

## GENERAL STORAGE AND TRANSPORT PROTOCOLS FOR COLLECTED SAMPLES

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### *Papahānaumokuākea Marine National Monument*

This protocol applies to the storage and transport of commonly collected samples (i.e. terrestrial samples, coral, fish, and invertebrates) in the Papahānaumokuākea Marine National Monument. It was developed to ensure proper precautions in the handling of biological samples under the IATA and DOT Federal guidelines for safe transport of biological material (Packing Instruction 650). Separate protocols may exist for individual species or activities.

#### **A. Diseased metazoan samples:**

1. Diseased samples or those in which a parasite or pathogen is suspected will be collected and placed into individual plastic ziplock or whirl pak bags and sealed until return to base station (research vessel or field camp). Bags will be immediately labeled, or pre-labeled bags will be used.
2. Separate equipment will be used to sample health-compromised versus healthy organisms and these tools will be soaked in a freshwater bleach solution for at least ten minutes and rinsed in fresh water between dives (see Disease and Introduced Species protocol for additional collection equipment and disinfection requirements).
3. Sealed plastic bags with diseased samples will be processed immediately or stored on ice in a cooler or other leak-proof container until return to base station.
4. Specimens will not be released or exposed to environments beyond the collection location (island/atoll).
5. Upon return to the ship or field camp, collection tools used for diseased samples will be disinfected with 10% bleach by soaking in a freshly made solution for a minimum of ten minutes, followed by a thorough freshwater rinse and air-drying.
6. Collected specimens will be processed as soon as possible and placed in a clearly labeled primary storage container.
7. Processing and storage containers are dependent on preservation method:

#### Chemically preserved:

8. Specimens will be chemically fixed or preserved in one of the following (in sufficient concentration to fix all tissues):
  - Ethyl alcohol
  - Isopropyl alcohol
  - Methyl alcohol
  - DMSO
  - DNA extraction buffer
  - Z-fix
  - Formaldehyde/formalin
  - Glutaraldehyde
  - Acetone
  - Bouin's fixative
  - Helly's fixative

9. Specimens in primary containers (the first zip-lock or whirl-pak bag) will be double contained in an additional whirl-pak bag, ziplock plastic bag, or plastic jar with a label identifying the collector, site and contents between the primary and secondary containers.
10. Double-contained samples will then be placed in action packer, cooler, or other leak-proof packaging (providing triple containment) capable of surviving a 1.2 m drop without rupture, and clearly labeled on the outside as to the PI and contents for future transport off the ship. This leak-proof packaging must have sufficient absorbent material to contain the entire fluid volume contained in the cumulative sample volume if a leak should occur.
11. Specimens will be preserved and stored in primary and secondary containment, as outlined above, as soon as possible upon return to the ship or field camp, and will remain in preservative in unopened secondary containment until return from NWHI into a BSL-2 facility.
12. All sample containers will be sealed and the outside surface of the leak-proof cooler or action packer will be decontaminated with bleach solution prior to transport off the ship directly to the receiving laboratory.

Frozen:

13. Immediately upon returning to the base station, seawater remaining in whirl-pak storage bags will be decanted into a container. This water will be processed by an MSD or similar sewage treatment process.
14. The specimens will not be removed from the whirl-pak storage bag. Instead, the bag will be resealed, disinfected, clearly labeled, and placed in secondary containment such as ziplock freezer bags. The secondary containers will also be clearly labeled.
15. Samples will be stored frozen in the absence of any buffer aboard the research vessel.
16. The work area will be decontaminated with a 10% bleach solution.
17. After freezing, samples will not be removed, thawed, or opened while aboard the research vessel.
18. When it is time for transport from base station, the samples (still in secondary containment) will be placed in a cooler loaded with icepacks in order to keep them frozen. The cooler will then be sealed for transport.
19. All coolers or action packers will be sealed and the outside surface decontaminated with bleach solution prior to transport off the ship directly to the receiving laboratory.

**B. Non-diseased metazoan or other multicellular samples:**

20. See Disease and Introduced Species protocol for collection equipment and disinfection requirements.
21. Processing and storage containers are dependent on preservation method:

Chemically preserved:

22. Specimens will be chemically preserved in one of the following:

Ethyl alcohol  
 Isopropyl alcohol  
 Methyl alcohol  
 DMSO  
 DNA extraction buffer  
 Z-fix  
 Formaldehyde/formalin  
 Glutaraldehyde  
 Acetone  
 Bouin's fixative  
 Helly's fixative

23. Specimens will be double contained in plastic vials or bottles, glass bottles, whirl-pak or ziplock bags; and placed in action packer or cooler. Blood or blood components should be contained using a primary container, absorbent material, a secondary container, and an outer container that is leak-proof.
24. Specimens will be preserved and stored prior to leaving collection location and will remain in preservative until return from NWHI.

Frozen:

25. Specimens (tissues or whole organisms) will be double contained in plastic bottles, glass bottles, or whirl-pak bags; or larger plastic bags and placed in action packer, cooler, or other leak-proof packaging.

Dried:

26. Terrestrial plants or their parts may be pressed and dried and then transported in a closed container.
27. When appropriate, terrestrial arthropods may be pinned and dried and transported in appropriate closed containers.
28. Coral skeletal samples (e.g., for taxonomic verification studies) will be soaked in commercial bleach solution to remove tissues, air dried, stored in whirl-pak bags, and placed in action packer, or cooler. Remaining bleach solution should not be discarded, but stored in plastic or glass bottles, properly labeled as "waste".

*Papahānaumokuākea Marine National Monument**REVIEWERS:*

*Dr. Roger Fujioka, Researcher, Water Resources Research Center, University of Hawaii*  
*Dr. Drew Harvell, Professor of Ecology and Evolutionary Biology, Cornell University*  
*Dr. Cheryl Woodley, Research Microbiologist, NOAA/National Ocean Service*