

## **The Marine Environmental Specimen Bank (Marine ESB): A Research and Environmental Monitoring Resource**

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**Abstract**—The Marine Environmental Specimen Bank (Marine ESB) was established in 2001 by the National Institute of Standards and Technology (NIST) at the Hollings Marine Laboratory (HML) in Charleston, South Carolina, USA. The Marine ESB is designed to cryogenically bank environmental specimens as part of ongoing research and monitoring programs conducted in the marine and coastal environments. Banked specimens include: mussels, oysters, fish tissues, marine sediments, marine mammal tissues, bird eggs and feathers, and sea turtle tissues. Because of the unique goals of the U.S. government agency projects providing these specimens, special features have been considered in the design of the bank facility. The Marine ESB consists of approximately 80 m<sup>2</sup> of non-metallic, certified ISO Class 5 and 240 m<sup>2</sup> of ISO Class 7 clean room space, along with 60 m<sup>2</sup> of support, ante rooms and office space. The Marine ESB contains liquid nitrogen (LN<sub>2</sub>) vapor phase freezers (−150°C) and −80°C ultra-cold upright freezers for sample storage, and equipment for preparing frozen samples for chemical analysis. A Reference Material Production (RMP) Facility, for preparing Certified Reference Materials (CRM) and analytical control materials, occupies part of the ISO Certified Class 7 clean room space and also uses cryogens in its operations. Special safety features and emergency preparedness plans are in place to ensure successful operations of the RMP facility and Marine ESB. This paper discusses the development of the Marine ESB, including the overall design of the facility, as well as describes current specimen banking projects.

**Keywords:** environmental specimen bank, marine animal, retrospective analysis, biospecimen

### INTRODUCTION

The National Institute of Standards and Technology (NIST) has conducted long-term biological and environmental specimen banking for over 25 years through programs associated with two NIST facilities, the National Biomonitoring

Table 1. Projects of the National Biomonitoring Specimen Bank (NBSB) at the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA. EPA, Environmental Protection Agency; NOAA, National Oceanic and Atmospheric Administration; IAEA, International Atomic Energy Agency; USDA, U.S. Department of Agriculture; FDA, Food and Drug Administration; DOI, Department of the Interior.

Dates	Specimens	Program or Agency
1979–1994	Human livers	Joint EPA/NIST Pilot Environmental Specimen Bank Program
1976–1978 1983–1992	Mussels and oysters	EPA/NOAA Mussel Watch, and NOAA National Status and Trends Program
1985–1992	Fish livers and muscle	NOAA National Status and Trends Program
1985–1992	Marine sediments	NOAA National Status and Trends Program
1986	Human food specimens	Nutrients in Human Diet Project (IAEA/USDA/FDA)
1986	Human blood serum	National Cancer Institute
1987–1995	Marine mammal tissues* from Alaska	Alaska Marine Mammal Tissue Archival Project (DOI)
1990–1995	Marine mammal tissues* from the Lower-48 States	National Marine Mammal Tissue Bank Program, and Marine Mammal Health and Stranding Response Program (NOAA)
1994	Fish (whole)	NOAA
1994	Human blood spots	Department of Defense

\*Liver, kidney, fat, muscle, brain.

Specimen Bank (NBSB), established at NIST in 1979 in Gaithersburg, Maryland and the Marine Environmental Specimen Bank (Marine ESB), established in 2001 at the Hollings Marine Laboratory in Charleston, South Carolina. The NBSB was the result of a pilot Environmental Specimen Bank Program that was sponsored by the U.S. Environmental Protection Agency (EPA) to collect and cryogenically bank human liver specimens for monitoring contaminant trends and future retrospective analyses for new analytes of interest (Wise and Zeisler, 1984). The success of the human liver project led to the additional programs at the NBSB, which were developed during the 1980's and 1990's in collaboration with other U.S. Government Agencies (Table 1). Various types of biological and environmental specimens were collected, including mussels and oysters, human food specimens, human blood serum, human blood spots and marine mammal tissues.

In 1995, NIST established a temporary ESB facility at a National Oceanic and Atmospheric Administration (NOAA) Laboratory in Charleston, SC. The temporary ESB became the lead facility for all on-going NBSB projects involving the banking of marine specimens. In 2001, the Hollings Marine Laboratory

Table 2. Projects of the Marine Environmental Specimen Bank (Marine ESB) at the National Institute of Standards and Technology (NIST), Hollings Marine Laboratory, Charleston, SC. EPA, Environmental Protection Agency; NOAA, National Oceanic and Atmospheric Administration; IAEA; DOI, Department of the Interior.

Dates	Specimens	Program or Agency
2004–present	Mussels and oysters	EPA/NOAA Mussel Watch
1995–present	Marine mammal tissues* from Alaska	Alaska Marine Mammal Tissue Archival Project (DOI)
1995–present	Marine mammal tissues* from the Lower-48 States	National Marine Mammal Tissue Bank Program, and Marine Mammal Health and Stranding Response Program (NOAA)
1999–present	Egg contents	Seabird Tissue Archival and Monitoring Project (DOI)
2003–present	Egg contents and feathers	American Peregrine Falcon Project (DOI)

\*Liver, kidney, fat, muscle, blood, milk, skin.

(HML), a collaborative research laboratory involving five partners, NOAA, NIST, the South Carolina Department of Natural Resources (SCDNR), the College of Charleston, and the Medical University of South Carolina (MUSC), was completed and the Marine ESB established by NIST at the HML. All of the samples and associated equipment that were acquired from 1995–2001 were moved from the temporary ESB facility into the Marine ESB.

The Marine ESB and NBSB facilities function as a single environmental specimen bank program but the Marine ESB is specifically devoted to the cryogenic banking of well-documented environmental specimens collected as part of ongoing research and monitoring programs conducted in marine and coastal environments (Pugh *et al.*, 2008). The current projects developed through the NBSB and Marine ESB provide a resource of specimens that are used to: 1) address questions regarding temporal and geographic trends in environmental contamination; 2) evaluate the feasibility of long-term storage of environmental samples under various conditions; 3) address new analytes of interest and improved analytical techniques; and 4) look at changes in ecosystem structure and function, genetic separation of populations of animals, and the health status of marine animals.

#### CURRENT PROJECTS OF THE MARINE ESB

Marine animal specimens archived at the Marine ESB are collected through various projects in collaboration with other government agency research and monitoring programs and/or non-profit organizations (Table 2). Banking for two NOAA programs, the National Marine Mammal Tissue Bank (NMMTB) and the National Status and Trends (NS&T)—Mussel Watch Program began prior to the construction of the Marine ESB. Therefore, collections for these projects prior to

2001 are stored at the NBSB facility but all activities, including current collections from these projects, as well as new specimen banking projects that began after 2001 are maintained at the Marine ESB.

#### *National Status and Trends Mussel Watch Program*

NOAA's NS&T Mussel Watch Program began in 1986 and is the longest continuous contaminant monitoring program in U.S. coastal waters. Mussels, oysters, fish, and sediment are collected to quantify the long-term trends in concentrations of selected contaminants in biological indicator organisms and environmental matrices of coastal and estuarine areas in the U.S. (Wise *et al.*, 1993). Samples are collected routinely from over 300 U.S. coastal sites and analyzed for over 100 organic and inorganic contaminants. In addition to collecting mussel and oyster samples for real-time analysis, samples are also collected following NIST protocols and shipped to the NBSB for banking (Lauenstein *et al.*, 1987). Although suspended in 1992, the banking component of Mussel Watch was reinstated in 2004. The protocols were updated and the lead for banking assumed by the Marine ESB. To date, the Marine ESB has received specimens from over 150 collection sites.

#### *National Marine Mammal Tissue Bank (NMMTB)*

In 1987, NOAA, in collaboration with NIST began the NMMTB for the long-term cryogenic archival of marine mammal tissues (Lillestolen *et al.*, 1993; Becker *et al.*, 1999). The NMMTB is an important component of NOAA's Marine Mammal Health and Stranding Response Program (MMHSRP). In 1992 it was legally established by the Marine Mammal Health and Stranding Response Act (Public Law 102-587). Specimens are collected for the NMMTB through two projects, the Alaska Marine Mammal Tissue Archival Project (AMMTAP) and the MMHSRP.

NIST began the AMMTAP in 1987 in collaboration with the U.S. Minerals Management Service (MMS), and later with support by the U.S. Geological Survey (USGS) and NOAA. Standard collection and banking protocols were developed by NIST for blubber (or fat), liver, kidney, and muscle collected from animals taken by Alaska Natives during subsistence hunts (Becker *et al.*, 1988, 1991, 1993). In 1990 these same protocols were modified for use by NOAA's MMHSRP to collect marine mammal specimens throughout the coastal U.S. from individual and mass strandings, unusual mortality events, and from animals incidentally caught in commercial fishing operations (Becker *et al.*, 1999). Over 20 government and academic agencies, non-profit organizations, and stranding network volunteers now collect tissues for the NMMTB. Currently there are over 3,500 specimens collected from 1,235 animals from 46 species of cetaceans, pinnipeds, and fissipeds.

#### *Seabird Tissue and Archival Monitoring Project*

In 1999 NIST, USGS, and the U.S. Fish and Wildlife Service (USFWS),

began the Seabird Tissue and Archival Monitoring Project (STAMP). STAMP is a systematic, long-term program (decades) to identify and track anthropogenic contaminants in Alaskan seabirds over time (York *et al.*, 2001). Five species of seabird eggs are collected annually from 44 seabird colonies located throughout the Alaska Maritime National Wildlife Refuge (AMNWR) and other areas (York *et al.*, 2001). The Bureau of Indian Affairs (BIA) joined this collaboration in 2004, and additional financial support for the project was provided by the North Pacific Research Board in 2005. Over 1,400 egg specimens have been collected by AMNWR, USGS, USFWS Office of Migratory Bird Management (MBM), and University of Alaska-Fairbanks (UAF) biologists and local coastal community residents using standard protocols developed by NIST (York *et al.*, 2001, Vander Pol *et al.*, 2009).

#### *Bottlenose dolphin and beluga whale health assessment project*

In 2002, NIST in collaboration with NOAA, the Chicago Zoological Society, and Mote Marine Laboratory developed protocols for the collection, processing and banking of blubber, skin, blood (whole blood, plasma, and serum), and milk from live bottlenose dolphins (*Tursiops truncatus*) captured and released as part of ongoing animal health assessment studies throughout the coastal U.S. In 2008, these protocols were adopted by NOAA for collecting and banking beluga whale (*Delphinapterus leucas*) samples collected during health assessment studies in Alaska. Currently, there are over 3,000 specimens stored at the Marine ESB.

#### *American peregrine falcon project*

Through a 2003 agreement between the USFWS and NIST, egg contents and feather specimens from American peregrine falcons (*Falco peregrines anatum*) are collected throughout the U.S. and banked for future chemical analyses. These specimens are collected in support of the *Monitoring Plan for the American Peregrine Falcon: A Species Recovered Under the Endangered Species Act* as a part of the post-delisting monitoring plan (USFWS, 2003). In the final delisting rule, the potential threat that environmental contaminants pose to the sustained recovery of the American peregrine falcon warranted a contaminant monitoring component of the plan (USFWS, 2003). Presently, 120 egg contents and feather specimens collected throughout the seven designated monitoring regions have been archived at the Marine ESB.

### THE MARINE ENVIRONMENTAL SPECIMEN BANK FACILITY

#### *Clean air laboratories*

The Marine ESB facility occupies 390 m<sup>2</sup> of space inside the Hollings Marine Laboratory (Fig. 1) and consists of high-efficiency particulate air (HEPA)-filtered clean air laboratories (ISO Certified Class 5, 6, and 7 clean rooms) and associated office space. The clean room space is divided into three interconnected laboratories; an ISO Class 5 Clean Room, ISO Class 7 Freezer Room, and ISO

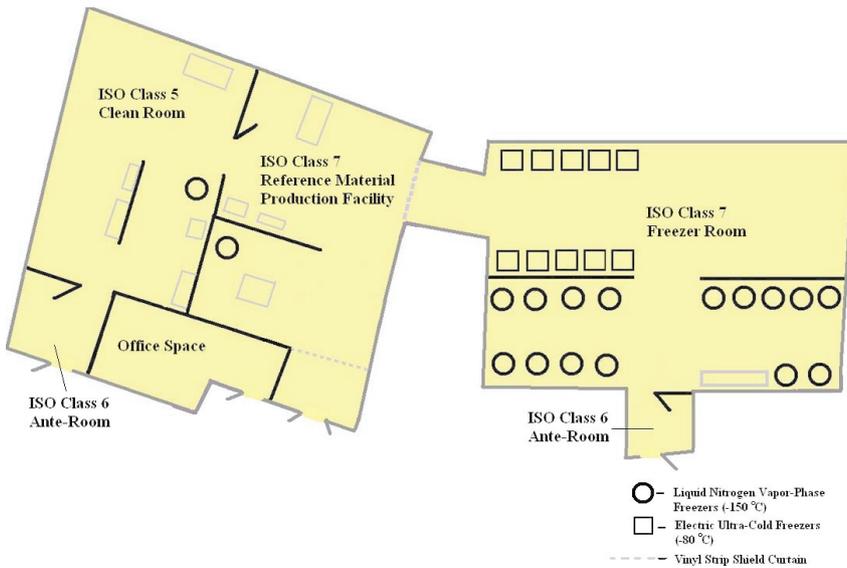


Fig. 1. Marine Environmental Specimen Bank floorplan.

### Class 7 Reference Material Production (RMP) Facility.

The ISO Class 5 Clean Room is used to prepare and cryogenically homogenize specimens for analyses. The cryogenic homogenization procedure transforms a solid frozen tissue into a powder and provides identical (i.e. homogenous) sample aliquots. This process has been previously described in detail (Zeisler *et al.*, 1983; Pugh *et al.*, 2007). Supplies and equipment needed for the cryogenic homogenization process and supplies needed for field collections kits (i.e. Teflon jars, cryovials, and titanium knives) are also cleaned in the ISO Class 5 Clean Room. The procedures for cleaning have previously been described (Pugh *et al.*, 2007).

The ISO Class 7 Freezer Room consists of liquid nitrogen vapor-phase freezers ( $-150^{\circ}\text{C}$ ) and mechanical upright freezers ( $-80^{\circ}\text{C}$ ). Specimens that are collected for long term banking are archived in the liquid nitrogen vapor-phase freezers while reference materials and control materials are stored in the mechanical upright freezers. The Marine ESB was constructed to accommodate 26 liquid nitrogen vapor-phase freezers and ten mechanical upright freezers. A data entry station is also located in the Freezer Room so that biological data and storage location information for each specimen can immediately be logged into the computerized tracking system after the specimens are stored in the freezers.

The ISO Class 7 RMP Facility is located within the Marine ESB, between the ISO Class 5 Clean Room and the ISO Class 7 Freezer Room. Large quantities of frozen materials (i.e. mussels, fish tissue, marine mammal tissue) are cryogenically

prepared to create certified standard reference materials, and analytical control materials for interlaboratory comparison exercises. The frozen materials are cryogenically homogenized using a large volume vibrating cryomill that generates a fresh, frozen powder material. The reference or control materials can also be freeze-dried using a large capacity freeze-drier that is also located in the RMP Facility.

There are two ISO Class 6 ante-rooms that provide direct access to the ISO Class 5 Clean Room and the ISO Class 7 Freezer Room. The ante-rooms are used by Marine ESB personnel to change into proper “clean” garments before entering the clean air laboratories and provide space to properly clean all materials and supplies that are brought into the main rooms following strict protocols (Pugh *et al.*, 2007).

### *Special features*

The Marine ESB has a number of unique features that maintain the highest quality specimens. These characteristics are consistent with the overall NIST philosophy for environmental specimen banking.

Individual collection and banking protocols are carefully developed for each project. Although each protocol is unique to the individual project, all insure sample integrity and minimize inadvertent contamination during sample collection, processing, and banking. The specimen collection requirements that have been considered when designing collection procedures for specimens selected for banking have been discussed in detail (Wise and Koster, 1995; Pugh *et al.*, 2008). The standard protocols are published as NIST internal reports and are updated if changes in the protocols are warranted.

A computerized sample inventory database system is used to track each specimen within each freezer. All data associated with a specimen including field data (i.e. collection location, collection date, specimen identification number, etc.), processing data (i.e. processing location, processing date, etc.), and analytical data are maintained in the database system.

A security system and electronic monitoring of freezer temperatures and room conditions (temperature, percent humidity, and percent oxygen) are maintained on a 24 hour-a-day/7 days-a-week system. The room temperature, percent humidity, and percent oxygen are monitored for safety and to insure that clean room certifications are maintained. If preset conditions are exceeded, the monitoring system will alarm and a phone identification system will be enabled to alert the Marine ESB personnel of the alarm. This system ensures that trained personnel will be able to correct the problem or error within the Marine ESB before any damage has occurred to the freezer(s) and/or specimens.

All freezers are connected to a liquid nitrogen bulk tank located outside of the building, with a vacuum jacketed insulated in-line piping system. This ensures that the pipeline is kept at cryogenic temperatures with minimum liquid loss. A telemetry system on the liquid nitrogen bulk tank provides the commercial liquid nitrogen supplier with daily readings on the tank to ensure automatic liquid

nitrogen delivery when required. There is also a separate liquid nitrogen fill station near the bulk tanks so that biological dry shippers and dewars can be filled as needed for shipping and receiving specimens.

### *Safety features*

There are a number of safety features, equipment, and protocols that are used throughout the Marine ESB. General safety laboratory protocols include Chemical Hygiene Plan, Chemical Waste Management Plan, Bloodborne Pathogens Plan, and Emergency Preparedness Plan. These and other safety protocol requirements are described in detail in Pugh *et al.* (2007).

All of the liquid nitrogen vapor-phase freezers have auto-fill capabilities and the upright mechanical freezers have a liquid nitrogen back-up system installed on each freezer. All freezers and associated equipment are connected to an uninterruptible power supply (UPS) so that if there is a power loss, the equipment will automatically be connected to the building's back-up diesel generator. The freezers are also connected to the 24 hour-a-day/7 day-a-week environmental monitoring control system that was previously described.

Duplicate specimens from within a project are stored in different freezers to ensure that in the case of a freezer failure or catastrophic event (i.e. hurricane, fire, flood, etc.), the entire specimen or set of specimens is not destroyed. Also, the computerized data associated with each specimen is backed-up on a daily basis.

### SUMMARY

The Marine ESB is a research and environmental monitoring resource that allows researchers to answer questions on trends in newly recognized environmental contaminants and to verify past analytical results. Through ongoing environmental monitoring projects, marine specimens, including marine mammal tissues and blood, mussels and oysters, and bird egg contents and feathers, are collected and cryogenically stored following carefully designed standard protocols. The design of the Marine ESB, including the HEPA-filtered clean air laboratories, the cryogenic homogenization system for sample preparation, the computerized sample inventory and tracking system, computerized security and monitoring systems and redundancy to minimize sample loss due to equipment or system failure are features that insure the safety and stability of the samples for 50–100 years. In the future the Marine ESB will incorporate new projects with specimens from additional species of seabirds and from sport fish species, marine sediments from additional locations, and other specimens used for genetics, biotoxin exposure, and infectious disease research.

*Disclaimer*—Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the procedure employed. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best for the purpose.

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