Human microbiome sample aliquoting

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1 Abstract

The goal is to subaliquot the specimen from each stool hat into 5 small aliquots (25- 500mg) in 2mL screw-cap tubes and 1 large aliquots (1 g) in 15mL short polycarbonate screw-cap vials for more efficient storage of each sample. DNA will be extracted from one of the smaller aliquots. It is important to keep everything cold when handling the stool preparation (-80C).

2 Materials and equipment

2.1 Materials

- Disposable Aluminum Dishes with Fluted Sides [Fisher Scientific, 08-732-109]
- GEN Standard Aluminum Foil Roll [Office Depot, 3994463]
- Axygen 2.0mL Self-Standing Screw Cap Tubes [Fisher Scientific, 14-222-626]
- Nalgene™ General Long-Term Storage Cryogenic Tubes [Fisher Scientific, 03-337-7B]
- ECO Spatula, Double-Ended, with Spoon & Scoop [VWR, 89233-128]
- Absorbent Underpads with Waterproof Moisture Barrier [VWR, 56616-022]
- PORTRAIT CRYO LABELS-1ML 2.75 [Fisher Scientific, NC9569413]
- Ziploc Storage Bags, 1 Gallon [Office Depot, 507271]
- Professional Standard Cone-Style Surgical Mask [Fisher Scientific, 19-166-977]
- Cardinal Health Fluid Resistant Cover Gowns [Fisher Scientific, 19-160-257]
- PolyWear™ Smooth Disposable Gowns [Fisher Scientific, 18-567]
- SPEARLAB CRYOGENIC FOAM [Fisher Scientific, 50-005-3]
- Cryo/Freezer Boxes - 100 [Fisher Scientific, 03-395-465]
- Cryo/Freezer Boxes - 81 [Fisher Scientific, 03-395-464]
- Misc (Gloves, Iodine, Bleach, EtOH, dry ice, LN_2)

2.2 Equipment

- Sensitive digital scale
- Biological safety cabinet (non-recirculating)
- Freezer (-80C)
- Custom mortar, pestle, mold to shape aluminum foil and tube racks
- Containers for dry ice and cleaning solution

3 Safety precautions

3.1 Human specimen handling

All procedures should be performed in a biosafety cabinet that vents to the HVAC system or to a carbon filter to protect the user from exposure to any potentially infectious agents and to limit the level of odor released to the lab. Universal precautions should be followed when handling human samples considered potentially infectious. Minimal protective clothing requirements are lab coat, gloves and safety glasses.

3.2 Waste

Dispose of all waste as biohazardous material.

3.3 Specific precautions

Nitrogen gas can cause suffocation without warning. Store and use liquid nitrogen only in a ventilated area. In closed areas, excessive nitrogen gas reduces the concentration of oxygen and can result in asphyxiation. The use of oxygen monitoring equipment is strongly recommended. Contact with the skin or eyes may cause serious freezing injuries. Always wear the appropriate protective clothing, i.e cryo-gloves and face shield.

4 Method

4.1 Specimen handling

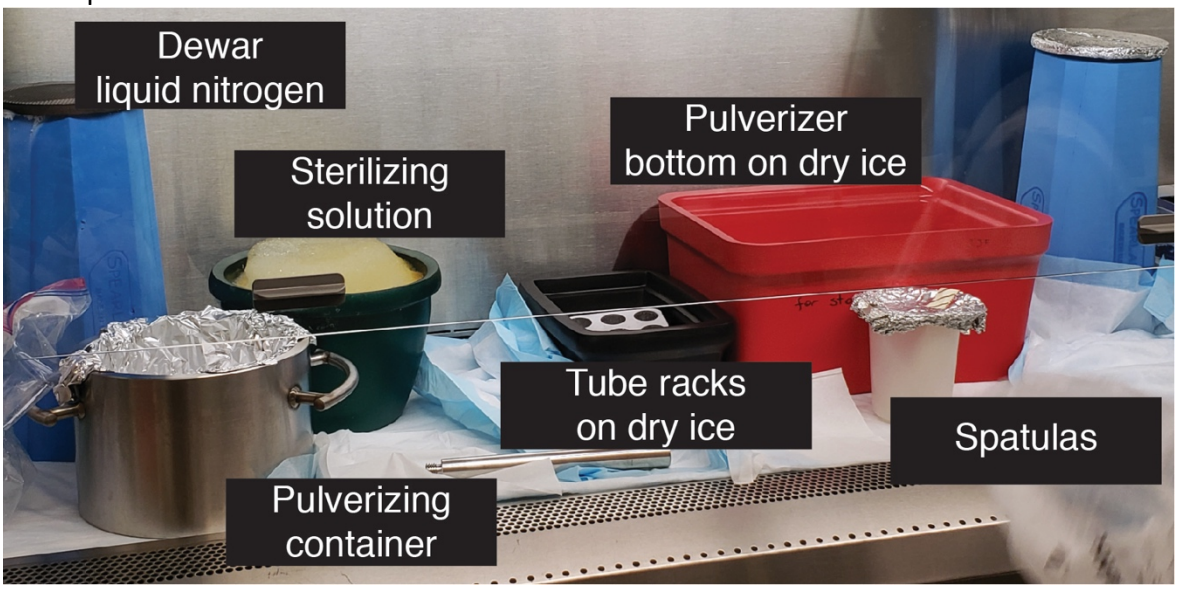
Keep the specimen at -80C to minimize the death of strict anaerobes that could result from exposure to an oxygen rich environment. Secondly, stool becomes sticky and difficult to manipulate if it is not kept solid to the point of being brittle.

4.2 Preparation

1. Pre-weigh all labels and barcoded tubes (5x Axygen 2.0mL Self-Standing Screw Cap Tubes, 1x Nalgene™ General Long-Term Storage Cryogenic Tubes)
2. Autoclave spatulas.

4.3 Prepare workspace

Workspace:



3. Fill dewar with liquid nitrogen.
4. Prepare sterilizing solution by diluting Wescodyne 100x in cold water and pour into designated container.
5. Place pulverizer bottoms on dry ice.
6. Place tube racks on dry ice.
7. Prepare 70% Ethanol (150 ml H_2O and 250 ml ethanol)

4.4 Pulverize stool sample (for each sample)

Mold:

8. Shape aluminum foil on mold and place in steel pulverizing container.
9. Place disposable aluminum dish in steel pulverizing container.
10. Transfer fecal sample into the pulverizing container.
11. Pour LN_2 over the stool to make it increasingly brittle.
12. Strike the stool with the pestle until there are numerous large fragments, then pulverize some of the larger fragments until a granular powder (~1-2 mm pieces) is generated.
13. Transfer into tubes as follows

a) Axygen Tube 1 (~50 – 100 mg). 

b) Axygen Tube 2 & 3 (~100 – 200 mg) 

c) Axygen Tube 4 & 5 (~200 - 500 mg) 

d) Long-Term Storage fill (~4 – 5g)

4.5 Post-processing

14. Place pestle in diluted Wescodyne solution for at least 10 minutes and subsequently rinse with 70% ethanol and air dry (pestle can now be use for next sample).
15. Post weight all tubes and store at -80C.

4.6 Disinfecting Biosafety Cabinet

16. Remove all dry ice and sterilizing containers and clean in the sink with bleach (1Tbsp of bleach in 3.7L water) for 2 minutes.
17. Clean biosafety hood from left (sample processing area) to right (“clean” area) by removing used pads, spray working area with 70% isopropyl alcohol, wipe down and put a new clean pad on the surface.
18. Return all cleaned containers, close hood and turn on UV light for 15 minutes.