

Appendix V. Support Team Processing Guidelines Form

	Analysis	Processing	Preparation to Ship
H-P Tissue	Molecular (protein/DNA)	Time sensitive- IN SHADED OR DARK AREA: Rinse mucus by swishing in seawater, dab on Bounty, place in new pre-labeled Whirlpak™	Wrap in aluminum foil, label with waterproof marker or pre-printed cryotags, place in dry shipper
D-P Tissue	Histology (fixative)	Immediately preserve by transfer to a 50 cc polypropylene tube with approximately 25 mL of an appropriate fixative, such as Z-fix (Anatech Ltd.) diluted 4:1 with sterile ASW (35ppt) * 1:10 tissue: fixative ratio	Hold at ~25°C (Store in cooler) DO NOT FREEZE
H-B Tissue	Microbiology	Keep in Whirlpak™ add sterile artificial sea water if needed, keep at ambient temperature in cooler filled with local seawater	Upon return to shore, homogenize tissue and skeleton with sterile mortar and pestle <ul style="list-style-type: none"> - Flash freeze half of homogenate - Culture bacteria with other half of homogenate
U-B Tissue	Molecular and Microbiology	Epicenter type swab- break off tip and put in cryovial, FTA type swab wipe on card, then break tip and store in 15 cc tube or cryovial	Put cryovials in dry shipper, store card at ambient temp. in Ziploc or other container
D-B Tissue	Microbiology	Should be placed in a container, such as a cryogenic vial	Immediately flash freeze in a liquid nitrogen dry shipper
H-S Swab	1/2 Molecular	Should be kept at ambient seawater temperature, possibly in screw top vials	Culture on media as soon as possible (2-3 hours)
U-S Swab	1/2 Microbiology	Top-side- Invert tube- shake- loosen cap and decant water into 2 mL cryovial Leave gap ~2 cm between sediment sample and cap	Cap sediment tightly and freeze in dry shipper Store liquid at ambient temperature and plate in lab
D-S Swab	Microbiology & Molecular	Transfer from syringe to 2.0 mL cryogenic vial	Place in dry shipper
H-M Mucus	1/2 Molecular		
U-M Mucus	1/2 Microbiology		
D-M Mucus	Microbiology & Molecular		
H-Sediment	1/2 Molecular		
D- Sediment	1/2 Microbiology	Transfer from syringe to 2.0 mL cryogenic vial	Keep at ambient temperature for culture dependent methods
H-Water			
D- Water			