



Human fecal sample collection (Cryo)

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1 Abstract

The goal is to collect fecal samples in sufficient amounts and in a manner that is compatible with various downstream assays including metagenomics and live microbial culturing. Live microbial culture of anaerobes and aerobes allows for functional characterization of these microbes in in vitro assay and in vivo (gnotobiotic) animal models and whole genome assembly including genome annotation. This requires a cold-chain samples collection and overnight shipment. Note that Thermo packs frozen at -23°C and then placed in a well-insulated container typically rises about 0.5°C per hour at an ambient temperature of 25.3°C. Samples should be kept as much as possible below -15°C and post-processing should take place under cryogenic conditions (see MTCP002-Stool_Processing).

2 Materials and equipment

2.1 Materials

- EasySampler Stool Collection Kit [Alpco, 58-EZSAMPLER]
- ThermoSafe Multi-Purpose Foam Container [Fisher Scientific, 03-530-79]
- Biohazard Specimen Transport Bags [Fisher Scientific, 01-800-05]
- Covidien Precision Stool Collector [Fisher Scientific, 14-375-156]
- ThermoSafe U-tek™ Phase Change Materials [Fisher Scientific, 03-528-5]
- Ziploc® Storage Bags, 1 Gallon [Office Depot, 507271]
- Wraparound Labels [FreezerWorks, LBL-02WR1]
- Ribbons [FreezerWorks, RIB-004]

2.2 Equipment

- Freezer (-80°C)

3 Safety precautions

3.1 Human specimen handling

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.

4 Method

4.1 Kit components

#	Description	Vendor	Catalog	Picture
1	EasySampler® collection hat from EasySampler Stool Collection Kit	Alpco	58-EZSAMPLER	
2	Pair of disposable gloves from EasySampler Stool Collection Kit	Alpco	58-EZSAMPLER	
3	ThermoSafe Multi-Purpose Foam Container	Fisher Scientific	03-530-79	
4	Biohazard Specimen Transport Bags	Fisher Scientific	01-800-05	
5	Covidien Precision Stool Collector	Fisher Scientific	14-375-156	
6	ThermoSafe U-tek™ Phase Change Materials	Fisher Scientific	03-528-5	
7	Two Ziploc® Storage Bags, 1 Gallon	Office Depot	507271	

Table 1. Kit components

4.2 Kit assembly instructions

- 1) Label Covidien Precision Stool Collector (Table 1, component 6) with printed barcoded-Freezerworks label.
- 2) Combine into Ziploc bag “A” the following:
 - (a) EasySampler collection hat from EasySampler Stool Collection Kit (Table 1, component 1)
 - (b) Pair of disposable gloves from EasySampler Stool Collection Kit (Table 1, component 2)
 - (c) Biohazard Specimen Transport Bags (Table 1, component 5)
 - (d) Barcode-labelled Covidien Precision Stool Collector (Table 1, component 6)
 - (e) Return label

- 3) Combine into Ziploc bag "B" the following:
 - (a) Stool collection instructions (see Appendix 1)
 - (b) Package collection instructions (see Appendix 2)
 - (c) Dietary Questionnaire (see Appendix 3)

- 4) Combined into the ThermoSafe Multi-Purpose Foam Container the following:
 - (a) Ziploc bag "A"
 - (b) Ziploc bag "B"
 - (c) Two ThermoSafe U-tek Phase Change Materials (Table 1, component 7)

4.3 Storage

This requires a cold-chain samples collection and overnight shipment. Note that Thermo packs frozen at -23°C and then placed in a well-insulated container typically rises about 0.5°C per hour at an ambient temperature of 25.3°C. Samples should be kept as much as possible below -15C and post-processing should take place under cryogenic conditions (see MTCP002-Stool_Processing).

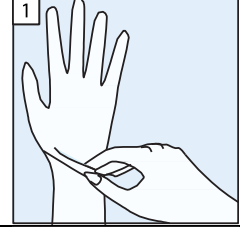

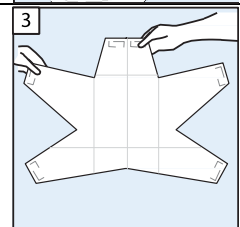
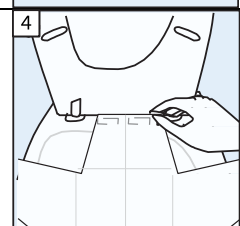
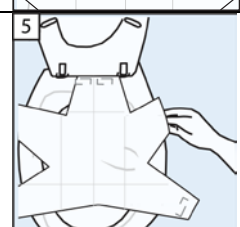
Appendix 1 – Stool collection instructions

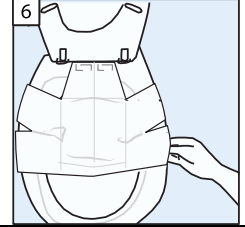
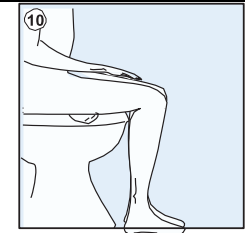


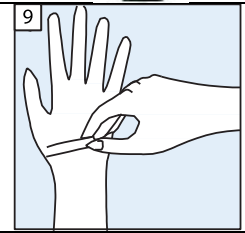
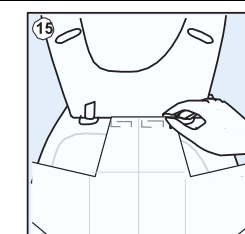

Stool collection instructions

The packaging kit includes:

- Styrofoam box
- Ziploc bag "A" containing: Stool collection instructions, Packaging instructions and Dietary Questionnaire
- Ziploc bag "B" containing:
 - EasySampler collection hat
 - Pair of disposable gloves
 - Biohazard Specimen Transport Bags
 - Barcode-labelled Covidien Precision Stool Collector
 - Envelope/Package Sealing Tape Strips






****Before you collect your sample, place all gel packs in your freezer for at least 12 hours****

	<p>Put on disposable gloves.</p> <p>Note: Gloves are not for patient examination or surgical use.</p>
	<p>Lift toilet seat and wipe off the surface of the toilet bowl rim.</p>
	<p>Unfold the EasySampler collection hat containing five flaps. Orient the larger (REAR) flap towards the back of the toilet with the tape side down.</p> <p>Note: Before affixing the EasySampler to the toilet bowl, bend the paper along the lines for better flexibility.</p>
	<p>Remove the protective covering from the tape and adhere the REAR flap of the EasySampler onto the back of the toilet rim.</p>
	<p>Remove the protective covering from the two MIDDLE flaps and adhere them to the outside of the toilet bowl as far back as possible.</p> <p>Note: EasySampler should not touch the surface of the water.</p>

	<p>The protective covering from the two FRONT flaps and adhere them to the outside of the toilet bowl.</p>
	<p>Make sure the EasySampler is taut and in the shape of a bowl. If not, re-orient the flaps for the correct positioning. Lower the toilet seat once the EasySampler is oriented correctly.</p> <p>Note: Tape can be removed from toilet and re-adhered.</p>
	<p>Have a bowel movement.</p> <p>Note: Make sure stool does not come in contact with the toilet water or urine. If necessary, tilt EasySampler and slowly drain urine away from bowel movement before collecting a sample.</p>
	<p>Collect stool sample using the Covidien Precision Stool Collector spoon into the SAMPLE container.</p> <p>Note: Be careful not to contaminate outside surface of sample container.</p>
	<p>Screw the cap onto the sample container with the spoon is attached to the screw cap.</p>
	<p>Remove gloves and properly dispose of material. Wash hands to prevent contamination.</p>
	<p>Remove EasySampler flaps from toilet bowl and rim. Flush both the EasySampler and excess stool down the toilet.</p> <p>Note: When using water saving toilets use a full flush. Repeat if necessary.</p>
	<p>Place sealed container into biohazard bag and close bag. Place the biohazard bag containing the sample container in Ziploc bag "B" and close the bag. Store in freezer (-20C) or immediately prepare shipment.</p>

Appendix 2 – Packaging instructions

Packaging Instructions

 <p>1</p>	<p>Take the gel packs and place them in your freezer for at least 12 hours.</p>
 <p>2</p>	<p>Remove the gel packs from your freezer and place one gel pack in the bottom of the Styrofoam box.</p>
 <p>3</p>	<p>Place the sealed Ziploc bag "B" containing your collected stool in the biohazard bag on top of the frozen gel packs in the Styrofoam container.</p>
 <p>4</p>	<p>Place another frozen gel pack on top of the sample bag so that the bag is completely surrounded.</p>
 <p>5</p>	<p>Place the Styrofoam lid on the Styrofoam container and place the Ziploc bag "A" with your completed <u>dietary questionnaire</u> on the top or side.</p>
<p>Contact the research team for pickup or prepare shipment.</p>	

Appendix 3 - Questionnaire

Questionnaire