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Preparing Microbiome Samples for Cryo Shipment

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ABSTRACT

This is a generic protocol written for any collaborators or contributing institutions sending samples to the Rich Lab at Otterbein University for microbiome or other genetic analyses. The protocol is written for samples that have already been frozen. They may be stored in any stabilizing solution or dried.

ATTACHMENTS

FedEx Dry_Ice_Label.pdf
Dry_Ice_Job_Aid.pdf

PROTOCOL CITATION

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GUIDELINES

Use proper aseptic technique during this procedure. Do not open sample vials, and make sure you are wearing lab gloves the entire time. Also consider wearing cryo-gloves of some kind to protect your hands from cold burns.

MATERIALS TEXT

If you are transferring samples to new tubes:

- Sterile, disposable tweezers
- 10% bleach solution and kimwipes for cleaning and sterilizing
- Biological Safety Cabinet or some secure area for dealing with biological material
- Sterile, RNase/DNase free tubes (1.5 - 2 mL)
- Biohazard disposal bag
- PPE: gloves, lab coat, optional face mask

For shipment:

- Freezer boxes with lids (can be cardboard or other cryo-safe material)
- Styrofoam cooler with lid
- Dry ice
- Sturdy cardboard box
- Fedex Hazardous materials Labels
- Fedex shipment labels
- PPE: lab gloves, cryo-gloves, lab coat

SAFETY WARNINGS

If you open any of the tubes then treat them as biohazards. Wear proper PPE at all times. That includes proper gloves to protect you from ultra low temps.

BEFORE STARTING

Make sure all samples are stored in the smallest, most secure and clearly-labeled vessels possible. This will keep the shipment cheaper, safer, and ensure that all samples remain frozen. To ensure that this is the case, I am including an optional stage at the beginning of this protocol for transferring samples to smaller tubes and boxes before preparing the shipment. If you carry out this procedure, please be extra careful about aseptic technique. If possible, carry out these steps inside an operational biological safety cabinet or some other enclosure with a HEPA filtration system. Do not let any tools cross-contaminate DNA/RNA between samples. If you need to reuse a tool for two samples, wash it using a 10% bleach solution. Remember that ethanol will not sterilize genetic material.

If samples are already in the proper storage containers for shipment, be sure that you have made arrangements for the time and date of pickup with Dr. Rich or with Fedex. Pre-cool all of the materials, including the box, in a **-80 °C** freezer (or the coldest option available), and do not add the dry ice and close the box until just before shipment.

1 **On ice** Keep all samples on cooling blocks or ice during these procedures.

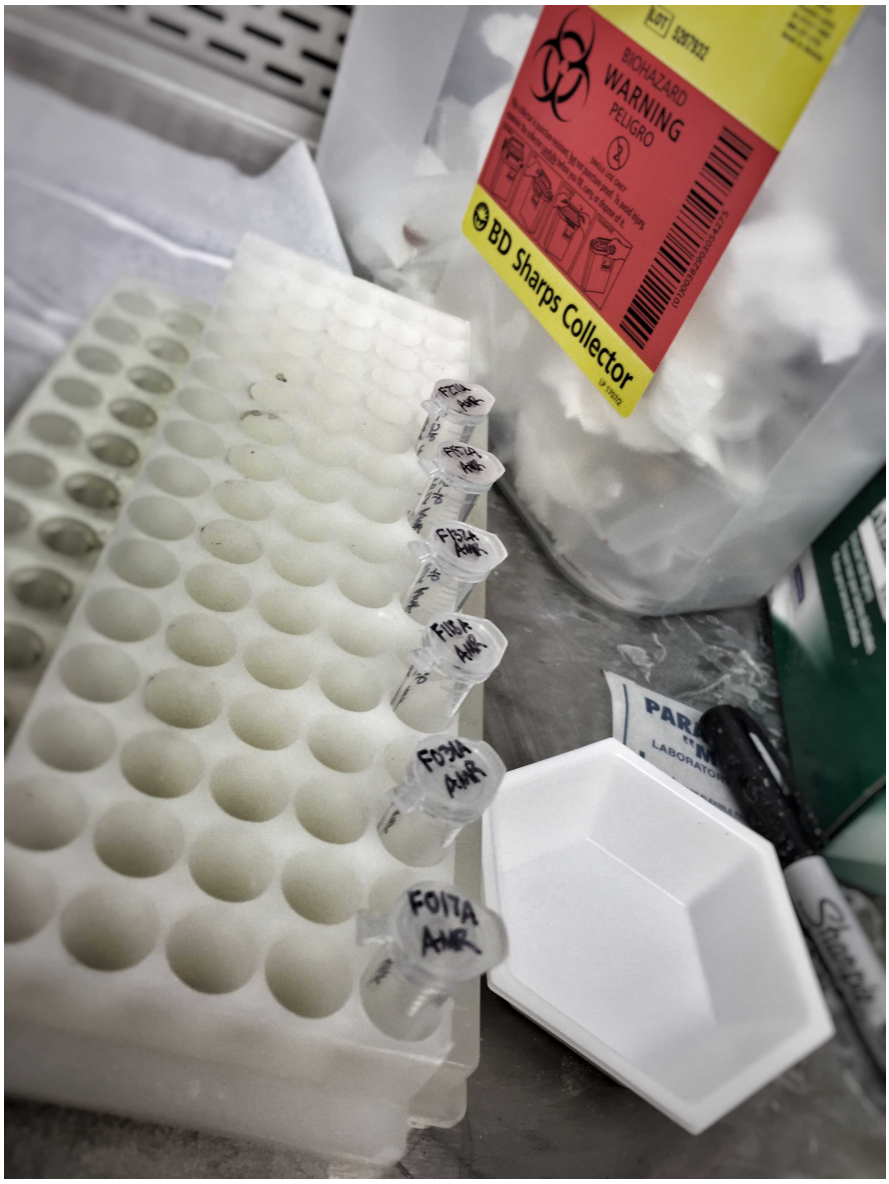
Step 1 includes a Step case.
New Tubes
Shipment Prep

step case

New Tubes

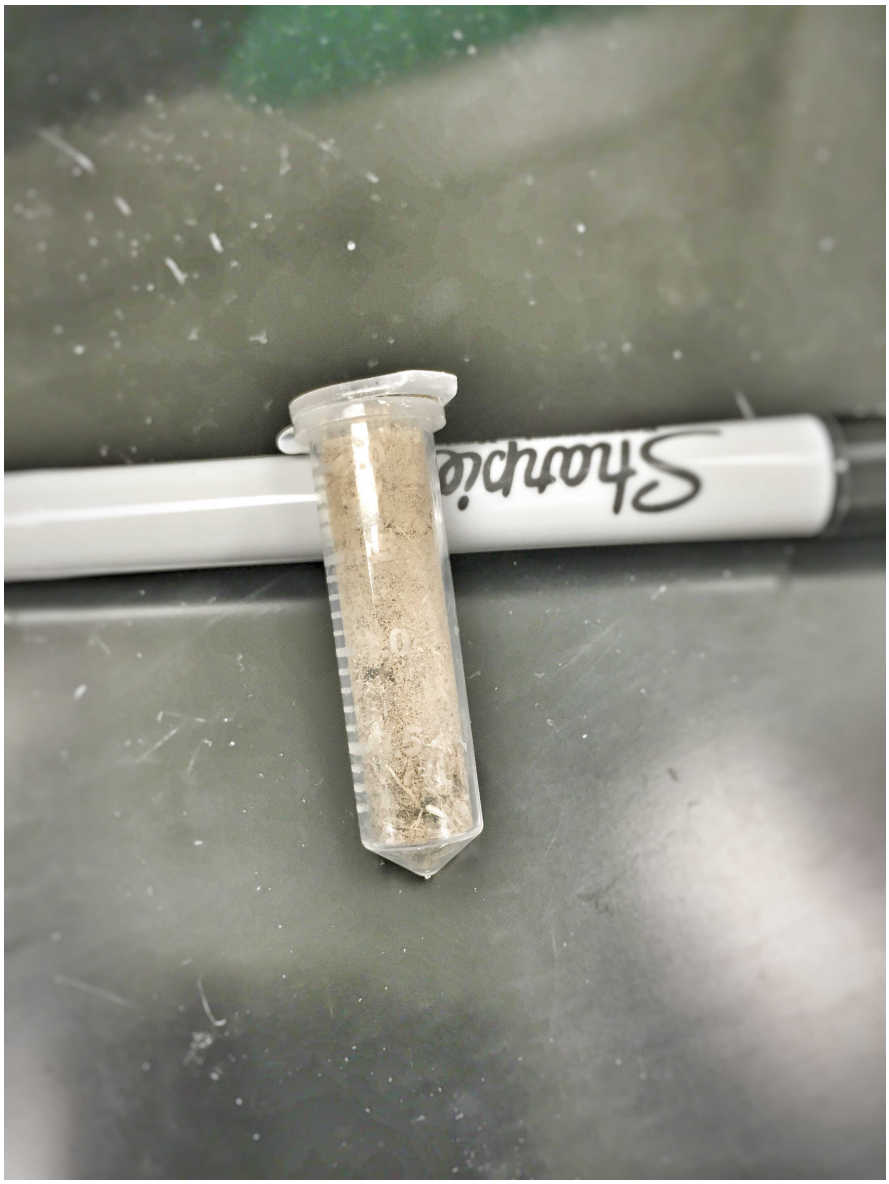
2 Label sterile, RNase/DNase-free tubes for each sample that you will be transferring. You should label them using a thin-tip permanent marker, and copy the information on the previous tube. If that information is not visible, add your initials and the date. Try to make out the sample ID # and add a ? if necessary.

Make sure there are labels on the side and the top of each tube. This ensures that the sample will still be labeled if we lose the cap and that you will be able to easily see the sample number on the tops of tubes looking down into the storage box.



Be careful not to touch the inside of the lids or the tubes, even with your gloved hands. Also try not to touch your face or hair while wearing the gloves, and don't cough or lean over the open tubes. If you need to touch something that might contaminate your gloves, then change out a new pair.

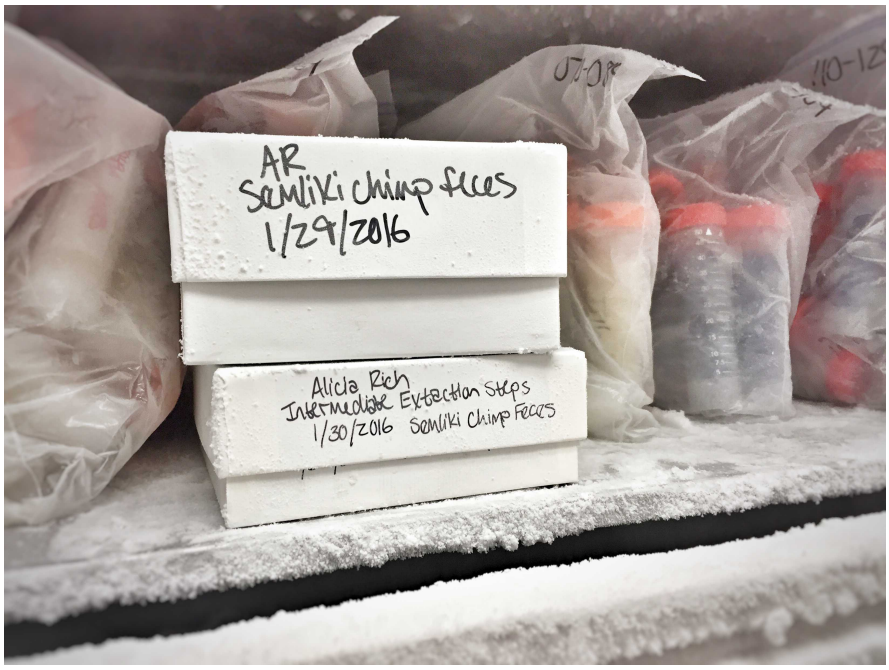
3 Use the sterile tweezers to carefully transfer all of the material from an old tube into the new, fully labeled tube. Again, take care not to touch your finger to the sample or the inside of the lid. Do not use the same tweezers for more than one sample, or if you must, then clean the tweezers between samples with a 10% bleach solution.



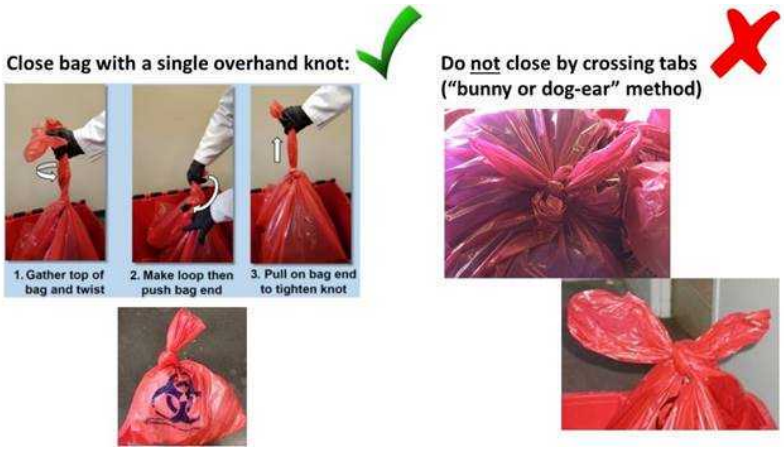
3.1 As soon as you complete one sample, cap both tubes. Put the old tube into a biohazard disposal bag. Put the new tube back into the freezer immediately or keep it on ice.

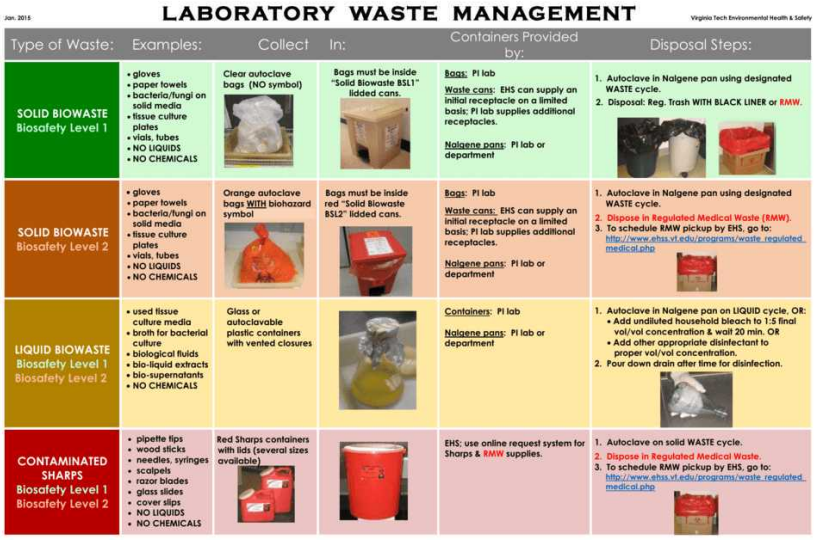
The most important thing is that you do not transfer genetic material from one sample to the next when you do this. Consider rubbing a few drops of the 10% bleach solution on your gloves between samples or change gloves if you think you inadvertently touched any sample material before you move to the next one.

3.2 After you finish all of the tubes, carefully arrange them in the sample box that I provided and double check that all of the caps are labeled with the sample #. Put this box into the freezer until shipment.



3.3 Properly dispose of the biohazard bag with the old tubes. This will depend on your lab's protocols, but you may need to put autoclave tape on the bag, autoclave it for sterilization, and then put it in the trash for disposal. Only materials that may have remaining biological materials on them are considered biohazard waste requiring autoclaving before disposal.





LABORATORY WASTE MANAGEMENT

Jan. 2015

Virginia Tech Environmental Health & Safety

Type of Waste:	Examples:	Collect In:		Containers Provided by:	Disposal Steps:
SOLID BIOWASTE Biosafety Level 1	• gloves • paper towels • bacteria/fungi on solid media • tissue culture plates • vials, tubes • NO LIQUIDS • NO CHEMICALS	Clear autoclave bags (NO symbol)	Bags must be inside "Solid Biowaste BSL1" lidded cans.	Bags: PI lab Waste cans: EHS can supply an initial receptacle on a limited basis; PI lab supplies additional receptacles. Nalgene pans: PI lab or department	1. Autoclave in Nalgene pan using designated WASTE cycle. 2. Disposal: Reg. Trash WITH BLACK LINER or RMW.
SOLID BIOWASTE Biosafety Level 2	• gloves • paper towels • bacteria/fungi on solid media • tissue culture plates • vials, tubes • NO LIQUIDS • NO CHEMICALS	Orange autoclave bags WITH biohazard symbol	Bags must be inside red "Solid Biowaste BSL2" lidded cans.	Bags: PI lab Waste cans: EHS can supply an initial receptacle on a limited basis; PI lab supplies additional receptacles. Nalgene pans: PI lab or department	1. Autoclave in Nalgene pan using designated WASTE cycle. 2. Dispose in Regulated Medical Waste (RMW). 3. To schedule RMW pickup by EHS, go to: http://www.ehss.vt.edu/programs/waste_regulated_medical.php
LIQUID BIOWASTE Biosafety Level 1 Biosafety Level 2	• used tissue culture media • broth for bacterial culture • biological fluids • bio-liquid extracts • bio-supernatants • NO CHEMICALS	Glass or autoclavable plastic containers with vented closures		Containers: PI lab Nalgene pans: PI lab or department	1. Autoclave in Nalgene pan on LIQUID cycle, OR: • Add undiluted household bleach to 1:5 final vol/vol concentration & wait 20 min. OR • Add other appropriate disinfectant to proper vol/vol concentration. 2. Pour down drain after time for disinfection.
CONTAMINATED SHARPS Biosafety Level 1 Biosafety Level 2	• pipette tips • wood sticks • needles, syringes • scalpels • razor blades • glass slides • cover slips • NO LIQUIDS • NO CHEMICALS	Red Sharps containers with lids (several sizes available)		EHS; use online request system for Sharps & RMW supplies.	1. Autoclave on solid WASTE cycle. 2. Dispose in Regulated Medical Waste. 3. To schedule RMW pickup by EHS, go to: http://www.ehss.vt.edu/programs/waste_regulated_medical.php

4 Proceed to the steps for sample shipment.