (DOI) as cooperator for establishing and managing this specimen bank.

The National Marine Fisheries Service (NMFS), Office of Protected Resources (OPR), is the NOAA line organization responsible for the NMMTB. The NMMTB is a component of NMFS Marine Mammal Health and Stranding Response Program (MMHSRP). Through an agreement between NMFS and the National Institute of Standards and Technology (NIST), the NMMTB is maintained at the NIST Biorepository, located at the Hollings Marine Laboratory in Charleston, South Carolina, as part of the National Marine Analytical Quality Assurance Program. The U.S. Fish and Wildlife Service (USFWS), the former Minerals Management Service (MMS), and the U.S. Geological Survey Biological Resources Division (USGS/BRD) are the DOI agencies cooperating in the NMMTB. These Federal agencies, NMFS, USFWS, USGS/BRD, and NIST, are defined as the NMMTB Collaborating Agencies.

Additional information on the history of the MMHSRP and the NMMTB can be found on the following NMFS website:
<https://www.fisheries.noaa.gov/national/marine-mammal-protection/national-marine-mammal-tissue-bank>

The scientific community, contributors, and principal investigators can request specimens from this repository for retrospective analyses investigating environmental trends of contaminants and other analytes of interest. The NMMTB collects, processes, and stores specimens from specific species (e.g., Atlantic bottlenose dolphins, Atlantic white sided dolphins, pilot whales, harbor porpoise) obtained from mass strandings; animals that have been trapped, injured or killed incidental to commercial fisheries; animals taken for subsistence purposes; animals from which biopsies have been obtained; and animals from unusual mortality events.

Specimen Description

Each specimen consists of two duplicates, ≤ 150 g each (denoted "A" and "B"), and these duplicates are banked in the NMMTB in separate liquid nitrogen vapor-phase freezers and are maintained at ≤ -150 °C. If a portion of a specimen is requested for analysis, the "B" specimen can be cryogenically homogenized and aliquoted into approximately 20 subsamples of <6 g. The "A" specimen remains as a bulk specimen and will only be homogenized after all portions from the corresponding "B" has been depleted and there is sufficient justification to homogenize the

remaining material. Thus, 50 % of each specimen is available to the scientific community for research and scientific evaluations consistent with the goal of the NMMTB and 50 % is intended for long-term storage. If a blood sample is collected, it is processed immediately into multiple aliquots. The aliquots are split with half of them being stored in one liquid nitrogen vapor-phase freezer and the other half in a different liquid nitrogen vapor-phase freezer.

Each “B” specimen is divided into three categories (Table 1). Category 1, which is 10 % of the homogenized material, is reserved for baseline analyses. Category 2 consists of 50 % of the specimen and is reserved for use by the specimen collector(s)/contributor(s). Category 3 constitutes 40 % of the specimen and is available to the scientific community for research that is consistent with the goal of the NMMTB and the MMHSRP.

If an “A” specimen is eventually homogenized, it is divided into the following four categories. Category 1 consists of 10 % of the specimen for baseline analyses. Category 2 consists of 25 % of the specimen reserved for use by the specimen contributors. Category 3 consists of 25 % of the specimen available to the scientific community. Category 4 contains the remaining 40 %, which is intended as a permanent archive. Category 4 will not be used unless a very high need can be identified by NOAA and DOI. Combining the A and B specimens, the specimen allocations for each use are as follows: Category 1 = 10 %, Category 2 = 37.5 %, Category 3 = 32.5 %, and Category 4 = 20 %.

Table 1. Relative amounts of specimen for each NMMTB category

(Category) Use	Specimen A = 150 g		Specimen B = 150 g	
	Long-Term Archival Weight (g)	Portion of Specimen (%)	Available for Homogenization Weight (g)	Portion of Specimen (%)
(1) Baseline Analyses	15	10%	15	10%
(2) Contributor	37.5	25%	75	50%
(3) Scientific Community	37.5	25%	60	40%
(4) Long-Term Archival	60	40%		

ACCESS PROCEDURES

Category 1, Baseline Analyses

Baseline analyses may be conducted by a Federal Laboratory to establish values of analytes of interest for a specimen. These baseline values may be used to monitor specimen stability during storage and, for QA purposes, to compare with analytical data being generated on the same specimens (and/or similar specimens from the same individual animals) by collaborating monitoring and research programs. The baseline database may also be used in NMMTB planning and development (i.e., evaluating collection protocols, evaluating sampling procedures required for adequate statistical analysis, etc.). NIST and NMFS, in consultation with USGS/BRD,

USFWS, and the Contributor(s) have the primary responsibility for baseline analyses, the details of which will be documented in the annual work statement of each agency.

Category 2 and 3 Applicants

The scientific community, contributors, and principal investigators for research can request specimens for retrospective studies. The Contributor is an organization that contributes specimens, funds, or personnel for collecting or archiving specimens in the NMMTB (see below under the section, Major Contributors, To-Date). A principal investigator is an individual primarily responsible for the taking, importation, export, and any related activities conducted under a permit issued for scientific research or enhancement purposes.

Of particular importance in determining whether a specimen request by the scientific community will be granted is the justification that the use being proposed is consistent with the goals of the NMMTB (page1, paragraph1) and the MMHSRP; and comparable specimens necessary to accomplish the goals of the proposed research could not be readily obtained from other sources. The goals of the MMHSRP are to facilitate the collection and dissemination of reference data on marine mammals and health trends of marine mammal populations in the wild; to correlate the health of marine mammals and marine mammal populations in the wild with available data on physical, chemical, and biological environmental parameters; and to coordinate effective responses to unusual mortality events.

The applicant should keep in mind that only a very limited amount of a specimen may be available and that the NMMTB emphasizes the intended use of banked specimens for retrospective studies.

How To Apply:

1. Applicants must submit the completed NMMTB Specimen Request Form Package, to the NIST Biospecimen Science Group Leader (identified below under the section, Principal Representatives of the NMMTB Collaborating Agencies).
2. The following specific information must be included in the request:
 - a. A clear and concise study plan which includes a statement of the proposed use of the banked specimens. The applicant must justify the use of the banked specimens and demonstrate that the proposed use is consistent with the goals of the NMMTB and the MMHSRP (described above);
 - b. A copy of the applicant's scientific research permit. The applicant must demonstrate that the proposed use of the banked specimens is authorized by the permit;
 - c. Name of principal investigator, official title, and affiliated research or academic organization;
 - d. Table 1. Individual Specimen Information for Tissues Requested from the National Marine Mammal Tissue Bank, completed which includes specific specimen data and quantity desired;

- e. Research facility where analyses will be conducted. The applicant must demonstrate that the research facility will follow the Analytical Quality Assurance (AQA) program, which was designed to ensure the accuracy, precision, level of detection, and intercompatibility of data resulting from chemical analyses of marine mammal tissues. The AQA consists of annual interlaboratory comparisons and the development of control materials and standard reference materials for marine mammal specimens. Standard Reference Materials® for use in the analysis of marine mammal specimens can be purchased from the NIST
- f. Verification that funding is available to conduct the research;
- g. Estimated date for completion of research, and schedule/date of subsequent reports;
- h. Signed copy of the NMMTB Agreement Form which indicates that (1) all research/findings based on use of the banked specimen will be reported to NIST, the NMMTB/MMHSRP Program Manager, and the contributor; and (2) specimen that are used/released for genetic analyses (DNA sequencing) are required to archive sequences in the National Center for Biotechnology Information's GenBank. Sequence accessions in GenBank should document the source, citing an animal field identification number that identifies the animal; and
- i. Agreement that credit and acknowledgment will be given to U.S. Fish and Wildlife Service (USFWS), U.S. Geologic Service (USGS), NMFS, NIST, former MMS, the NMMTB, and the collector for use of banked specimens. The applicant shall insert the following acknowledgment in all publications, abstracts or presentations based on research using the banked specimen:

The specimens used in this study were collected by [the contributor] and provided by the National Marine Mammal Tissue Bank, which is maintained at the NIST Biorepository at the Hollings Marine Laboratory, Charleston, SC and which is operated under the direction of NMFS with the collaboration of USGS, USFWS, former MMS, and NIST through the Marine Mammal Health and Stranding Response Program (and the Alaska Marine Mammal Tissue Archival Project [if the specimens are from Alaska]).

3. Upon submission of a complete application package, the NIST Biospecimen Science Group Leader will send the Request Form Package to the following entities which will function as the review committee:

- a. Appropriate Federal agency (NMFS or USFWS) marine mammal management office for that particular species, and
- b. Representatives of the NMMTB Collaborating Agencies (NMFS, USFWS, USGS Biological Resources Division, and NIST). If no member of the review committee is an expert in the field that is related to the proposed research activity, any member may request an outside review of the proposal, which may be outside of NMFS or USFWS but within the Federal government.

4. The NIST Biospecimen Science Group Leader will send the request and attached study plan to any contributor(s) of the specimen. The contributor(s) of the specimen may submit comments on the proposed research activity to the NIST Biospecimen Science Group Leader who will provide comments to the MMHSRP Program Manager.

5. Review committees for requests involving species managed by the DOI will be chaired by the USFWS Representative of the NMMTB Collaborating Agencies. All other review committees will be chaired by the NIST Biospecimen Science Group Leader in coordination with the MMHSRP Program Manager.

6. Recommendations on the request and an evaluation of the study plan will be provided by each committee chair to the NIST Biospecimen Science Group Leader

7. The MMHSRP Program Manager will make the final decision on release of the specimens based on the advice provided by the review committee, comments received from any contributor(s) of the specimen within the time provided in Section 4, and determination that the proposed use of the banked specimen is consistent with the goals of the MMHSRP and the NMMTB. The NIST Biospecimen Science Group Leader will send a written decision to the applicant and send copies to all review committee members. If the specimens are released, the response will indicate whether the specimens have been homogenized and, if not, the homogenization schedule.

The average time for review of the request and the mailing of the written response to the requester will be 90 working days from receipt of the request by the committee chair. However, the NIST Biospecimen Science Group Leader will respond in writing no later than 90 days following receipt of the letter of request.

8. Shipping and homogenization costs related to the use of any specimens from the NMMTB will be borne by the applicant.

9. The applicant will dispose of any remaining specimen after the research is completed unless the requester submits another request for research and receives approval in accordance with the procedures listed above. The request must be submitted within three months after the original project has been completed.

Category 4, Long-Term Archival

Specimens of Category 4 cannot be withdrawn until all other categories of a specimen have been depleted. When Category 4 specimen are withdrawn, the same review process will be followed as described for Category 3 with the final determination made jointly by the Representatives of the NMMTB Collaborating Agencies and specific Contributors.

PRINCIPAL REPRESENTATIVES OF THE NMMTB COLLABORATING AGENCIES

Sarah Wilkin
MMHSRP Program Manager
U.S. Department of Commerce
NOAA/NMFS/PR2
1315 East-West Highway
Silver Spring, Maryland 20910-3282

Rebecca S. Pugh
Leader, Biospecimen Science Group
U.S. Department of Commerce
NIST
Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, South Carolina 29412

U.S. Department of the Interior
USGS Biological Resources Division
Alaska Science Center
Bldg. SSMC-3, Room 13752
1011 East Tudor Road
Anchorage, Alaska 99503

Monty Kodis
U.S. Department of the Interior
USFWS

DEPARTMENT OF THE INTERIOR REPRESENTATIVES FOR SPECIMEN ACCESS REVIEW

For walrus, N sea otter, polar bear:

USGS Biological Resources Division
Alaska Science Center
1101 E. Tudor Rd
Anchorage, Alaska 99503

U.S. Fish and Wildlife Service
Marine Mammals Management
Alaska Science Center
1101 E. Tudor Rd
Anchorage, Alaska 99503

For Manatees:

Robert K. Bonde, Ph.D., Sirenia Project
USGS Biological Resources Division
Florida Caribbean Science Center
412 NE 16th Avenue, Room 250
Gainesville, Florida 32601

Jim Valade, Lead Recovery Biologist and/or
Species Expert
U.S. Fish and Wildlife Service
Jacksonville Field Office
6620 South Point Drive, Suite 310
Jacksonville, Florida 32216-0958

For southern sea otter:

USGS Biological Resources Division
Western Fisheries Research Center
6505 NE 65th Street
Seattle, Washington 98113

U.S. Fish and Wildlife Service
Ventura Field Office
24993 Portola Rd, Suite B
Ventura, CA 93003

DEPARTMENT OF COMMERCE REPRESENTATIVES FOR SPECIMEN ACCESS REVIEW

For cetaceans and pinnipeds (except walrus), depending on region or species managed:

NMFS
Alaska Regional Office
Protected Resources Division
P.O. Box 21668
Juneau, Alaska 99802

NMFS,
Alaska Fisheries Science Center y
National Marine Mammal Laboratory
7600 Sand Point Way N.E.
Seattle, Washington 98115-6349

NMFS
Southwest Fisheries Science Center
Protected Resources Division
8604 La Jolla Shore Drive
La Jolla, California 92037-1508

NMFS,
Northeast Regional Office
Protected Resources Division
One Blackburn Drive
Woods Hole, Massachusetts 01930-2298

NMFS
Southeast Fisheries Science Center
Protected Resources and Biodiversity Branch
Miami, Florida

NMFS
Northwest Fisheries Science Center
Environmental Conservation Division
2725 Montlake Blvd., East
Seattle, Washington 98112-2097

NON-FEDERAL CONTRIBUTORS, TO-DATE:

North Slope Borough Department of Wildlife Management, Barrow, Alaska
The Marine Mammal Center, Sausalito, California
Minerals Management Service, Alaska OCS Environmental Studies Division, Anchorage, Alaska
St. Paul Tribal Government, St. Paul Island, Alaska
Kawerak, Inc., Natural Resources Division and Eskimo Walrus Commission, Nome, Alaska
Kotzebue, IRA, Kotzebue, Alaska
Alaska Sea Life Center, Seward, Alaska
Alaska Department of Fish and Game, Juneau, Alaska
New England Aquarium, Boston, Massachusetts
University of North Carolina at Wilmington, North Carolina
Hubbs Sea World Research Institute, Orlando, Florida
Sea World, San Diego, California
Hawaii Pacific University, Waimanalo, Hawaii
University of Hawaii, Honolulu, Hawaii

Appendix E. National Marine Mammal Tissue Bank Specimen Request Form



National Marine Mammal Tissue Bank

Specimen Request Form

Principle Investigator(s): _____

Investigator(s) Title: _____

Affiliated Institution(s): _____

Address: _____

E-mail: _____ Phone: _____

1) Requirements for Specimens:

Total Number of Specimens Requested: _____

Total Number of Animals Requested: _____

Total Number of Each Specimen Type Requested: Liver _____ Blubber _____

Kidney _____ Blood _____

Other _____

List other type: _____

Title of Project: _____

Research Location: _____

Type of Research: _____

Estimated Date of Completion: _____

Attach the following items:

- 1) A description of the proposed research including a justification as to the use of the banked specimens and how this is consistent with the goals of the NMMTB and the MMHSRP;
- 2) Copy of the scientific research permit;
- 3) Verification that funding is available to conduct the research;
- 4) Table 1 filled out completely;
- 5) A signed copy of the National Marine Mammal Tissue Bank Agreement Form

Table 1. Individual Specimen Information for Specimens Requested from the NMMTB.

	Field ID	NMMTB Storage ID	Species	Common Name	Tissue Requested	Year Collected	Sex	Age Class	Pregnant or Lactating	Size of Sample Needed (i.e. 1mL, 2 g, 5 g)
ex.	MMES2004100SC	NM10L101C	Tursiops truncatus	Bottlenose dolphin	Liver	2004	F	Adult	No	2 g
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										



National Marine Mammal Tissue Bank Agreement Form

I, _____, agree to the following conditions upon acceptance of my proposed research on specimens taken from the National Marine Mammal Tissue Bank:

- 1) Research/findings based on use of the banked specimens will be reported to the National Marine Mammal Tissue Bank, Marine Mammal Health and Stranding Response Program Manager, and the specimen contributor;
- 2) Specimens that are used/released for genetic analyses (DNA sequencing) are required to archive sequences in the National Center for Biotechnology Information's GenBank. Sequence accessions in GenBank should document the source, citing the NMMTB Field ID number that identifies the animal;
- 3) Credit and acknowledgment will be given to U.S. Fish and Wildlife Service (USFWS), U.S. Geological Survey (USGS), National Marine Fisheries Service (NMFS), National Institute of Standards and Technology (NIST), the (former) Mineral Management Service (MMS), the National Marine Mammal Tissue Bank (NMMTB), and the specimen collector for use of the banked specimens. I shall insert the following acknowledgment in all publications, abstracts or presentations based on research using the banked tissue:

The specimens used in this study were collected by [the contributor] and provided by the National Marine Mammal Tissue Bank, which is maintained at the NIST Biorepository at the Hollings Marine Laboratory, Charleston, SC, and which is operated under the direction of NMFS with the collaboration of USGS, USFWS, former MMS, and NIST through the Marine Mammal Health and Stranding Response Program (and the Alaska Marine Mammal Tissue Archival Project [if the specimens are from Alaska]).

Signature

Date

Affiliation