NISTIR 8281

Dolphins In Human Care Sampling Protocol and Sample Entry Assistant (SEA) for Tissue Archival and Analyte Analysis

Colleen E. Bryan
Jared M. Ragland

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8281

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

NISTIR 8281

Dolphins In Human Care Sampling Protocol and Sample Entry Assistant (SEA) for Tissue Archival and Analyte Analysis

Colleen E. Bryan
Jared M. Ragland
Chemical Sciences Division
Material Measurement Laboratory

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8281

January 2020

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

National Institute of Standards and Technology
Walter Copan, NIST Director and Undersecretary of Commerce for Standards and Technology

National Institute of Standards and Technology Interagency or Internal Report 8281
Natl. Inst. Stand. Technol. Interag. Intern. Rep. 8281, 33 pages (January 2020)

This publication is available free of charge from:
https://doi.org/10.6028/NIST.IR.8281

ABSTRACT

The National Institute of Standards and Technology (NIST) Pacific Marine Mammal Health Assessments (PMMHA) were initiated in 2010 as an expansion of NIST marine mammal health assessment activities that began in 2002 with bottlenose dolphin (*Tursiops truncatus*) wild populations. As part of PMMHA, NIST began a new collaboration to parallel wild population studies in a controlled setting by archiving and analyzing tissues sampled from dolphins in human care. As of this report, approximately 5,130 blood and milk sample aliquots from 20 dolphins in human care, along with associated dietary prey items, have been archived at the NIST Biorepository. When these samples are collected, valuable information associated with each sample is recorded; this vital step ensures the highest quality interpretation is available from analytical results. The Sample Entry Assistant (SEA) was developed to replace traditional paper data sheets and notebooks with a means for electronic data collection and automatically generated chain of custody documentation to reduce the potential for loss of, and increase accessibility to, sample information. This report provides (1) the standardized protocol for collecting, processing, and archiving blood and milk samples from dolphins in human care and their dietary prey items; (2) key points when teaching and executing the protocol; (3) an outline of the SEA platform; and (4) highlights of the components associated with the data tool.

KEY WORDS

Animals in Human Care; Biobanking; Blood; Data Tool; Dolphin; Milk; Prey; Standardized Protocol; Tissue Archival; Tissue Sampling.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The National Institute of Standards and Technology (NIST) has a history of involvement in marine mammal health assessment related work in collaboration with NOAA National Marine Fishery Service, Chicago Zoological Society's Sarasota Dolphin Research Program, and Dolphin Quest including: (1) support for the banking of marine mammal tissues in the NIST Biorepository, (2) designing standardized sample collection protocols, (3) providing technical assistance in the field, (4) development of standard reference and control materials (SRMs), (5) implementation of inter-laboratory comparison exercises using marine mammal tissues, and (6) analyzing samples for trace elements, organic pollutants, chemicals associated with metabolomics, and hormones. Pacific Marine Mammal Health Assessments (PMMHA), which began in Fall 2010, are an expansion of NIST marine mammal health assessment collaborations that began in 2002 with bottlenose dolphin (*Tursiops truncatus*) wild populations. As part of PMMHA, NIST began collaborating on health assessment studies for dolphins in human care with Dolphin Quest (DQ) at the Oahu (DQO) and Hawaii (DQH) facilities in October 2010. The NIST role has been to (1) develop and transfer a standardized protocol for sample collection and processing methods to archive samples; (2) develop and implement the Sample Entry Assistant (SEA) data tool; and (3) conduct studies assessing environmental and anthropogenic stressors that may impact the long-term health of bottlenose dolphins in human care. Since the research collaboration between NIST and DQ began, blood and milk samples, along with dietary prey samples, have been successfully collected and archived from hundreds of sampling events. While research on a managed dolphin population is valuable to address health concerns that affect animals in human care, it also provides an opportunity to conduct studies in a controlled setting, which can be used to better address questions about wild populations. Bottlenose dolphins have been proposed as a good sentinel species for monitoring coastal ecosystem health and potential human exposure threats [1]. The dolphins in human care at DQ are key in conveying conservation concerns affecting wild dolphin populations to the general public.

This report provides (1) the standardized protocol for collecting, processing, and archiving samples from dolphins in human care and their dietary prey items; (2) key points when teaching and executing the protocol; and (3) an outline of the SEA platform and highlights the components associated with the data tool.

COLLECTION AND ARCHIVAL OF DOLPHIN IN HUMAN CARE SAMPLES

One way to monitor an ecosystem is to study biomarkers, chemical exposures, and infectious agents in sentinel species, or organisms that are indicators of the environment. Sentinel species are ideal for biomonitoring because they can provide early warnings of ecosystem changes, and potential impacts to humans [2, 3]. Bottlenose dolphins are an ideal sentinel species because they share many ecological and life history traits with humans. Some of these include long life spans; similar reproductive cycles, such as giving birth to one offspring at a time and length of birthing intervals; fat stores that can concentrate toxicants; and top-predators feeding on similar food sources, such as fish and squid [1, 4, 5]. Bottlenose dolphins also often inhabit coastal waters that are in close proximity to human activities.

Since it is challenging to control for variability in wild populations, studies using animals in human care can reduce some uncertainties that can impact sample collection and analysis results [6]. Some variables that may not be known or cannot be controlled when sampling individuals during health assessments from free-ranging bottlenose dolphin populations include: time of last meal before sample collection; exact diet composition and source; reproductive history; health history; and migratory history or home range. Dolphins in human care have known location history, age, sex, reproductive status, health status and history, medications, supplements, and diets. Daily feeding times for dolphins in human care are also well documented, allowing fasted blood collection to prevent levels for some measurements from being affected by a recent meal. Since dietary information is available and diet samples can be collected for dolphins in human care, dietary exposures can be directly measured and modelled. Additionally, repetitive sampling can be done from the same individual within a defined time period and over the course of their lifetime.

Dolphin in human care samples that are archived in the NIST Biorepository are collected and processed according to detailed standardized protocols and stored cryogenically in liquid nitrogen vapor phase freezers (approximately -150 °C), which makes NIST Biorepository samples specifically useful for analytical analysis of organic contaminants, trace elements, and other chemical and biological analytes. Standardized protocols utilized across projects and collaborators allows sample analysis to be comparable over time and between marine mammal populations of the same species. Since the NIST Biorepository is a long-term storage repository, dolphin in human care samples are available for research questions involving emerging contaminants, health concerns that arise in the future, or retrospective analyses.

SAMPLE ENTRY ASSITANT (SEA) OVERVIEW

Maintaining proper context for samples in any collection is vital for meaningful interpretation of analytical results. Traditionally such information has been recorded on field data sheets or in laboratory notebooks. While this can be adequate, it leaves open many sources of data fragility, from the legibility of handwriting to the physical integrity of the paper upon which these are written, to accessibility and usability of recorded data. Transferring such information to electronic formats improves these aspects, but if the information is captured in physical form other integrity considerations exist such as transcription errors. For these reasons, NIST has been exploring approaches for direct electronic capture of sample information. The SEA data tool was developed in 2017 and put into full use during 2018 to electronically capture sample data from any web-enabled device via Google Forms. Using Google Apps and scripted routines in gscript (a form of javascript), data entry is restricted to valid values, preprocessed, and stored securely. Once the SEA electronic form is submitted, a digital chain of custody document is created and sent via email to DQ and NIST personnel within seconds. Documentation accompanies each set of samples through the preservation and shipping process and, if a form is damaged or destroyed in transit, or if any data entry errors are identified, documentation can be quickly reissued. Leveraging such a tool for scripted data preprocessing, verification, and document generation from templates has enhanced all aspects of data integrity associated with the DQ collaboration. Additional details on the Sample Entry Assistant are provided in a separate section below.

MANAGEMENT SYSTEM

Facilities that have marine mammals in the United States must have a valid United States Department of Agriculture (USDA) license or registration as required under the Animal Welfare Act (AWA). Dolphin Quest has an active USDA exhibitor license, Certificate No. 95-C-0037 (renewed annually), which permits samples to be collected for collaboration with NIST.

NIST Research Protections Office/Animal Care and Use Coordinator (RPO/ACUC) implemented in the Fall of 2018 for all activities to be conducted or supported by NIST that use vertebrate animal samples or data must be in compliance with NIST Directives for the Humane Care and Treatment of Vertebrate Animals (NIST Policy 5510, NIST Order 5511, and NIST Procedures 5511.01). This requires NIST personnel to complete training in animal welfare protections relevant to their role and for each research activity to submit the appropriate forms with supporting documents for review and approval. Dolphin in human care collaborations with NIST, as of October 2018, are now required to adhere to these guidelines and requirements. Since dolphin in human care NIST collaboration samples are biological specimens that are collected from live vertebrate animals, sample collection requires submission and approval of the "NIST Vertebrate Animals Research Administrative Review Initial Request Form and Checklist" along with supporting documents and annual re-approval.

NIST researchers or the dolphin in human care sample collector can request archived samples through written communication (e.g., email) with the lead of PMMHA (or NIST management appointed successor) and the collaborating collector. Communication to decide sample use should explain the scientific purpose of using the archived samples, methods, and data reporting (e.g., presentations, publications) with proposed authors. Additionally, dolphin in human care sub-samples archived in the NIST Biorepository that are requested for a research activity by a NIST Principal Investigator are considered by NIST RPO/ACUC as Excluded Specimens since they are pre-existing specimens in an established repository that were collected prior to the submission of the request. Before obtaining sub-samples from the NIST Biorepository, the "Excluded Animal Specimens/Data Request" form must be submitted and approved by NIST RPO/ACUC.

PROJECT OBJECTIVES AND GOALS

- Develop NIST standardized sampling protocol for dolphins in human care.
- Develop SEA, pilot test, and put into full operation.
- Teach collaborators NIST standardized sampling protocol and SEA.
- Collect and process fasted dolphin blood samples at least quarterly.
- Collect and process milk samples as available from lactating adult female dolphins within a day of when a paired blood sample is collected.
- Collect representative samples from each new catch date of each fish species fed to the dolphins in human care at collaborating facilities.
- Archive dolphin in human care whole blood, plasma, serum, milk, and internal organ tissue samples (if an animal passes away), and prey items in the NIST Biorepository.
- Analyze identified subsamples of dolphin in human care whole blood, plasma, milk, and internal organ tissue samples (if an animal passes away), and prey items for trace elements,

organic contaminants, metabolomics, and other chemical markers of interest approved by NIST and collaborators.
- Integrate chemical measurements and metabolic profiles with animal life history, medical, and diet information.
- Compare dolphin in human care findings with wild population study results.

SAMPLING PROTOCOL FOR DOLPHINS IN HUMAN CARE

The protocol for collecting, processing, and archiving dolphin in human care tissues was developed to parallel NIST wild bottlenose dolphin health assessment sampling protocols so that comparable supplies and methods would not introduce bias for potential study comparisons. Collection and processing procedures are included below for dolphin blood, dolphin milk, and prey items as well as sample labeling, storage, and shipping instructions. The following protocol serves as standard operating procedures for future dolphin in human care sampling efforts. Some information included in the protocol has been customized for the collaboration with Dolphin Quest and NIST. The protocol will be refined and tailored throughout the life of the project for additional collaborators and if a supply or method is identified as critical to change or modify.

Overview: Blood collection from all dolphins participating in the project should occur quarterly within a one week window of the planned collection date. In addition, milk samples should be collected within a day of the paired blood sample. Since feeding may affect circulating levels of contaminants and other biomarkers, a fasted blood sample collection should occur in the morning prior to the first feeding of the day. Prey items should be collected each time a new catch of a fish species is acquired at the facility.

Blood Collection and Processing

Preface: The tops of other blood tubes can be a source of trace element contamination. Lavender-top Sarstedt blood tubes, which can be used for trace element analysis, are collected **FIRST** to minimize contamination from other tubes. (**See *NOTE* below**)

Trace Elements: minimum whole blood 2 mL
maximum whole blood 7.5 mL

Organic Contaminants and Metabolomics:
minimum whole blood 5 mL (≈ 2.5 mL plasma)
maximum whole blood 10 mL (≈ 5 mL plasma)

Serum Banking: minimum whole blood 4 mL (≈ 2 mL serum)
maximum whole blood 9 mL (≈ 4.5 mL serum)

Collection Materials:

- 4 mL or 7.5 mL lavender-top Sarstedt EDTA KE blood tubes (1/animal)
- 10 mL green-top BD Vacutainer sodium heparin blood tubes (1/animal)
- 4.9 mL or 9 mL red-top Sarstedt serum clotting activator blood tubes (1/animal)
- Alcohol wipes
- Blood sampling device (double ended butterfly needle 21G or 23G) (1/animal)
- Quick data card and pencil
- Plastic container to hold supplies for each animal

Collection Procedure:

- Label blood tubes with the animal's Research ID in pencil.
- Set the vacuum in the lavender-top EDTA KE whole blood and red-top serum clotting activator tubes by pulling the plunger out until it locks into place and then snap off plunger.
- Wipe the blood collection site on the fluke with an alcohol wipe.
- **BLOOD TUBE COLLECTION ORDER:**
    1. Lavender-top Sarstedt EDTA KE blood tube
    2. Green-top BD Vacutainer sodium heparin blood tube
    3. Red-top Sarstedt serum clotting activator blood tube
- Record time of collection and fill out quick data card with pencil.
- After collection, invert the lavender-top and green top blood tubes 8-10 times to mix the blood with the anticoagulants.
- Place blood tubes in container for each animal and take to lab for processing.

Blood Collection and Processing (*continued*)

Processing Materials:

- Centrifuge
- Powder free vinyl gloves
- Tex wipes (TechniCloth Wipers (Blend))
- Decon-Ahol Spray or High Purity isopropanol in clean plastic squirt bottle
- Blood storage vial kit (barcodes, 2 mL self-standing external thread plastic cryovials, 7 mL pre-cleaned Teflon jar and Nomex tab (lid label)) (1/animal)
- Cryogenic or permanent marker
- 7 mL Teflon jar with colored water as reference for 2.5 mL plasma aliquot
- GC grade acetone and hexane (or isooctane) Teflon bottle rinsed glass Pasteur pipettes and bulbs
- Plastic pipettes
- Test tube rack
- Cryovial holder
- Biohazard and sharps container
- Freezer (-20 ºC; -80 ºC or LN_2 vapor phase)
- Electronic device with internet access to SEA electronic data sheet
- NIST deionized water (18.3 MΩ cm) in Teflon bottle (NIST Teflon Water)
- NIST deionized water (18.3 MΩ cm) in plastic bottle (NIST non-Teflon Water)

Processing Procedure:

Note: Every effort should be made to process blood samples within **4 hours** of collection.

- Record time blood processing begins.
- Put on a clean pair of powder free vinyl gloves.
- Spray work surface with Decon-Ahol or High Purity isopropanol in squirt bottle and wipe off with a Tex wipe.
- **Lavender-top Sarstedt EDTA KE whole blood tubes:**
    1. Gently rock tubes to mix whole blood.
    2. Wearing clean gloves unscrew the blood tube top and pour **1 mL** aliquots of whole blood into 2 mL cryovials. Aliquots should not be removed from these vials for contamination may occur.
    3. Add the date to the pre-printed barcodes and wrap the appropriate barcode around each cryovial in the order the aliquots were poured off (Whole Blood 1, Whole Blood, 2, Whole Blood 3, etc.). For more detailed instructions see the labelling instructions on the kit.
- **Green-top BD Vacutainer sodium heparin plasma blood tubes:**
    1. Centrifuge at 1300 × *g* for 10 minutes (adjust for each individual centrifuge) to separate plasma from red blood cells and other cellular material.
    2. Record hemolysis (pink serum) or lipemia (milky serum) on the electronic data sheet, if present.
    3. Put on clean powder free gloves.

4. Attach a reusable bulb to a clean glass Pasteur pipette, transfer a **2.5 mL** aliquot of plasma into a 7 mL Teflon jar (use reference jar for volume) and the remaining plasma into 2 mL cryovials in **1 mL** aliquots. Aliquots should not be removed from these vials for contamination may occur.
5. Add the date to the label on the Nomex Tab in the recessed lid of the Teflon jar. Add the date to the pre-printed barcodes and wrap the appropriate barcode around each cryovial in the order the aliquots were pipetted (Plasma 1, Plasma 2, Plasma 3, etc.). For more detailed instructions see the labelling instructions on the kit.

- **Red-top Sarstedt serum clotting activator blood tubes:**
    1. After blood collection, store tubes in the refrigerator in an upright position for at least 30 minutes.
    2. Centrifuge at 2500 × *g* for 10 minutes (adjust for each individual centrifuge) to separate serum from red blood cells and other cellular material.
    3. Record hemolysis (pink serum) or lipemia (milky serum) on the data sheet, if present.
    4. Put on clean powder free gloves.
    5. Using a clean plastic pipette; transfer **1 mL** aliquots of serum into 2 mL cryovials. Aliquots should not be removed from these vials for contamination may occur.
    6. Add the date to the pre-printed barcodes and wrap the appropriate barcode around each cryovial in the order the aliquots were pipetted (Serum 1, Serum 2, Serum 3, etc.). For more detailed instructions see the labelling instructions on the kit.
- **After processing all blood tubes:**
    1. If less than 1 mL of whole blood, plasma, or serum is sub-sampled into the last cryovial, note the partial sub-sample volume of each for the electronic data sheet.
    2. Record the blood tube lot number information for the electronic data sheet before discarding the tubes.
    3. Freeze all blood samples in -20 °C (only short-term), -80 °C or LN_2 freezer.
- **Fill out the SEA electronic data sheet record for each animal.**

***NOTE* If the protocol is not followed or blood tubes are collected out of sequence please make note in the SEA electronic data sheet record.** Blood tubes (6 of each type and lot number) that are collected out of protocol sequence along with non-provided blood sampling devices (6 each) should be sent with samples to NIST to measure background contamination.

Blanks for blood will be made at NIST:

Collect three field blanks for each type of blood tube collected.

Lavender-top lot# _____ made on _____ (ddmmmyy)
Green-top lot# _____ made on _____ (ddmmmyy)
Red-top lot# _____ made on _____ (ddmmmyy)

Blanks should be made in the same manner as samples were collected. One blank set will be made with NIST Teflon water and another set will be made with NIST non-Teflon water:

- Using a fresh blood sampling device, draw deionized water from the Teflon bottle into a lavender-top blood, then a green-top blood tube, and finally a red-top blood tube. Invert

the lavender-top and green top blood tubes 8-10 times as done with the blood sample to mix the water with the anticoagulants.

- Follow the steps in the Processing Procedure Section above with water blanks in the blood tubes
- Label the sample storage containers for the blanks according to the Label Section.
- Repeat the steps above two more times using new tubes and blood sampling devices. Label these appropriately Blank A, B, and C for each set of blanks.

NOTE: A new set of blanks will need to be made when a different blood tube lot number is used.

Blood Collection and Processing Summary

Collection Order	Blood Tube Type and Additive	Blood Tube Volume	# of blood tubes per animal	Blood Fraction	Storage Container/ Aliquot Volume	Short-term storage	Long-term storage
1	Lavender-top Sarstedt EDTA KE	4 mL or 7.5 mL	1	Whole Blood	2 mL cryovials/ 1 mL aliquots	-20 ºC freezer	-80 ºC or LN_2 freezer
2	Green-top BD Vacutainer Na Heparin	10 mL	1	Plasma	7 mL Teflon Jar/ 2.5 mL aliquot 2 mL cryovial/ 1 mL aliquots	-20 ºC freezer	-80 ºC or LN_2 freezer
3	Red-top Sarstedt Clot activator	4.9 ml or 9 mL	1	Serum	2 mL cryovials/ 1 mL aliquots	-20 ºC freezer	-80 ºC or LN_2 freezer

Milk Collection

Preface: The major sources of contamination of the milk sample will arise from the sample collection device and carry-over of previous milk samples if the device is re-used between animals. The milk collection device should not be shared among animals and a new device should be used each time a milk sample is collected.

Sample Volume Requirements: minimum milk 2 mL
maximum milk 15 mL

Materials:

- Milk sampling apparatus (1/animal) (dispose of apparatus after use)
- Powder free vinyl gloves
- 2 mL self-standing external thread plastic cryovials
- 7 mL Teflon jars and Nomex tabs (lid labels)
- 7 mL Teflon jar with colored water as reference for 2.5 mL milk aliquot
- Cryogenic or permanent marker
- Freezer (-20 ºC, -80 ºC, or LN_2 vapor phase)
- Electronic device with internet access to SEA electronic data sheet
- NIST deionized water (18.3 MΩ cm) in Teflon bottle (NIST Teflon Water)

Procedure:

- Record time milk collection begins.
- Place 10 mL syringe without plunger over the dolphin's mammary slit.
- Apply gentle pressure at the base of the open syringe against the mammary slit.
- Begin pumping milk by pulling up on the plunger of the 20 mL syringe, which will draw milk up from the 10 mL syringe through the tubing into the 20 mL syringe.
- Record time milk processing begins if different than blood processing.
- Put on clean gloves; dispense milk directly from the 20 mL syringe into:
    - **Less than 6 mL collected** – multiple 2 mL cryovials (**1 mL** aliquots)
    - **Greater than 6 mL milk collected** - one 7 mL Teflon jar (**2.5 mL** aliquot, use reference jar for volume) and multiple 2 mL cryovials (**1 mL** aliquots)
    - **Aliquots should not be removed from vials to avoid contamination**
- Label the Nomex Tab according to the Label Section and place it in the recessed lid of the Teflon jar. Also, label the cryovials according to the Labeling Section.
- After processing, freeze milk samples in -20 °C (only short-term), -80 ºC or LN_2 freezer.
- Fill out the SEA electronic data sheet record for each animal.

Blanks for milk:

- Using a clean, unused sampling apparatus, pour a volume of NIST Teflon water into the device that is comparable to a milk sample.
- Dispense the deionized water into a 7 mL Teflon jar and 2 mL cryovials using the volumes in the above procedure.
- Label the storage containers for the milk blanks according to the Labeling Section.
- Collect three field blanks in this manner using a new sampling apparatus for each set of blanks. Label these appropriately Blank A, B, and C for each set of blanks.

Made blanks on _____ (ddmmmyy)

Prey Item Collection

Materials:

- Teflon 12" X 12" bags
- Zip ties
- Tyvec Tags

Procedure:

- Obtain a representative sample of each fish species that are fed to the dolphins every time a new catch is acquired for use. This should be 3 to 5 fish per species. These samples should be collected after the fish are pulled and partially thawed in the refrigerator. They should not come from fish that have been completely thawed in the sink in water.
- Place representative samples of each fish species into separate Teflon bags. Label Tyvec tag with fish species, date collected at Dolphin Quest, fish catch date, kcals/lb, and institution location (DQO or DQH).
- Place fish samples back in the freezer until they are shipped.

Blood and Milk Sample Labeling

All samples should be labeled following a standard procedure.

Blood storage vials should be labeled with the pre-printed barcodes in each animal kit according to the labelling instructions on the kit. Kits should be used in time point sequential order for each animal.

If labels are hand written, each vial label should include the following information:

Research ID
Date (ddMmmyy)
Institution and Location of Animal (or sample collected)
Sample Type and Aliquot Number

Abbreviations: Institution – DQ = Dolphin Quest; Location – O = Oahu, H = Hawaii
Sample Type – WB = Whole Blood, P = Plasma, S = Serum, M = Milk
Water Blank – TW = Teflon Water , NTW = non-Teflon Water

ANIMAL Example:	83H1 09Mar10 DQH WB 1	BLANK Example:	TW Blank A 09Mar10 DQH WB 1

Interim Storage

Dolphin Quest Oahu – Notify the NIST point of contact at Hawaii Pacific University (HPU) to pick up the samples and signed paperwork within a week of collection for transport on wet ice in a cooler and interim storage at HPU in their -80 °C or LN_2 vapor phase freezer.

Dolphin Quest Hawaii – Within a week of collection, transport samples on wet ice in a cooler and signed paperwork to The Marine Mammal Center Ke Kai Ola (TMMC KKO) for interim storage in the NIST -80 °C freezer.

Shipping

All samples will be shipped in an LN_2 shipper (provided by NIST). Place samples in Teflon bags, secure shut with zip tie, and place filled bag(s) in LN_2 vapor shipper. All samples must be shipped UPS or Federal Express, Priority Overnight using the NIST account to the following address:

NIST Biorepository
Hollings Marine Laboratory
331 Fort Johnson Road
Charleston, SC 29412

Please provide a copy of the printed electronic data sheets along with the shipment.

Please contact one of the following NIST Biorepository personnel prior to shipping to ensure that one of them will be there to accept the shipment.

Amanda Moors (amanda.moors@nist.gov)

843-460-9814 (work)

Jennifer Ness (Jennifer.ness@nist.gov)

843-460-9815 (work)

If you have questions about the protocol or sampling, contact:

Colleen Bryan (colleen.bryan@nist.gov)

843-460-9784 (work) **843-425-7753 (cell phone)**

Additional Protocol Details

- Each dolphin in human care that is sampled for the NIST collaboration is assigned a unique research identification code (Research ID). Use of Research IDs were implemented for dolphins at collaborating facilities so that their show name or NOAA ID in the NOAA Fisheries National Inventory of Marine Mammals could not be linked due to concerns for information requests under the Freedom of Information Act (FOIA).
- Barcode labels are pre-printed for each blood sub-sample with specific animal and sample identification fields (Figure 1). Text fields are used to easily identify a sample and barcodes can be scanned into a sample tracking database.
- In order to be able to pre-print barcode labels for each animal blood storage vial kit, a chronological time series must be assigned since the date of sampling is unknown. The chronological time series are named "Time Point 1 (TP1), TP2, TP3, TP4, etc." for each Research ID and the sequence starts over each calendar year (Figure 1).

Figure 1. Example of barcode label printed for each blood sub-sample.

- The supplies referred to as Teflon in the protocol and SEA are a generalized category of non-stick products made of fluoropolymers, which are resistant to solvents, acids, and bases and can withstand cryogenic storage conditions (-150 °C). The Teflon supplies used for sample processing and storage are made of the following fluoropolymers:
    - 7 mL jars – perfluoroalkoxy alkane (PFA)
    - Squirt bottles – fluorinated ethylene propylene (FEP) body; Ethylene tetrafluoroethylene (ETFE) screw cap and draw tube
    - Bottles with water for blanks – FEP body; ETFE screw cap
    - Bags – FEP

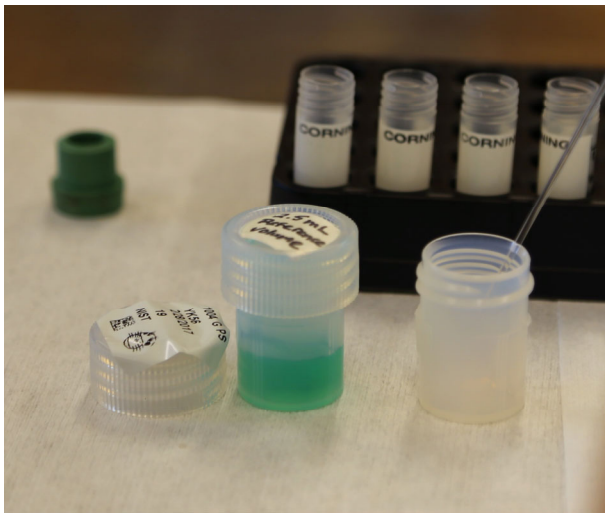


Figure 2. Teflon reference jar with 2.5 mL colored water used to estimate plasma volume as it is dispensed into a new pre-cleaned jar.

- Teflon jars for plasma and milk are pre-cleaned at NIST using the Marine Environmental Specimen Bank: Clean room and specimen bank protocols [7].
- Since the 7 mL Teflon jars used for plasma and milk aliquots do not have graduation lines indicating volume, a reference jar that contains 2.5 mL of water with food coloring is provided to use during processing to estimate a plasma or milk aliquot as it is dispensed into a new pre-clean Teflon jar (Figure 2).
- The glass Pasteur pipettes used for plasma are pre-cleaned at NIST by rinsing them three times with GC/Pesticide Residue Analysis Grade acetone and three times with GC/Pesticide Residue Analysis Grade hexane or isooctane and wrapping in hexane rinsed aluminum foil. The pipettes should remain in the foil pouch until one is removed at a time for plasma processing.

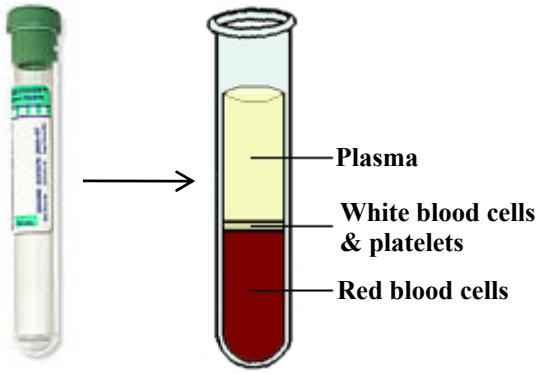


Figure 3. Blood compartment separation for plasma in sodium heparin blood tubes.

- If a pipette tip (glass or plastic) touches anything but the intended sample, it should be considered contaminated and be discarded.
- If the rubber reusable bulb for pipetting plasma gets plasma inside, the bulb should be discarded.
- If the plasma or serum and the cellular material interface is disturbed while pipetting aliquots, the blood tube can be spun in the centrifuge again to separate the blood compartments (Figure 3).
- Whole blood, plasma, serum, and milk cryovials should be sequentially filled and labelled (Figures 4 and 5). The last sub-sample of each type should always contain the volume less than 1 mL if enough material is not available for a full 1mL sub-sample.

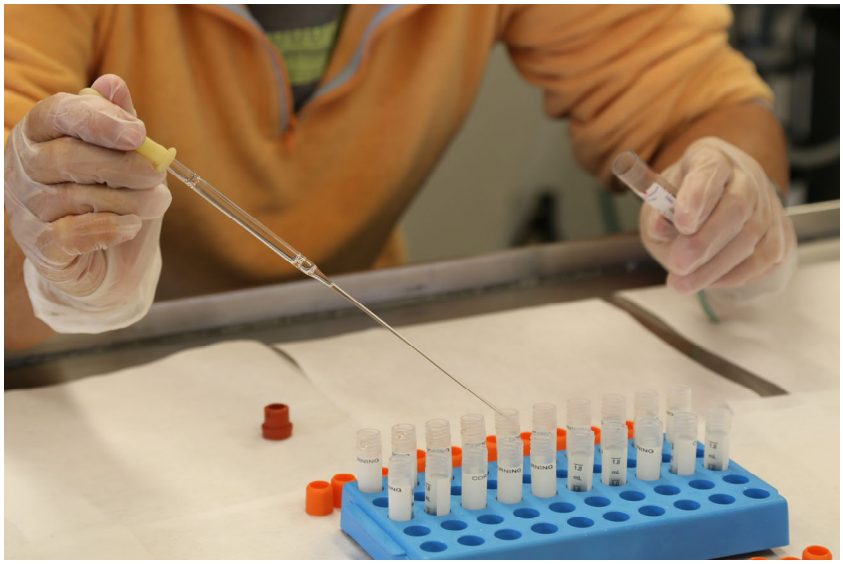


Figure 4. Cryovials in cryovial rack are being fill sequentially with 1 mL aliquots of plasma as they are pipetted out of the sodium heparin blood tube.

- Ensure all Teflon jars and cryovials are tightly capped before freezing since they can loosen due to contraction at freezing temperatures.
- NIST Teflon water is in a pre-cleaned Teflon bottle and NIST non-Teflon water is in a pre-cleaned low density polyethylene (LDPE) plastic bottle. Both types of bottles used to make blanks are filled with high purity deionized water (18.3 MΩ·cm) from the NIST Charleston Inorganic Laboratory.
- Teflon bottles, Teflon squirt bottles, and LDPE bottles are pre-cleaned at NIST by rinsing 5 times with high purity deionized water, filling with 3 % Ultra Trace Elemental Analysis grade nitric acid, allowing the acid solution to sit for a minimum of 4 hr, rinsing again 5 times with high purity deionized water, and allowing them to dry under a positive pressure laminar flow hood.

NIST Sample Labelling Instructions:

1. Before removing label from backing, write collection date as DD MMM (example: 01Feb) before printed year with permanent marker
2. Peal label from backing; place the printed section of the label on cryovial/Teflon jar tab first; wrap clear label section around cryovial/Teflon jar tab
3. Only place labels on cryovials that contain samples
4. Insert Teflon jar tab into lid
5. Place all unused labels and in original bag to send back to NIST when samples are shipped

Figure 5. NIST sample labelling instructions included with each blood storage vial kit.

Figure 6. Dolphin milk sampling apparatus.

- Each one time use disposable milk sampling apparatus is constructed with a sterile 10 mL syringe without the plunger and a sterile 20 mL syringe with the plunger connected together with Tygon 2275 High Purity Tubing (3.2 mm I.D. x 6.4 mm O.D. x 1.66 mm wall) and placed in a new resealable plastic bag (Figure 6).
- Prey items are labelled with Tyvec tags that are tear-proof, weatherproof, and chemical resistant. Once prey are in a new Teflon bag and the information has been written on the tag with a permanent marker or cryopen, the bag is secured by placing a zip tie through the reinforced hole of the tag and firmly tightening the zip tie so the bag is air tight (Figure 7).

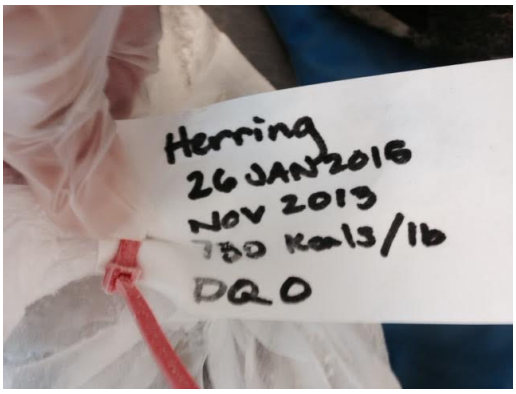


Figure 7. Prey item Tyvec tag label secured with zip tie to close Teflon bag with samples.

SAMPLE ENTRY ASSISTANT (SEA)

Chain of custody documentation is important in establishing the history of any given sample prior to accession into the NIST Biorespository. Field data sheets provide the main mechanism by which sample metadata are recorded as part of that accession. Recording of information by hand on paper logs or data sheets, while still the industry standard, can result in loss of vital information through physical destruction, illegibility, inaccurate transcription, or other means. While NIST and our collaborators have done an excellent job in the past with this aspect, identifying another avenue to record sample information needed to be established to reduce the overhead associated with quality assurance and control from such manual processes. Web-based form entry has many advantages over classic paper forms. Preservation of data is instantaneous, generation and transmission of documentation can easily be automated, forms can be quickly and easily extended as project needs change, and data can be shuttled from one platform to another (e.g. Freezerworks) easily. The ongoing collaboration with Dolphin Quest was appealing to examine the technical and practical feasibility of electronic forms for a variety of reasons: investigators were open to using the platform, samples are collected at commercial facilities with consistent internet coverage, metadata collection was well defined, and data types collected covered a good range for testing. The Dolphin Quest Sample Entry Assistant (SEA) was developed to serve as proof-of-concept for replacing paper collection sheets with electronic data collection forms driven by Google Apps. This platform is hoped to provide a robust conceptual framework to enhance data collection activities across a variety of projects within the NIST Biorepository.

SEA was developed in spring of 2017, pilot tested over the course of six months making improvements and incorporating user inputs, and put into full operation on January 29, 2018. The basics of the SEA are to: (a) collect information in a simple curated manner through a web-based Google Form available from anywhere; (b) automatically collate data; (c) automatically produce chain of custody documentation; and (d) send such documentation via email as a .pdf for wet signature and transmission alongside sample shipments from the field to the NIST Biorepository.

SEA Instructions

Dolphin Quest SEA is available to anyone with the web address and does not require an account with Google (Figure 8). Progressing through the form is very straightforward. Questions are presented in a series of thematic sections. Depending on the answer to some questions (e.g. “Is this a new patient?”), users will be redirected to different sections. Some questions are limited to controlled vocabulary answer options such that users select the appropriate answer from a drop down box or option list. Questions expecting numerical answers are limited to reasonable numerical answers or ranges. Any question required to be answered are indicated with a red asterisk and must be answered in order to proceed to the next page. Answers for certain questions have been prepopulated specifically for Dolphin Quest to increase usability and decrease the chance of erroneous data entry. Users progress through the form to answer each set of questions and click “Submit” at the end. At any point before clicking “Submit”, the user can click the “Back” button to change or review prior answers. Upon form submission, a chain of custody document is generated automatically (Figure 9) and emailed to appropriate personnel (Figure 10).

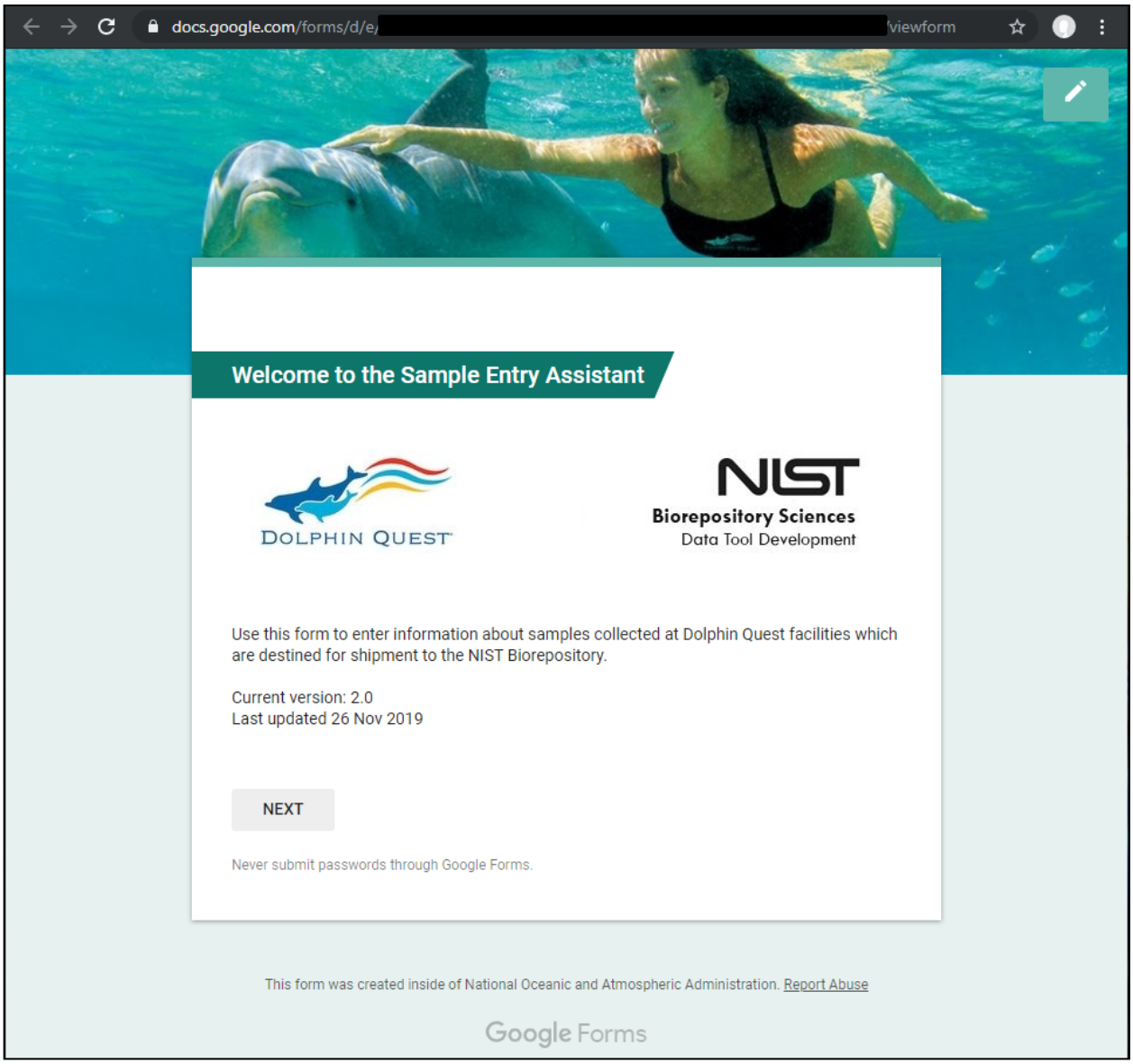
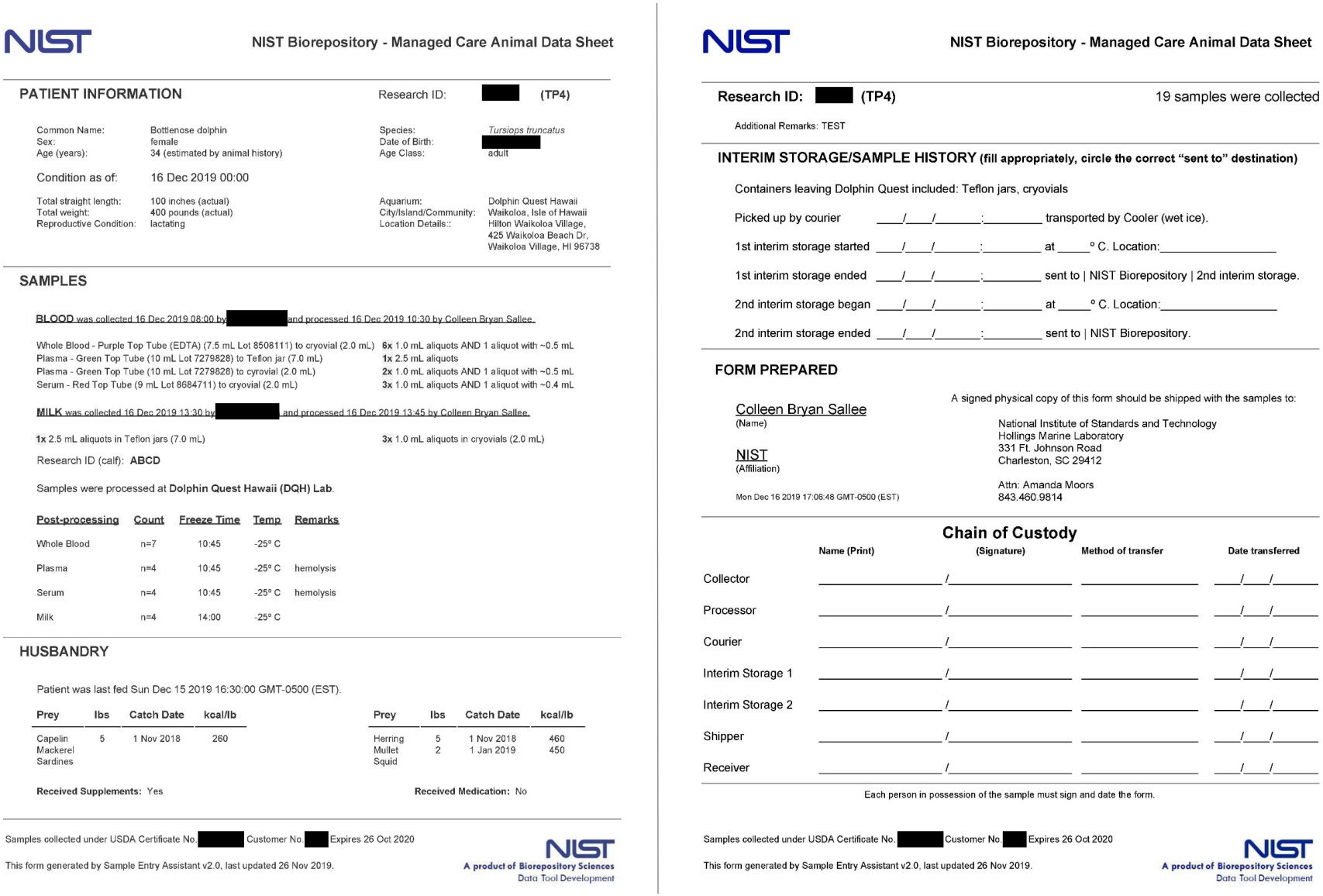


Figure 8. Sample Entry Assistant landing page for the Google Form, through which collectors enter information. Users click through a series of screens to provide all sample metadata necessary for submission to the NIST Biorepository.



NIST Biorepository - Managed Care Animal Data Sheet

PATIENT INFORMATION

Research ID: [redacted] (TP4)

Common Name:	Bottlenose dolphin	Species:	*Tursiops truncatus*
Sex:	female	Date of Birth:	[redacted]
Age (years):	34 (estimated by animal history)	Age Class:	adult

Condition as of: 16 Dec 2019 00:00

Total straight length:	100 inches (actual)	Aquarium:	Dolphin Quest Hawaii
Total weight:	400 pounds (actual)	City/Island/Community:	Waikoloa, Isle of Hawaii
Reproductive Condition:	lactating	Location Details::	Hilton Waikoloa Village, 425 Waikoloa Beach Dr, Waikoloa Village, HI 96738

SAMPLES

BLOOD was collected 16 Dec 2019 08:00 by [redacted] and processed 16 Dec 2019 10:30 by Colleen Bryan Sallee.

Whole Blood - Purple Top Tube (EDTA) (7.5 mL Lot 8508111) to cryovial (2.0 mL)	**6x** 1.0 mL aliquots AND 1 aliquot with ~0.5 mL
Plasma - Green Top Tube (10 mL Lot 7279828) to Teflon jar (7.0 mL)	**1x** 2.5 mL aliquots
Plasma - Green Top Tube (10 mL Lot 7279828) to cyrovial (2.0 mL)	**2x** 1.0 mL aliquots AND 1 aliquot with ~0.5 mL
Serum - Red Top Tube (9 mL Lot 8684711) to cryovial (2.0 mL)	**3x** 1.0 mL aliquots AND 1 aliquot with ~0.4 mL

MILK was collected 16 Dec 2019 13:30 by [redacted] and processed 16 Dec 2019 13:45 by Colleen Bryan Sallee.

1x 2.5 mL aliquots in Teflon jars (7.0 mL)

3x 1.0 mL aliquots in cryovials (2.0 mL)

Research ID (calf): **ABCD**

Samples were processed at **Dolphin Quest Hawaii (DQH) Lab**.

Post-processing	Count	Freeze Time	Temp	Remarks
Whole Blood	n=7	10:45	-25° C	
Plasma	n=4	10:45	-25° C	hemolysis
Serum	n=4	10:45	-25° C	hemolysis
Milk	n=4	14:00	-25° C	

HUSBANDRY

Patient was last fed Sun Dec 15 2019 16:30:00 GMT-0500 (EST).

Prey	lbs	Catch Date	kcal/lb
Capelin	5	1 Nov 2018	260
Mackerel			
Sardines			

Prey	lbs	Catch Date	kcal/lb
Herring	5	1 Nov 2018	460
Mullet	2	1 Jan 2019	450
Squid			

Received Supplements: Yes

Received Medication: No

Samples collected under USDA Certificate No. [redacted] Customer No. [redacted] Expires 26 Oct 2020

This form generated by Sample Entry Assistant v2.0, last updated 26 Nov 2019.

NIST A product of Biorepository Sciences Data Tool Development

NIST Biorepository - Managed Care Animal Data Sheet

Research ID: [redacted] **(TP4)** 19 samples were collected

Additional Remarks: TEST

INTERIM STORAGE/SAMPLE HISTORY (fill appropriately, circle the correct "sent to" destination)

Containers leaving Dolphin Quest included: Teflon jars, cryovials

Picked up by courier ____/____/_____:_____ transported by Cooler (wet ice).

1st interim storage started ____/____/_____:_____ at _____° C. Location:_____

1st interim storage ended ____/____/_____:_____ sent to | NIST Biorepository | 2nd interim storage.

2nd interim storage began ____/____/_____:_____ at _____° C. Location:_____

2nd interim storage ended ____/____/_____:_____ sent to | NIST Biorepository.

FORM PREPARED

Colleen Bryan Sallee
(Name)

NIST
(Affiliation)

Mon Dec 16 2019 17:06:48 GMT-0500 (EST)

A signed physical copy of this form should be shipped with the samples to:

National Institute of Standards and Technology
Hollings Marine Laboratory
331 Ft. Johnson Road
Charleston, SC 29412

Attn: Amanda Moors
843.460.9814

Chain of Custody

	Name (Print)	(Signature)	Method of transfer	Date transferred
Collector	_____	_____	_____	____/____/_____
Processor	_____	_____	_____	____/____/_____
Courier	_____	_____	_____	____/____/_____
Interim Storage 1	_____	_____	_____	____/____/_____
Interim Storage 2	_____	_____	_____	____/____/_____
Shipper	_____	_____	_____	____/____/_____
Receiver	_____	_____	_____	____/____/_____

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

Samples collected under USDA Certificate No. [redacted] Customer No. [redacted] Expires 26 Oct 2020

This form generated by Sample Entry Assistant v2.0, last updated 26 Nov 2019.

NIST A product of Biorepository Sciences Data Tool Development

Figure 9. Example chain of custody generated by Sample Entry Assistant v2.0 customized to Dolphin Quest.

Dolphin Quest SEA - New Sample Entry Recorded for [redacted] on Mon Dec 16 2019

noreply@noaa.gov <noreply@noaa.gov>
Mon 12/16/2019 5:07 PM
To: [redacted]; Bryan Sallee, Colleen E. (Fed) <colleen.bryansallee@nist.gov>
Cc: Ragland, Jared M. (Fed) <jared.ragland@nist.gov>; Bryan Sallee, Colleen E. (Fed) <colleen.bryansallee@nist.gov>

1 attachments (122 KB)
DQ SEA - [redacted] on Mon Dec 16 2019.pdf;

For your records,

A new chain of custody has been created by Colleen Bryan Sallee from NIST for shipment to the NIST Biorepository.

Samples were collected for Research ID [redacted] (TP4).

Samples (n=19) were prepared for shipment including:

- 7 cryovials of whole blood
- 4 cryovials or Teflon jars of plasma
- 4 cryovials of serum
- 4 cryovials or Teflon jars of milk

Editable documents are available in the project folder for those with access. Copies of the Chains of Custody are attached to this correspondence and physical copies will be sent with the shipment.

Samples collected under USDA Certificate No.[redacted] Customer No.[redacted] Expires 26 Oct 2020.

Thank you,

DolphinQuest Sample Entry Assistant

A product of NIST Biorepository Sciences Data Tool Development

Figure 10. Example email generated by Sample Entry Assistant v2.0 customized to Dolphin Quest.

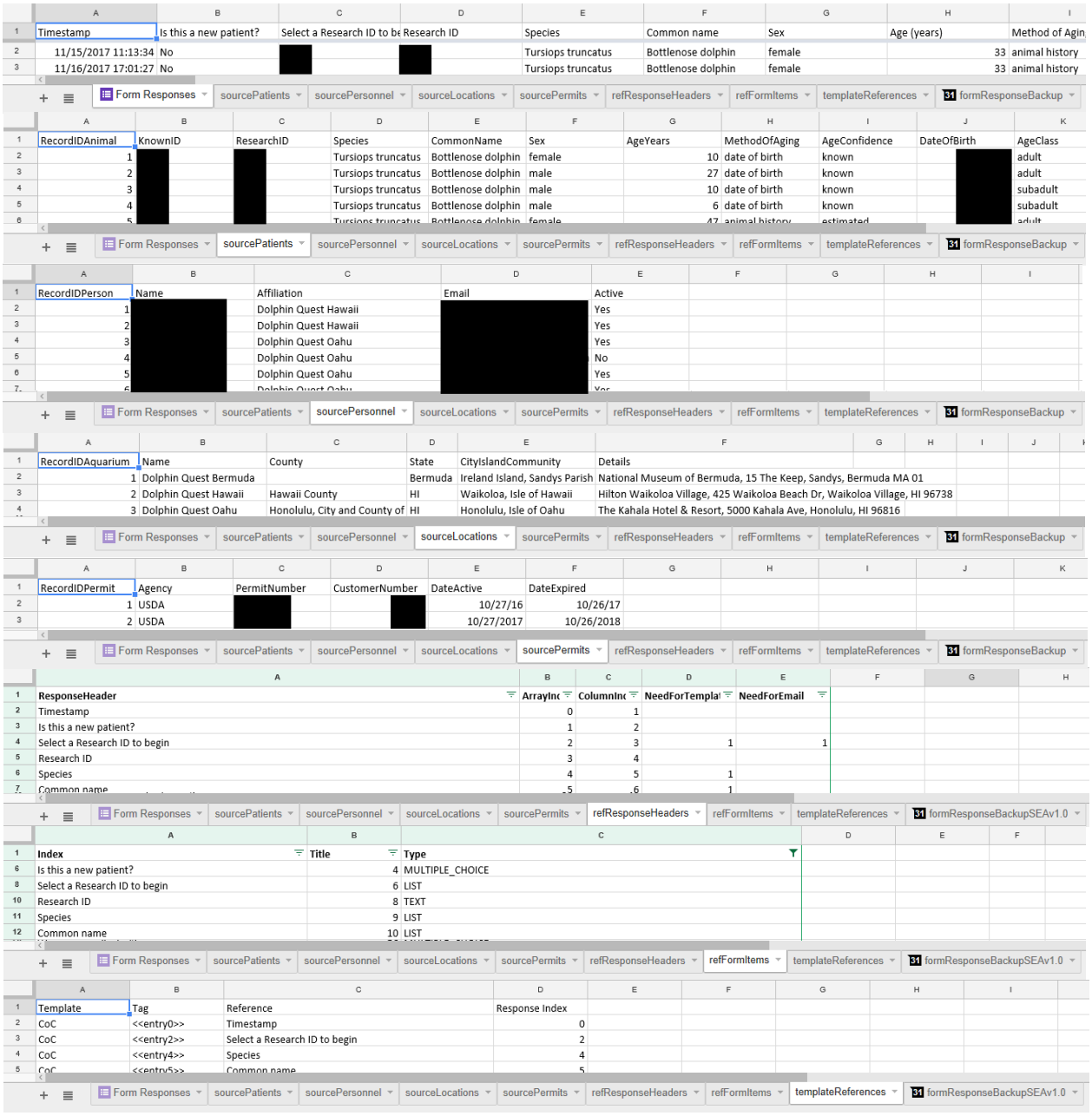


Figure 11. Example data structure within Sample Entry Assistant v2.0. One worksheet ("Form Responses") houses answers to questions submitted via the form (each column contains answers to a single question), and other worksheets (acting as lookup tables) contain information regarding unique patients, personnel, and facilities, or reference indices to ease maintenance and development. Construction of the data sources in this manner does not require a traditional relational database engine.

Additional SEA Details

Sample Entry Assistant is constructed on the Google Forms platform as a series of questions broken into sections; this implementation of the SEA framework for Dolphin Quest contains questions arranged in 42 sections. Each section presents as a single entry page. Users progress through the assistant in a workflow designed to minimize the number of inapplicable questions to increase usability and decrease time spent completing the form. Unfortunately, live feedback is unavailable in the Google Forms platform as code only executes upon form submission. Later answer options therefore, cannot depend upon prior answers. This is one example where full mobile application development would be superior. Users can, however, be redirected to different sections depending on answers. Expanded Patient Information (Form Section 5), for example, is only applicable if the user is adding a new patient. This section is skipped if samples apply to a patient that already exists. Answers to questions, where feasible, are limited to promote data consistency (e.g. names, numeric entry restriction, etc). Descriptions have been incorporated in some sections to aid the user in accurately answering the questions or to include appropriate details in a question answer. When submitted at the end of the form, answers to each question are automatically pushed into a corresponding spreadsheet (Figure 11). Those answers are then applied to the chain of custody (example Figure 9) and email templates (example Figure 10) through javascript functions triggered by form submission. Each chain of custody document is saved for persistence and archiving in a project-specific directory on Google Drive, converted to .pdf, and attached to an email for which addresses are predetermined by the SEA curator. Chains of custody can then be printed for wet signature and dating to accompany samples during transfer and shipment. Permission to edit and/or regenerate such chains of custody (e.g. in case a mistake is identified) can be controlled in fine-grained detail and requires only that the person(s) performing the editing have an account with Google. This combines the legal power of wet-signature chain of custody documentation with the power and benefits of web-based data entry (e.g. curation, consistency, data integrity, etc). Once records exist in the underlying data worksheet, they can easily be reformatted for direct import to other platforms such as Freezerworks, which is used by the NIST Biorepository for sample archival. Some level of maintenance is required on the part of the data tool custodian to reflect changes to the project, but this is relatively minor in the grand scheme of things and primarily limited to updates of permit information and other data collections that by necessity change as a project matures.

SEA Documentation Templates

Two templates are used in the Dolphin Quest SEA framework, both of which are effectively text files saved as Google Docs. The template for email is very simple, and intended to communicate only basic information regarding what samples were collected for which patient, how many, when, and by whom. This template includes HTML tags to increase readability; tags are stripped from the template to support non-HTML-compliant email clients or preferences.

The more involved of the two templates is the field data sheet with an attached chain of custody, which is the main purpose of the Dolphin Quest SEA tool. Previous paper data sheets with chains of custody for this project guided construction of the electronic template and, although formats were not able to be matched perfectly, the same information and general presentation were preserved. Nearly any current document can be recreated in this manner, though some more complicated formatting options are lacking in Google Docs. Additionally, to best meet the

needs of Dolphin Quest stakeholders, the flexibility of the underlying functions driving the SEA make modifications of this type relatively simple to deploy and customize for various purposes.

SEA Performance and Limitations

Feedback from collaborators includes that the SEA online form is straightforward and simple to follow. They can quickly choose from controlled dropdown menus and question notes are helpful. Dolphin Quest likes that once the form is submitted, the electronic datasheet with chain of custody is immediately generated and emailed to a set list of recipients. This allows staff to quickly review the document, flag a form input error, and request that the error is corrected before the paperwork is printed, signed, and samples leave the location with the chain of custody for interim storage. Feedback from NIST personnel involved with the project indicate data integrity has been greatly enhanced by eliminating data loss through legibility or fragility, providing transparency in data collection and transmission, streamlining of data custodial tasks, and enhancing data quality assurance and control protocols. For this project, 53 separate pieces of data are associated with each aliquot accessioned into the NIST Biorepository. Though many are conserved across aliquots for a given sample type and some are prepopulated during generation of sample labels, use of SEA ensures manual data entry of the remainder is unnecessary, greatly improving quality control. There have been no reported issues of inaccessibility or instability, and the very few minor changes necessary required minimal effort.

Training has been provided to staff at collaborating facilities, and most have had no issues using SEA thanks to the intuitive nature of the form interface. Some questions in the form were occasionally misconstrued by a user leading to inaccurate answers. This was resolved by reconstructing questions or adding notes below a question to ensure questions are as intuitive and specific as possible to the user.

Data collected through the SEA are generally of extremely high quality, and mistakes are easily reparable in a transparent manner. Altering data already collected to address mistakes is a much more targeted effort than using paper data sheets. Identifying mistakes is extremely simplified compared with laborious manual data entry, which represents a source of data fragility. If changes are necessary to unsigned chains of custody, those documents can be rapidly updated and reissued. If changes are necessary after documentation is signed, only the altered data points need to be addressed in the data file, leaving any changes to the signed documentation needing only an initial and overwrite in ink that no longer necessarily needs to be interpreted by other personnel.

Some limitations of the Google Apps platform used for SEA have also been identified. These could be remedied by moving to a more robust and customizable platform such as R Shiny or full mobile-native development. Desirable enhancements would include the capability to: (1) partially fill the form; (2) dynamically adjust questions and responses based on prior answers; (3) create save points; (4) adequately review in-process records; (5) improve support for multiple responses simultaneously; and (6) allow offline entry and synchronization.

SEA Benefits to NIST

Consistency in data collection and chain-of-custody documentation were the largest identified benefits of SEA to operations of the NIST Biorepository in supporting sample curation and archival for the ongoing dolphin in human care collaboration. Having documentation generated and distributed as a downstream part of a curated data capture process powered by scripting, ensures legibility, eliminates fragility, ensures data archival, and enhances communication. The import file for Freezerworks generated by SEA is also available to leverage during aliquot accession into the NIST Biorepository. Sample Entry Assistant provides several quality control aspects to flag errors. Already the use of SEA has captured data entry (from the field) or data management (within Freezerworks) errors, which would have represented critical failures of data integrity, and identified transcription mistakes during generation of sample labels. Resolving these errors absent use of SEA would have required significantly more effort and time both from NIST and collaborating personnel, assuming they were identified in the first place. Being able to flag sample shipments before they are received at the NIST Biorepository as likely having data issues, and having worked out rectification needs ahead of time, is a major improvement to quality assurance. Use of SEA as a quality assurance mechanism has enhanced all aspects of data integrity associated with this project.

PROJECT ACCOMPLISHMENTS

Sample Collection and Archival Summary

Since the NIST collaboration with the Dolphin Quest facilities began in fall of 2010, there have been 412 sampling events (01Oct2010 to 30Jun2019) from 20 bottlenose dolphins (7 female, 13 male) in human care. Blood was collected at 409 of the sampling events and milk was collected at 26 of the sampling events resulting in 5130 aliquots archived in the NIST Biorepository (Tables 1 and 2). Dolphins that participated ranged in age from 5 months to 48 years old at time of sample collection. This represents the largest and longest ongoing formal tissue archival project for bottlenose dolphins in human care. As the collaboration with Dolphin Quest enters its tenth year, the project has been able to sample the same animals over the course of their lives from being born at DQ as calves to sub-adulthood, becoming sexually mature, having offspring, and/or becoming established adults.

Prey items have been collected and archived in the NIST Biorepository for each new catch of species fed to the dolphins at DQ (Table 3). Amount and type of prey item being fed to the dolphin at time of blood and milk collection has been recorded to pair diet with animal samples.

Analysis Summary

NIST researchers along with NIST graduate students and guest researchers have examined dolphin in human care samples from the NIST biorepository for a number of analytes including:

- Total mercury – whole blood, plasma, serum, milk, prey items
- Perfluorinated alkyl acids – plasma, prey items

- Metabolomics – serum
- Bulk stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) – whole blood, prey items
- Percent lipid – prey items
- Oligosaccharides (analysis planned) – milk

Table 1. Number of sampling events for each tissue type collected (01Oct2010 to 30Jun2019) from DQH and DQO dolphins in human care

Year	Whole Blood	Plasma	Serum*	Milk*
2010	5	5	--	--
2011	65	63	46	0
2012	44	41	43	2
2013	36	32	35	4
2014	49	47	48	6
2015	50	50	50	5
2016	47	48	45	1
2017	51	50	49	5
2018	39	38	39	2
2019	18	18	16	1
Total	**404**	**392**	**371**	**26**

*Serum and milk collection started in 2011

Table 2. Total number of aliquots collected, processed, and archived (01Oct2010 to 30Jun2019) in the NIST Biorepository for dolphin in human care samples

Tissue Type	Number of Aliquots
Whole Blood	1742
Plasma	1221
Serum	1108
Milk	74
Total	**4145**

Table 3. Number of prey item catches collected and archived (01Oct2010 to 30Jun2019) in the NIST Biorepository for dolphin in human care

Prey Species	Number of Catches
Capelin	10
Herring	13
Mackerel	2
Mullet	3
Sardine	2
Smelt	1
Squid	12

Results of these analysis have been presented at scientific meetings (listed below), plan to be reported in manuscripts, and the oligosaccharides will be available in the NIST Libraries of Oligosaccharide Tandem Mass Spectra.

Research Study Presentations

Smith J.L., Jensen B.A., Campbell M., Bryan C.E. 2017. The Influence of Life History and Diet on Mercury Bioaccumulation and Biomagnification in Blood of Bottlenose Dolphins, *Tursiops truncatus,* Under Human Care. *International Conference on Mercury as a Global Pollutant*. Providence, Rhode Island.

Smith J.L. 2016. The Influence of Life History Traits and Diet on Bioaccumulation and Biomagnification of Mercury in Blood of Captive Bottlenose Dolphins (*Tursiops truncatus*). Quest Ocean Talks. Honolulu, Hawaii.

Smith J.L., Jensen B.A., Jones C.M., Campbell M., Bryan C.E. 2015. The influence of life history and diet on bioaccumulation and biomagnification of mercury in blood of bottlenose dolphins, *Tursiops truncatus*. *21st Biennial Conference on the Biology of Marine Mammals*. San Francisco, California.

Reiner J.L., Horton H.A., Bryan C.E. 2015. Perfluorinated alkyl acids in captive dolphins and their diet from Hawaii. *PACIFICHEM 2015*. Honolulu, Hawaii.

Schock T., Campbell M., Bryan C. 2015. Personalized and baseline environmental health of human managed bottlenose dolphin through metabolic profiling. *11th Annual International Conference of the Metabolomic Society*. San Francisco Bay Area, California.

Bryan C.E., Smith J.L., Balmer B.C., Wells R.S., Campbell M. 2014. Comparing blood mercury concentrations in captive and wild bottle nose dolphins (*Tursiops truncatus*). *45th Annual International Association for Aquatic Animal Medicine (IAAAM) Conference*. Gold Coast, Australia.

Moors A.J., Pugh R.S., Becker P.R., Bryan C.E., VanderPol S.S., Keller J.M., Kucklick J.R., Day R.D., Wise S.A. 2012. The U.S. Pacific Islands Region biorepository and environmental specimen bank. *Society of Environmental Toxicology and Chemistry 6th World Congress*. Berlin, Germany.

Pugh R.S, Moors A.J., Becker P.R., Bryan C.E., Vander Pol S.S., Keller J.M. 2012. Continued expansion of the Marine Environmental Specimen Bank into the U.S. Pacific Islands Region. *International Society for Biological and Environmental Repositories (ISBER) 2012 Annual Meeting*. Vancouver, British Columbia, Canada.

Bryan C.E., Pugh R.S., Moors A.J., Becker P.R. 2011. U.S. Pacific Island Region marine environmental biorepository marine mammal programs. *19th Biennial Conference on the Biology of Marine Mammals*. Tampa, Florida.

DISCLAIMER

Certain commercial products and instruments are identified in this paper to adequately specify the experimental procedures. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology. Nor does it imply that the items mentioned are the best for the intended purpose.

ACKNOWLEDGEMENTS

Dolphin Quest management are thanked for establishing the collaboration with NIST and in kind support for their staff and dolphins to participate in the project. Dolphin Quest Hawaii and Oahu staff are greatly appreciated for participating in protocol training; collecting and processing samples; enthusiasm for project involvement; and relaying research study information to guests at their facilities. Hawaii Pacific University and The Marine Mammal Center in Ke Kai Ola staff are thanked for coordinating sample interim storage and shipping to the NIST Biorepository. Julia Smith and DQH staff were key in pilot testing SEA and providing feedback. Amanda Moors is thanked for her support and input to be able to import information from SEA into Freezerworks and setting up the project in Freezerworks. The NIST Biorepository staff are thanked for preparing sample labels and supplies; aid with sample shipping; and archival of samples.

LITERATURE CITED

[1] Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC, Townsend FI, Stone R, Casper DR, Scott MD, Hohn AA, Rowles TK (2004) Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. *EcoHealth* 1:246-254.

[2] Aguirre AA , Tabor GM (2004) Introduction: Marine Vertebrates as Sentinels of Marine Ecosystem Health. *EcoHealth* 1(3):236-238. https://doi.org/10.1007/s10393-004-0091-9

[3] Grosell M , Walsh PJ (2006) Benefits from the Sea: Sentinel Species and Animal Models of Human Health. *Oceanography* 19(2):126-133.

[4] Bossart GD (2006) Marine Mammals as Sentinel Species for Oceans and Human Health. *Oceanography* 19(2):134-137.

[5] Ross PS (2000) Marine Mammals as Sentinels in Ecological Risk Assessment. *Human and Ecological Risk Assessment: An International Journal* 6(1):29-46. https://doi.org/10.1080/10807030091124437

[6] Ross PS (2002) The role of immunotoxic environmental contaminants in facilitating the emergence of infectious diseases in marine mammals. *Human and Ecological Risk Assessment* 8(2):277-292.

[7] Pugh RS, Ellisor MB, Moors AJ, Porter BJ, Becker PR (2007) *Marine Environmental Specimen Bank: clean room and specimen bank protocols. NISTIR7389* (USDOC, National Institute of Standards and Technology, Gaithersburg, MD).