

# **INCLUDE**

Down Syndrome Biorepository

## **Down Syndrome Cohort Development Program Collection Site Manual of Standard Operating Procedures**

# Overview of Manual of Standard Operating Procedures (SOPs)

The INCLUDE Down syndrome Biorepository (DS-Biorepository) is a federated biobank with the mission of improving the lives of people with Down syndrome by advancing biomedical research. The DS-Biorepository serves three essential functions: 1) providing streamlined sample collection using validated biospecimen collection and processing protocols and pre-assembled biospecimen collection kits, 2) hosting national (United States, U.S.) and international biospecimen catalogs from multiple biobanks in a centralized virtual biorepository, and 3) housing and distribution of biospecimens to accelerate collaborative research efforts.

This Manual of Standard Operating Procedures (SOPs) contains SOPs from the INCLUDE Down syndrome Biorepository (DS-Biorepository) relevant to the INCLUDE Down syndrome Clinical Cohort Coordinating Center (DS-4C), and the contributing research sites participating in the INCLUDE Down syndrome Cohort Development Program (DS-CDP). This manual is intended to serve as a starting point of reference for a manual of SOPs to be developed for the future DS-Biorepository and is subject to change. The SOPs included in the final manual will be selected and modified in accordance with the common protocol determined by the NIH, DS-4C, DS-CDP, and the DS-Biorepository.

# Version History

<b>DS-Biorepository</b> <i>Standard Operating Procedure</i>	
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## List of Abbreviations

<b>Abbreviation</b>	<b>Definition</b>
<b>BSC</b>	Biological safety cabinet
<b>CU</b>	University of Colorado
<b>CyTOF®</b>	Cytometry by time of flight
<b>DMSO</b>	Dimethyl sulfoxide
<b>DNA</b>	Deoxyribonucleic acid
<b>DS</b>	Down syndrome
<b>DS-4C</b>	Down syndrome clinical cohort coordinating center
<b>DS-CDP</b>	Down syndrome cohort development program
<b>EDC</b>	Electronic data capture
<b>EDTA</b>	Ethylenediamine tetraacetic acid
<b>FBS</b>	Fetal bovine serum
<b>GUID</b>	Globally unique identifier
<b>DS- Biorepository</b>	Down syndrome Biorepository
<b>INCLUDE</b>	INvestigation of Co-occurring conditions across the Lifespan to Understand Down syndromE
<b>LFB</b>	Lyse/fix buffer
<b>LIMS</b>	Laboratory information management system
<b>NIH</b>	National Institutes of Health
<b>PAX</b>	PreAnalytiX
<b>PBS</b>	Phosphate-buffered saline
<b>PID</b>	Participant ID
<b>QC</b>	Quality control
<b>RBC</b>	Red blood cell
<b>RNA</b>	Ribonucleic acid
<b>RUO</b>	Research use only
<b>SOP</b>	Standard operating procedure
<b>U.S.</b>	United States

# Manual Overview

## Purpose

The purpose of this protocol is to facilitate a streamlined process for biospecimen collection, storage, shipping, and processing. By following these protocols, biospecimens will be collected and handled uniformly, ensuring that data acquired from prospective uses are as comparable as possible, regardless of the collection site.

## Scope

This manual is broadly broken into four main sections: [Collection Kit Overview](#), [Biospecimen Collection](#), [Shipping Instructions](#), and [Quality Control](#). Biospecimen collection kits are assembled in the INCLUDE DS-Biorepository central lab, located at the University of Colorado Anschutz Medical Campus, and shipped to various collection sites. Using these kits and following the protocols outlined in the [Biospecimen Collection](#) section, the collection sites will at a minimum, collect and store tongue swabs, cells for downstream DNA isolation, cells stabilized for RNA isolation, and plasma. In addition to the minimum, these protocols will facilitate the collection of cryopreserved buffy coat, red blood cells, and whole blood fixed for immunophenotyping using Cytometry by time of flight (CyTOF®). Samples will be batch-shipped once a predetermined threshold of 4 participants (U.S. Sites) or 10 participants (international sites) have been collected, or 3 months have passed since the last shipment.

## Required Materials

Below is a list of the required materials for biospecimen collection, storage, and return shipment to the central biorepository. Once received by the central lab, these samples will be stored for prospective research use. Many of these materials are provided by the DS-Biorepository central lab as part of the pre-assembled kits. Materials provided in these kits, as well as materials and equipment required but not supplied, are detailed below and in their respective portions of the [Biospecimen Collection](#) section.

Material	Source	Vendor	Catalog #
1000 ul Barrier Tips, Sterile	To be provided by collection site		
10X Phosphate Buffered Saline (PBS)	Supplement Kit (for Child/Adult Kits)	Rockland	MB-008
200 ul Barrier Tips, Sterile	To be provided by collection site		
20 ul Barrier Tips, Sterile	To be provided by collection site		
BD Phosflow™ Lyse/Fix Buffer 5x	Supplement Kit (for Child/Adult Kits)	BD Bioscience	558049
Biospecimen Bag, Small	Child, Adult, Tasso Kits	Fisher Scientific	12-888-306
Biospecimen Bag, Medium	Child, Adult, Saliva	Fisher Scientific	23-700-205
Conical tubes, 15 mL	To be provided by collection site		

Conical tubes, 50 mL	To be provided by collection site		
Cryogenic Vials, 1.2 mL	Child, Adult Kits	Corning	430487
Dimethyl Sulfoxide (DMSO), Sterile Filtered	Supplement Kit (for Child/Adult Kits)	Sigma-Aldrich	D2438-50ML
Dry Ice	To be provided by collection site		
Dry Ice Label	Child, Adult Kits	Uline	S-2844
Fetal Bovine Serum-Heat inactivated (FBS)	To be provided by collection site	Gibco	16140071
Gauze	Tasso Kits	Fisher Scientific	22-362178
Gloves	To be provided by collection site		
Insulated Shipping Box	To be provided by collection site		
K2 EDTA Vacutainer, 10 mL	Adult Kits	BD Bioscience	366643
K2 EDTA Vacutainer, 4 mL	Child Kits	BD Bioscience	367861
Liquid Medical Waste Fluid Solidifier	Child, Adult Kits	Ecolab	LTSP3000
Matrix 0.5 mL Screw Top Tubes	Child, Adult, Tasso Kits	Thermo Scientific	3745-BR
Matrix Tube Caps, Colorless	Child, Adult, Tasso Kits	Thermo Scientific	4470
Matrix Tube Caps, Red	Child, Adult, Tasso Kits	Thermo Scientific	4470RED
Maxpar® Cell Staining Buffer	Supplement Kit (for Child/Adult Kits)	Standard BioTools	201068
Microcentrifuge Tubes, 1.5 mL	To be provided by collection site		
Microcentrifuge tubes, 2.0. mL	Tasso, Child, Adult Kits	Thermo Scientific	3463
OMNIgene Tongue Swab	Tasso, Child, Adult Kits	DNA Genotek	OMR-120
ORAgene™ Saliva Kit	Saliva Kits	DNA Genotek	OGR-600
PAXgene® Blood DNA Vacutainer, 2.5 mL	Child Kits	BD Bioscience	761165
PAXgene® Blood DNA Vacutainer, 8.5 mL	Adult Kits	BD Bioscience	761115
PAXgene® Blood RNA Vacutainer, 2.5 mL	Child, Adult Kits	BD Bioscience	762165
Red Blood Cell Lysis Buffer	Supplement Kit (for Child/Adult Kits)	Thermo Scientific	A1049201
Rigid shipping box (for return of samples)	To be provided by collection site		
RNAlater™ Stabilization Solution	Tasso Kit	Invitrogen	AM7020
Saliva Collection Sponge	Tasso, Child, Adult Kits	DNA Genotek	OG-AC1

Serological Pipets, 10 mL	To be provided by collection site		
Styrofoam box (return of samples)	To be provided by collection site		
Tasso+® EDTA Kit, RUO	Tasso Kits	Tasso	K1025-01-EDT-G01-RUO
Therapak™ Aquī-Pak™ Absorbent Sleeves	Child, Adult, Tasso, Saliva Kits	Fisher Scientific	22-130-043
UltraPure Distilled Water	Supplement Kit (for Child/Adult Kits)	Invitrogen	10977-015
UN3373 Label	All Kits	Uline	S-12521
Ziploc Bag, 12" x 15"	All Kits	Fiser Scientific	NC9870745

## Collection Kit Overview

The DS-Biorepository central lab will provide pre-designed and assembled biospecimen collection kits to collection sites. Each kit is designed for a single participant, and the materials provided in the kit are barcoded such that the kit ID barcodes will be linked to the participant of that kit.

**It is IMPERATIVE that barcoded sample collection tubes are not mixed between kits, used with different participants, or different participant visits.** For example, if a barcoded kit is used for a participant, every barcoded tube from **that same kit** must be used **for the same participant and same collection visit** or discarded after the visit is done. This ensures that the biorepository can properly track samples from a given participant visit. The only exceptions are the Child/Adult Supplement kits and Replacement Component kits which do not contain individually barcoded collection tubes. An overview of each collection tube can be viewed in the [Collection Kit Quick Reference Guide](#) in the appendix.

The following tables provide an overview of the intended participants. Detailed collection protocols for each kit can be reviewed in the [Biospecimen Collection](#) section.

## Collection Kit Demographics and Description

### Tasso, Child, Adult, Saliva, Remote Collection Kits

Participant Demographics	Tasso Kit	Child Kit	Adult Kit	Saliva Kit	Remote Kit
Weight requirement (kg)	3+	5-11	11+	0+	5+
Maximum blood draw (mL)	1	10-22	22-46	N/A	5
Age (Approx. years)	0+	0-10	10+	0+	0+

The preferred collection kits are the Child and Adult kits, which ensure harmonization between sample collections and that enough sample volume can be collected for prospective research uses.

The Tasso device kits are only intended for use in cases where a participant is unable or refuses to provide blood through venipuncture. The Tasso kits will yield a maximum of 1 mL of capillary blood. The Child and Adult kits provide materials for collecting multiple blood vacutainers. Not every vacutainer must be used to fulfill the minimum required samples. Safe blood draw maximums are determined by participant weight. The volumes above reflect 2 mL/kg/day. The saliva kit is an **optional** standalone kit that will enable collection of saliva as a backup source for DNA. The preferred source of DNA for all participants is blood.

Remote collection kits may be used for non-local participants who cannot provide samples directly to the research sites. These kits allow for collection of DNA and RNA through venipuncture as well as microbiome through tongue swabs. Collection of DNA and RNA will require a phlebotomist to perform a blood draw. The DS-Biorepository will provide the Remote kit to research sites, but the research sites will be responsible for coordinating remote collection events. After collection, the Remote kit samples should be returned to the collection site. These samples should then be batch shipped back to the DS-Biorepository as outlined in the shipping instructions.

Biospecimen Types	Collection Kits	Notes
DNA, RNA, Plasma, Microbiome, RBCs	 <p>Adult Kit    Child Kit    Tasso Kit</p>	<ul style="list-style-type: none"> <li>• One kit per participant</li> <li>• RBCs cannot be collected from the Tasso kit</li> </ul>
Viable Buffy Coat and CyTOF®-Ready Whole Blood	 <p>Child/Adult Supplement Kit</p>	<ul style="list-style-type: none"> <li>• One kit needed for 50 child or adult kits</li> <li>• Cannot be used alone (requires either an adult or child kit for sample collection)</li> </ul>
DNA	 <p>Saliva Kit</p>	<ul style="list-style-type: none"> <li>• Saliva kits should be used to collect DNA if the participant will not or cannot give a blood sample</li> </ul>
DNA, RNA, Microbiome	 <p>Remote Kit</p>	<ul style="list-style-type: none"> <li>• Remote kits may be used for non-local collection events.</li> </ul>

Figure 1. Biospecimens by Kit. Created with BioRender.com.

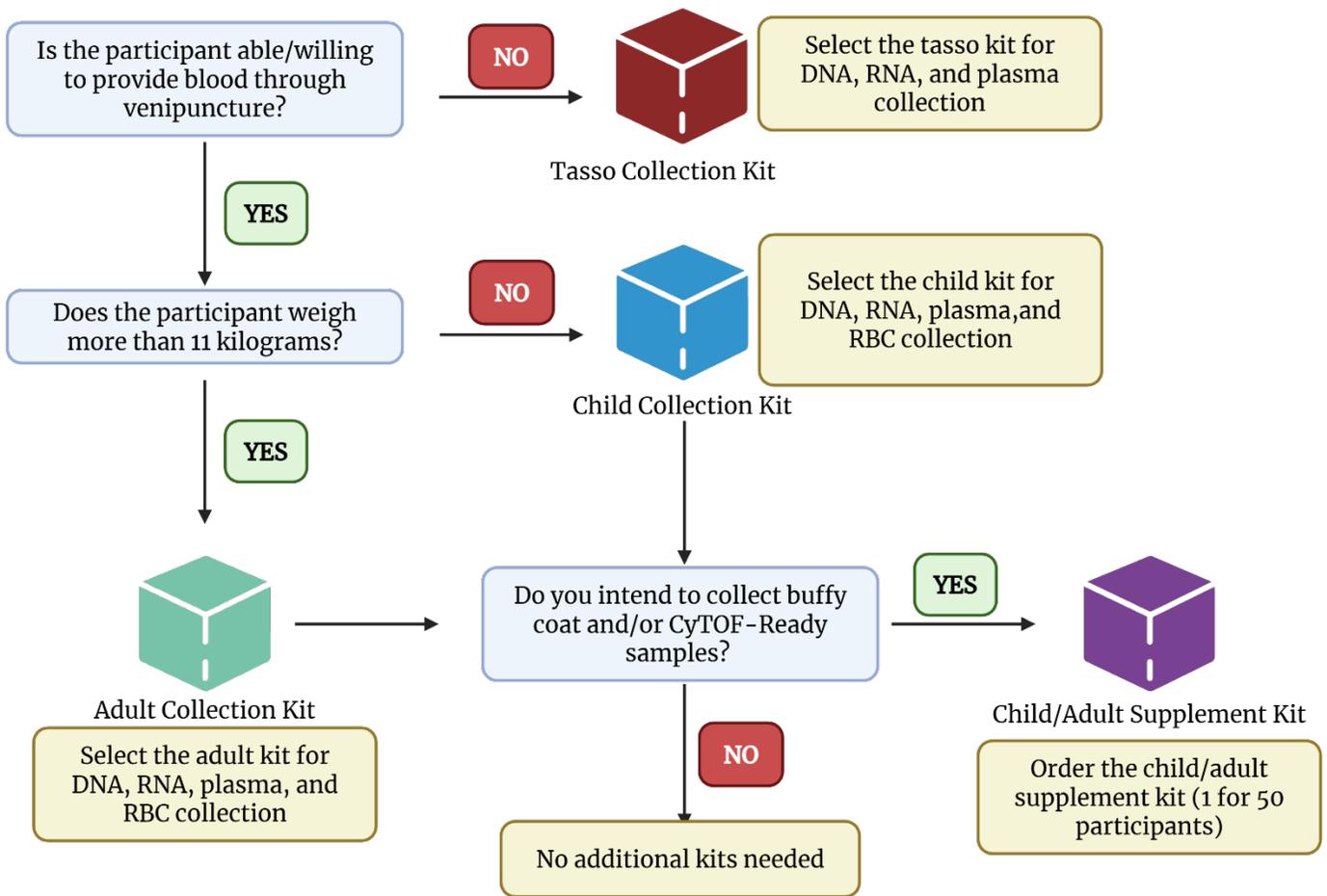


Figure 2. Tasso, Child, and Adult Kit Selection Guide. Created with BioRender.com.

## Replacement Component Kit

The replacement components are meant to be used if either a biospecimen collection tube (EDTA vacutainers, PAXgene® tubes, tongue swabs) or a biospecimen storage tube (15 mL conical of plasma, plasma matrix tubes, RBC matrix tubes, CyTOF®-ready microcentrifuge tubes, or buffy coat cryogenic vials) are damaged and need to be replaced. The replacement kit supports replacing tubes from the Tasso, Child, or Adult kits. Since the collection and storage tubes from each kit are barcoded with kit IDs, which are tied to specific collection events, **any replacement tubes needed must come from this kit rather than any of the other collection kits.**

If a tube needs to be used from the replacement kit, the collection site should track this event using the **replacement kit form**. On this form, indicate the kit ID and tube ID of the tube(s) that need to be replaced, and the tube ID(s) used to replace it. Attach a copy of this form to the kit specimen form used for biospecimen collection and processing. When both the kit specimen form and the replacement form are returned to the DS-Biorepository central lab with the biospecimens, the central lab will update the biospecimen tracking logs to include the replacement tube IDs as part of the specific collection event they were used for.

## Child/Adult Supplement Kit

These supplement kits are designed to provide labs with additional materials required for use with the Child or Adult kits. These materials facilitate the collection of cryopreserved buffy coats and

CyTOF®-ready samples. These materials include temperature-sensitive reagents, such as Fetal Bovine Serum (FBS), which are shipped separately for logistics. Each supplement kit contains enough materials for **50 participant visits**. The reagents are **NOT participant or visit-specific** and are intended for use during the blood processing steps of either the Child or Adult Kits.

## Kit Requests

Kits may be requested in advance through the DS-Biorepository website using the kit request portal. Alternatively, if the kit request portal is inaccessible, requests may be made through a webform on the DS-Biorepository website or via email to the DS-Biorepository staff. Multiple kits may be ordered in advance; however, collection sites should consider their anticipated enrollment schedules, as many of the kit components have a shelf life of 6 months.

## Kit Storage

### Tasso, Child, Adult, Saliva, Remote, Replacement Tube Collection Kits

- Store kit contents at room temperature.
- **For kit requests in the U.S.** Return shipping labels will be provided based on the number of kit requests. Collection sites should store the return shipment labels in a safe location until needed for return samples. **For international sites**, return shipping labels will be provided through World Courier.

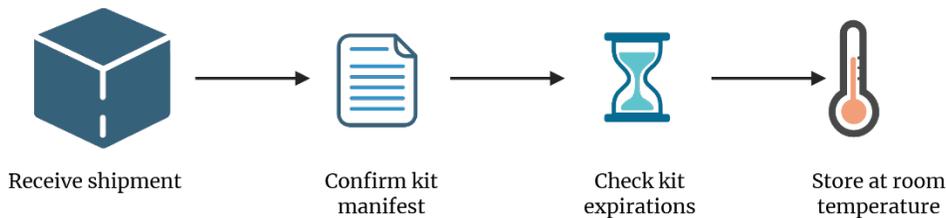


Figure 3. Tasso, Child, and Adult Kit Storage. Created with BioRender.com.

### Child/Adult Supplement Kit

- Store 10x PBS, H<sub>2</sub>O, and RBC Lysis buffer at room temperature.
- Store DMSO and 5x Lyse/Fix buffer at room temperature, protected from light.
- Store Maxpar® buffer at 4°C.
- Store FBS at 4°C for short-term (<1 month) or -20°C long-term.

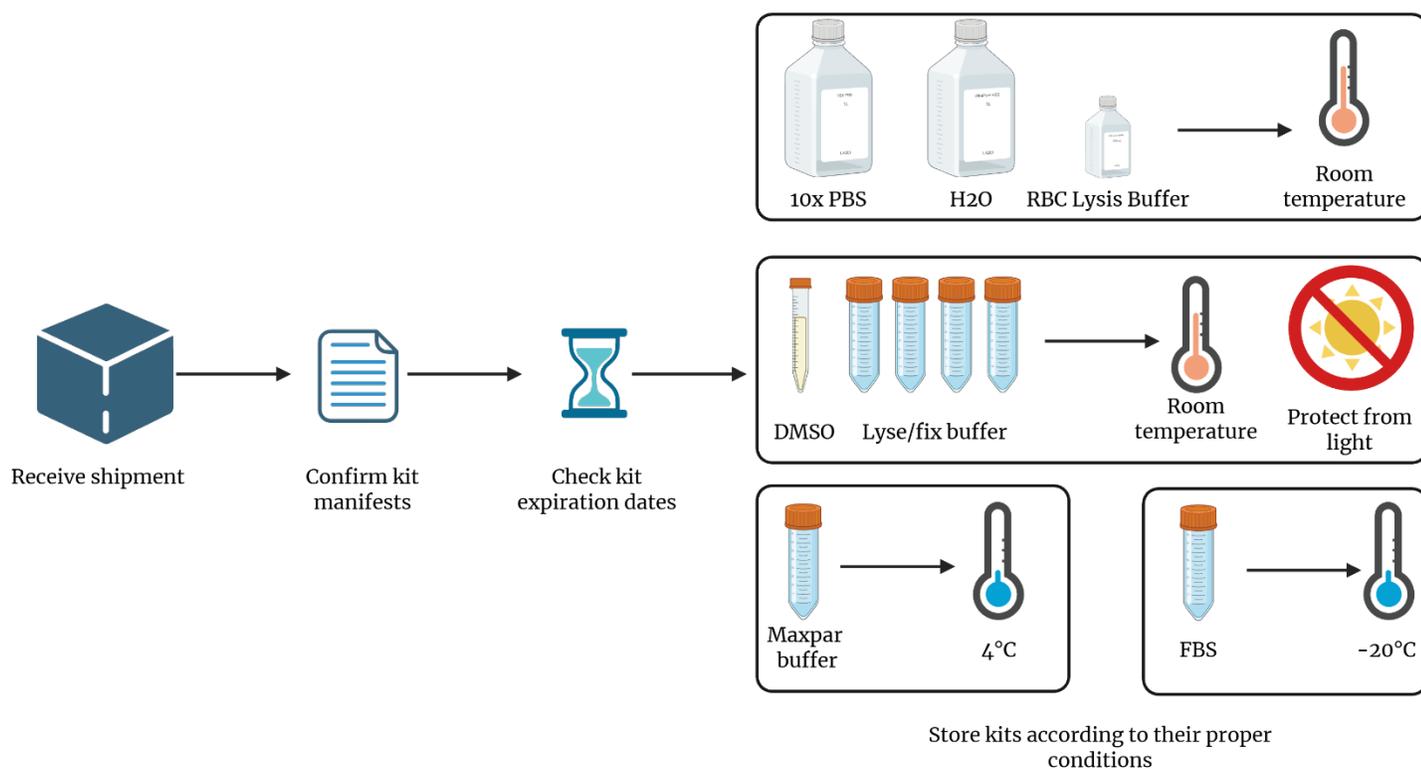


Figure 4. Storage of Child/Adult Supplement Kits. Created with BioRender.com.

## Biospecimen Collection

### Tasso Kit Instructions for Use

#### Materials

##### Materials supplied in Kits

- 1x Biospecimen Bag, Small (pre-labeled)
- 1x Biospecimen Bag, Small
- 1x DS-Biorepository Tasso Kit Biospecimen Form
- 1x Dry Ice Label
- 2x Gauze
- 1x Kit Contents Manifest
- 2x Matrix Tubes with Colorless Caps, 0.5 mL, for plasma (pre-labeled)
- 1x Microcentrifuge Tube for DNA Samples (pre-labeled)
- 1x Microcentrifuge Tube with RNAlater™ for RNA Samples (pre-labeled)
- 1x Omnigene Tongue Swab
- 2x Tasso+® EDTA Kit, RUO
- 1x Therapak™ Aquipak™ Absorbent Sleeve
- 1x UN3373 Shipping Label

##### Equipment (Not Provided)

- Class II A2 Biological Safety Cabinet
- -80°C Freezer
- Honeywell Xenon Ultra 1960g Barcode Scanner or Equivalent

- Adjustable Speed Microcentrifuge (Eppendorf 5417 or equivalent)
- P1000 Pipet
- P200 Pipet
- Timer

### *Consumables (Not Provided)*

- Microcentrifuge Tubes, 1.5 mL
- 1000 uL Barrier Pipet Tips, Sterile
- 200 uL Barrier Pipet Tips, Sterile
- Dry Ice

## Specimen Collection

### *Notes For Lab*

#### **Before Visit:**

- Check the expiration date on the tongue swab and Tasso devices to ensure they have not expired.
- Bring the kit biospecimen form to record collection details and note any issues during the sample collection.
- If a replacement tube is needed at any time, use a tube from the [replacement kit](#). Fill out the proper replacement kit form (provided in the replacement kits) and attach it to the biospecimen form.
- **Save the following materials for use in sample return shipments:**
  - 1x Biospecimen Bag, Small (pre-labeled)
  - 1x DS-Biorepository Tasso Kit Specimen Form
  - 1x Dry Ice Label
  - 1x Therapak™ Aquipak™ Absorbent Sleeve
  - 1x UN3373 Shipping Label
  - 1x Ziploc Bag, Large (bag containing all kit contents)

### *Notes For Clinical Coordinators*

#### **During Visit:**

- Meet with participant, obtain updated consent/assent if necessary.

### *Microbiome Tongue Swab Collection*

#### **Warning and Precautions**

- If the participant presents with oral lesions or bleeding, they should consult their healthcare professional before using this collection kit.
- This kit contains a liquid to stabilize the sample.
- Wash with water if stabilizing liquid comes in contact with eyes or skin. Do NOT ingest.
- Do NOT lay the sponge tip down or touch any other surface once removed from the packaging.
- Ensure the participant has not consumed food or drink, smoked, or chewed gum for 30 minutes before giving their sample.
- Small items may pose a choking hazard.

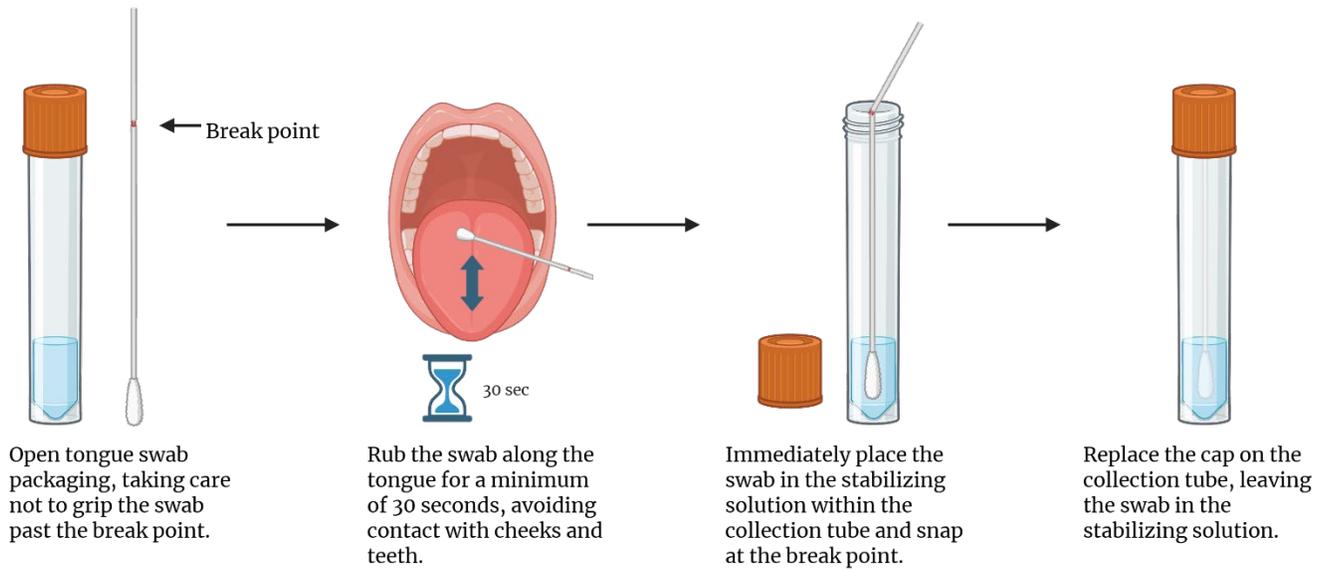


Figure 5. Tongue Swab Collection. Created with BioRender.com.

1. If the participant has eaten, drunk, smoked or chewed gum in the last 30 minutes, delay collection until 30 minutes has passed.
2. Complete the tongue swab section of the kit biospecimen form.
  - 2.1. Record time of last eating.
  - 2.2. Record time of last drink.
3. Collect microbiome samples using the tongue swab (OMR-120). For consistency in collection, the coordinator should administer the tongue swab.
  - 3.1. Wear gloves when handling the swab.
  - 3.2. Swab tongue only for 30 seconds by rolling swab front to back along the tongue. Note that only tongue is swabbed; avoid the cheeks.
  - 3.3. Place swab in collection tube and carefully break off handle. Be sure not to touch the outside of the tube or any other surface with the swab.
  - 3.4. Replace the cap on swab.
4. Record the swab collection on the provided kit biospecimen form.

## Blood

## TASSO+® EDTA Devices

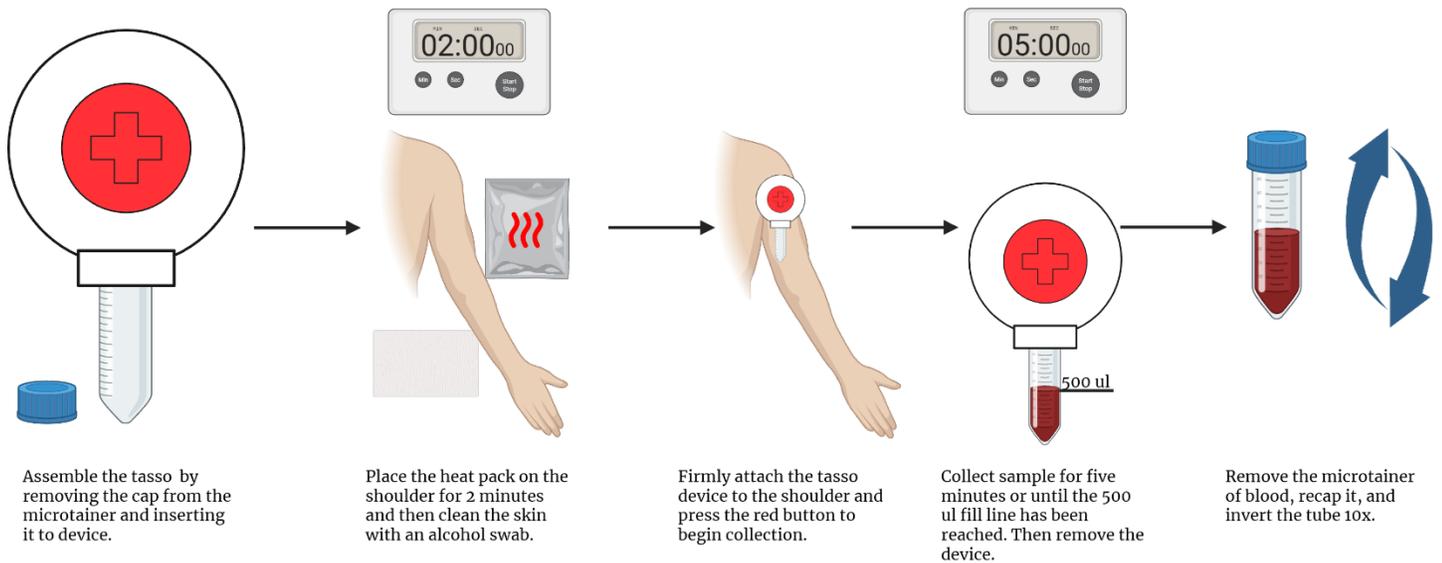


Figure 6. Tasso Collection Overview. Created with BioRender.com.

## 5. Begin whole blood collection with the Tasso+® EDTA device.

Collection video instructions can be found at: <https://www.tassoinc.com/tasso-plus-kit>

- 5.1. Remove the device from the packaging. Uncap the microtainer (purple top) tube, set the purple cap aside on a clean surface, and press the tube tightly onto the Tasso device with volume fill lines facing the same side as the red button.
- 5.2. Discard packaging in the trash bag.
- 5.3. Follow the instructions on the heat pack found in the kit to activate. Rub the heat pack on the skin of one shoulder for 2 minutes.
- 5.4. Clean the skin area with the alcohol prep pad provided in the kit.
- 5.5. Discard packaging and used prep pad in the trash bag.
- 5.6. Open the sterile gauze pad provided in the kit and set it aside on a clean surface.
- 5.7. Remove the clear plastic cover over the red button on the Tasso device and peel the paper tab from the side opposite the red button. Discard in the trash bag.
- 5.8. Stick the device firmly to the skin with the tube hanging directly below the Tasso device.
- 5.9. This is a gravity-dependent device so it must be upright to fill properly.
- 5.10. Prepare a 5-minute timer. After ensuring the participant is in a stable position, press the red button quickly and firmly until you hear a click, then release.
- 5.11. The device should be effectively stuck to the arm. If preferred, the administrator can let go of the device or hold it in place.
- 5.12. Note the time when the sample collection begins on the processing checklist.
- 5.13. Start the 5-minute timer and watch the device for filling progress.
- 5.14. Blood may not appear for several minutes. Monitor to make sure the blood does not fill the tube past the upper line, 500uL. When sufficiently filled, peel the device off the skin.
- 5.15. If the timer ends before the device is full, remove the device.

- 5.16. Press the sterile gauze pad to the collection site. Have the participant or a companion of the participant hold the gauze firmly in place.
  - 5.17. Use the Band-Aid provided in the kit as needed.
  - 5.18. Remove the tube from the device by pulling and twisting gently and place the Tasso device inside the clear processing waste bag.
  - 5.19. Snap the purple cap fully onto the tube until you hear a click, and flick the tube to get the blood moving, then invert 10 times to mix the sample with the anticoagulant.
  - 5.20. Mark on the kit biospecimen form that the Tasso EDTA was collected and note any issues.
  - 5.21. Place any packaging from the kit in the trash.
6. Repeat the above steps for the second Tasso device, placing the device on the opposite shoulder.
  7. Place oral and blood specimens in a clear transport bag and transfer to the processing lab.
    - 7.1. The lab team should be available for immediate processing of samples.
  8. Record the time of sample collection on the collection form.

## Sample processing

### *Equipment Preparation*

1. Start the biological safety cabinet (BSC) and allow it to run for 10 minutes before use.
2. Place the unlabeled small biohazard bag in the BSC to use for collecting waste.

### *Oral Specimen Processing*

3. Place the tongue swab upright at -80°C for a minimum of 2 hours, until it is frozen.
4. Once frozen, place in an absorbent sleeve, transfer to the large Ziploc bag the kit came in, and store at -80°C until ready for shipment.

### *Plasma Isolation*

5. Transfer the Tasso collection tubes to an adjustable speed microcentrifuge.
6. Centrifuge samples for 15:00 minutes at 700xg.
7. Carefully remove the tubes from the centrifuge, move them to the biosafety cabinet, and remove the purple cap.
  - 7.1. Blood should be separated into 3 distinct layers: plasma on top (yellow), buffy coat in the middle (white), and RBCs bottom (red).
  - 7.2. Be careful not to shake or invert the tube at all, as this can mix the layers.
8. Remove the 2.0 mL tubes from the white cardboard box and bring them into the biosafety cabinet along with the matrix tubes for plasma.

9. Using a P200 pipet and 200  $\mu$ L tips, aspirate plasma from the top surface of the plasma into a fresh 1.5 mL microcentrifuge tube (not provided), combining the plasma from both Tasso devices.
  - 9.1. Aspirate as much plasma as possible but leave the plasma closest to the buffy coat/RBC interface behind. It is better to isolate less plasma than to aspirate the other blood fractions into the plasma sample.
  - 9.2. To reduce the risk of contaminating plasma with the underlying blood layers, the p200 pipet volume may be set as low as 20  $\mu$ L and the plasma may be transferred incrementally.
  - 9.3. If any of the lower layers are pulled into the pipet tip, do not add them to the plasma tube.
    - 9.3.1. If the lower layers are disrupted while pipetting off the plasma, the tube can be re-centrifuged (700xg for 10 minutes) to separate the layers again. This extra spin can be done 2 additional times (3 times total) to ensure complete separation.
  - 9.4. Any used pipet tips can be discarded in a biohazard waste bag.
10. Using a P1000 pipet, gently mix the plasma and aliquot it into the matrix tubes with 250  $\mu$ L per tube.
11. Set the matrix tubes upright on the dry ice for at least 30 minutes until the plasma samples are frozen.
  - 11.1. Mark on the kit biospecimen form that plasma was collected and note any QC issues.
  - 11.2. Note the approximate volume of plasma collected.
12. Once frozen, transfer the plasma matrix tubes to the labeled small biospecimen bag and store at  $-80^{\circ}\text{C}$ .

#### *RNA stabilization*

13. Using a P1000 pipet and 1000  $\mu$ L pipet tips, transfer any remaining plasma, buffy coat, and red blood cells from ONE Tasso collection tube into the 2.0 mL microcentrifuge tube containing 1.3 mL of RNeasy<sup>TM</sup> (provided).
14. Cap the 2.0 mL tube and invert it 10 times to thoroughly mix the blood and RNeasy<sup>TM</sup> solution.
15. Mark that the RNA sample was collected on the kit biospecimen form and note any QC issues.

#### *Preparation of samples for DNA isolation*

16. Using a P1000 pipet and 1000  $\mu$ L pipet tips, transfer any remaining plasma, buffy coat, and red blood cells from the remaining Tasso collection tube into the provided empty 2.0 mL microcentrifuge tube.
17. Discard any remaining waste in the biohazard waste bag.
18. Transfer the RNA and DNA tubes to  $-80^{\circ}\text{C}$  and freeze upright, for at least 2 hours. Once frozen, transfer RNA and DNA tubes to the small biospecimen bag with the corresponding plasma sample.
19. Place the small biospecimen bag into the large Ziploc bag containing the tongue swab and store at  $-80^{\circ}\text{C}$  until ready for shipment.
  - 19.1. This kit bag now contains all biospecimens to be returned from this individual kit.

20. Record collection information from the kit biospecimen form into the online DS-4C REDCap form. Retain a completed copy of the kit biospecimen form to return with specimens as well.

## Child Kit Instructions for Use

### Materials

#### *Materials Supplied in Kits*

- 1x Biospecimen Bag, Small (pre-labeled)
- 1x Biospecimen Bag, Medium (pre-labeled)
- 1x Biospecimen Bag, Medium
- 1x Conical Tubes, 15 mL (pre-labeled)
- 3x Cryogenic Vials, 1.2 mL (pre-labeled)
- 1x DS-Biorepository Child Kit Biospecimen Form
- 1x Dry Ice Shipping Label
- 1x Kit Contents Manifest
- 3x K<sub>2</sub> EDTA Vacutainers, 4 mL
- 1x Liquid Medical Waste Fluid Solidifier
- 18x Matrix Tubes with Colorless Caps, 0.5 mL (pre-labeled)
- 6x Matrix Tubes with Red Caps, 0.5 mL (pre-labeled)
- 2x Microcentrifuge Tubes, 2.0 mL (pre-labeled)
- 1x Omnigene Tongue Swab (pre-labeled)
- 1x PAXgene<sup>®</sup> Blood RNA Vacutainer, 2.5 mL (pre-labeled)
- 1x PAXgene<sup>®</sup> Blood DNA Vacutainer, 2.5 mL (pre-labeled)
- 1x Therapak<sup>™</sup> Aquipak<sup>™</sup> Absorbent Sleeve
- 1x UN3373 Shipping Label

#### *Supplemental Materials (Supplement Kit)*

- 10x Phosphate Buffered Saline (PBS)
- BD Phosflow<sup>™</sup> Lyse/Fix Buffer
- DMSO
- Fetal Bovine Serum (FBS)
- Maxpar<sup>®</sup> buffer
- RBC Lysis Buffer

#### *Equipment (Not Provided)*

- Bead Bath or Water Bath Set to 37°C
- Class II A2 Biological Safety Cabinet
- Corning CoolCell Controlled-Rate Freezing Container or Equivalent
- -80°C Freezer
- Honeywell Xenon Ultra 1960g Barcode Sanner or Equivalent
- Liquid Nitrogen Dewar
- Liquid Nitrogen Safe Freezer Boxes
- Nutating Rotator
- P1000 Pipet
- Refrigerated Adjustable Speed Microcentrifuge (Eppendorf 5417r or Equivalent)

- Refrigerated Swinging Bucket Centrifuge with 15- mL Tube Adaptors (Allegra X-30r or Equivalent)
- Swinging Bucket Centrifuge with 15- and 50-mL Tube Adaptors (Allegra X-30 or Equivalent)
- Speed-Controlled Serological Pipet
- Timer
- Tube Racks

### *Consumables (Not Provided)*

- 1000 uL Barrier Pipet Tips, Sterile
- Conical Tubes, 50 mL
- Conical Tubes, 15 mL
- Dry Ice
- Liquid Nitrogen
- Microcentrifuge Tubes, 1.5 mL
- Serological Pipets, 10 mL
- Transfer Pipets, 3 mL (or 1000 uL Wide-Bore Barrier Tips, Sterile)

## Specimen Collection

### *Notes For Lab*

#### **Before Visit:**

- Check the expiration date on the tongue swab and vacutainers to ensure they have not expired.
- Bring the kit biospecimen form to record collection details and note any issues during the sample collection.
- If a replacement tube is needed at any time, use a tube from the [replacement kit](#). Fill out the proper replacement kit form (provided in the replacement kits) and attach it to the biospecimen form.
- **The following materials for use in sample return shipments:**
  - 1x Biospecimen Bag, Small (pre-labeled)
  - 1x Biospecimen Bag, Medium (pre-labeled)
  - 1x DS-Biorepository Child Kit Biospecimen Form
  - 1x Dry Ice Shipping Label
  - 1x Therapak™ Aquipak™ Absorbent Sleeve
  - 1x UN3373 Shipping Label
  - Ziploc bag, large (bag containing all kit contents)

### *Notes For Clinical Coordinators*

#### **During Visit:**

- Meet with participant, obtain updated consent/assent if necessary.

### *Microbiome Tongue Swab Collection*

#### **Warning and Precautions**

- If the participant presents with oral lesions or bleeding, they should consult their healthcare professional before using this collection kit.
- This kit contains a liquid to stabilize the sample.
- Wash with water if stabilizing liquid comes in contact with eyes or skin. Do NOT ingest.

- Do NOT lay the sponge tip down or touch any other surface once removed from the packaging.
  - Ensure the participant has not consumed food or drink, smoked, or chewed gum for 30 minutes before giving their sample.
  - Small items may pose a choking hazard.
1. If the participant has eaten, drunk, smoked, or chewed gum in the last 30 minutes, delay collection until 30 minutes has passed.
  2. Complete the tongue swab section of the kit biospecimen form.
    - 2.1. Record time of last eating.
    - 2.2. Record time of last drink.
  3. Collect microbiome samples using the tongue swab (OMR-120). For consistency in collection, the coordinator should administer the tongue swab.
    - 3.1. Wear gloves when handling the swab.
    - 3.2. Swab tongue only for 30 seconds by rolling swab front to back along the tongue. Note that only tongue is swabbed; avoid the cheeks.
    - 3.3. Place swab in collection tube and carefully break off handle. Be sure not to touch the outside of the tube or any other surface with the swab.
    - 3.4. Replace the cap on swab.
  4. Record the swab collection on the provided kit biospecimen form.

### *Blood Specimen Collection*

5. Let the phlebotomist know of any special instructions for the participant. i.e. nervous about BD, hard stick.
  - 5.1. If the participant is particularly small/slender, check with the phlebotomist that no more than 2.2 mLs/kg are drawn. The participant's weight collected during the medical exam may be used to determine the safe amount of blood to be drawn.
  - 5.2. Remove the necessary EDTA tubes according to weight.
6. Instruct the phlebotomist to perform venipuncture in the following order. Once filled, the vacutainer should be gently inverted 8-10 times to ensure proper mixing of stabilizer and the blood.
  - 6.1. **First**, EDTA vacutainers (purple tops)
  - 6.2. **Second**, PAXgene<sup>®</sup> RNA vacutainer (orange top)
  - 6.3. **Third** PAXgene<sup>®</sup> DNA vacutainer (blue top)

If a sample is missed due to difficulties with drawing blood, ask the participant and their study partner, if present, if they would be willing to return at a later date for collection of the missing sample.

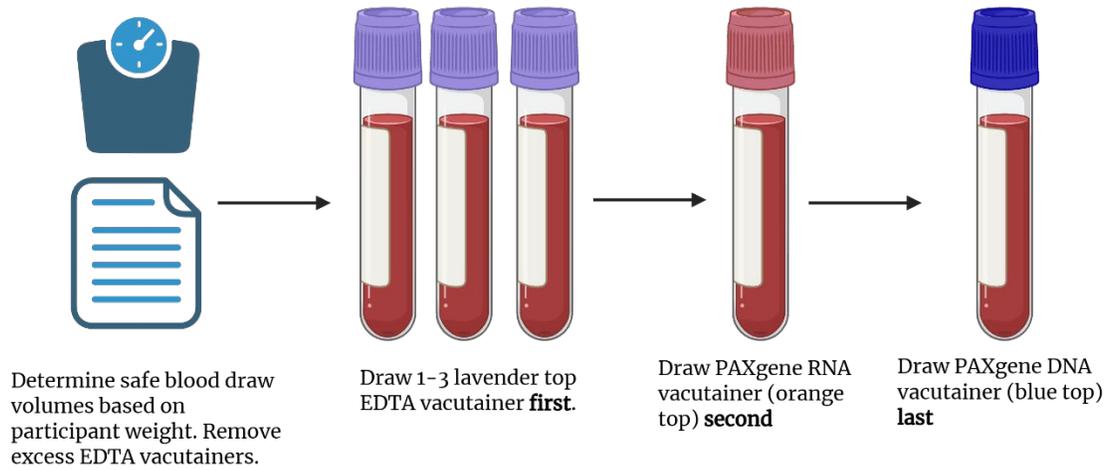


Figure 7. Proper Vacutainer Draw Order. Created with BioRender.com.

7. While the blood draw is being completed, fill out specimen collection form with times of blood draw, which tubes have been collected, any draw order or other blood draw issues (e.g. pulled with blood with syringe).
8. If additional sticks are necessary to complete the draw, the participant will be asked if this is ok. (No more than 3 attempts.)
9. Transfer oral and blood specimens to the processing lab.
  - 9.1. The lab team should be available for immediate processing of samples.

## Sample processing

### Equipment Preparation

1. Set one swinging bucket centrifuge to 4°C and the other to room temp.
2. Start the biological safety cabinet (BSC) and allow it to run for 10 minutes before use.
3. Place the unlabeled medium biospecimen bag in the BSC for waste collection. Pour a small amount of fluid solidifier into the bottom of the bag.
  - 3.1. During sample collection, if there is not enough solidifier to soak up the waste, add more to the biohazard bag.
4. Ensure the Corning CoolCell freezing container core (black ring) is at room temperature and set into the bottom of center cavity.

### Reagent Preparation

5. Prepare all buffers in advance in the BSC.
  - 5.1. 1x PBS

5.1.1. 5 mL of 10x PBS with 45 mL of Sterile ultrapure water.

5.2. Prepare 1 mL of freezing media (90% FBS, 10% DMSO) for every 4 mL vacutainer filled.

5.2.1. Ex: 0.9 mL of FBS with 0.1 mL of DMSO.

5.2.2. Keep at 4°C until used.

6. Bring 1X PBS to the proper temperature.

6.1. 20 mL of PBS at 4°C for CyTOF®-ready samples.

6.2. 30 mL of PBS at room temp for buffy coat processing.

7. Prepare two 15 mL conical tubes of 1x lyse/fix buffer (LFB) per donor for duplicate CyTOF® samples.

7.1. Each 0.5 mL volume of whole blood processed for CyTOF® requires 10 mL 1X lyse/fix buffer.

7.2. Mix 2mL of 5x LFB with 8mL of sterile ultrapure water in each 15mL conical.

7.3. Warm the 1X LFB by placing it in a 37°C bead bath for 15-30 minutes before processing.

#### *DNA, RNA, and Oral Specimen Processing*

8. Place the tongue swab upright at -80°C for a minimum of 2 hours, until it is frozen.

9. Once frozen, place the tongue swab in an absorbent sleeve, transfer to the pre-labeled medium biospecimen bag and store at -80°C until ready for shipment.

9.1. Place the medium biospecimen bag into the large Ziploc bag the kit arrived in. This will help keep the samples consolidated for return shipments.

10. Allow PAXgene® RNA and PAXgene® DNA vacutainers to sit upright at room temperature for 2 hours, transfer to -20°C overnight, then transfer to -80°C on the next business day.

10.1. When transferring tubes to -80°C place them in the absorbent sleeves along with the tongue swab samples and store them in the medium biospecimen bag until ready for shipment.

10.2. Place the medium biospecimen bag back into the large Ziploc bag the kit arrived in. This will help keep the samples consolidated for return shipments.

11. Record the collection vacutainers on the kit biospecimen form, including participant and visit information, date, and any collection notes.

12. Pull the proper number of pre-labeled tubes for biospecimen processing:

Tubes should never be mixed or used with those from another kit, even if they are of the same type. Discard any tubes not used in this collection.

- 18x 0.5 ml Matrix Tubes with Colorless Caps for Plasma
- 1x 15 ml Conical Tube for Extra Plasma
- 6x 0.5 ml Matrix Tubes with Red Caps for RBCS
- 3x 1.2 ml Cryovials for Buffy Coat
- 2x 2.0 mL Microcentrifuge Tubes for CyTOF®-Ready Samples

*Plasma, Buffy Coat, and RBC isolation*

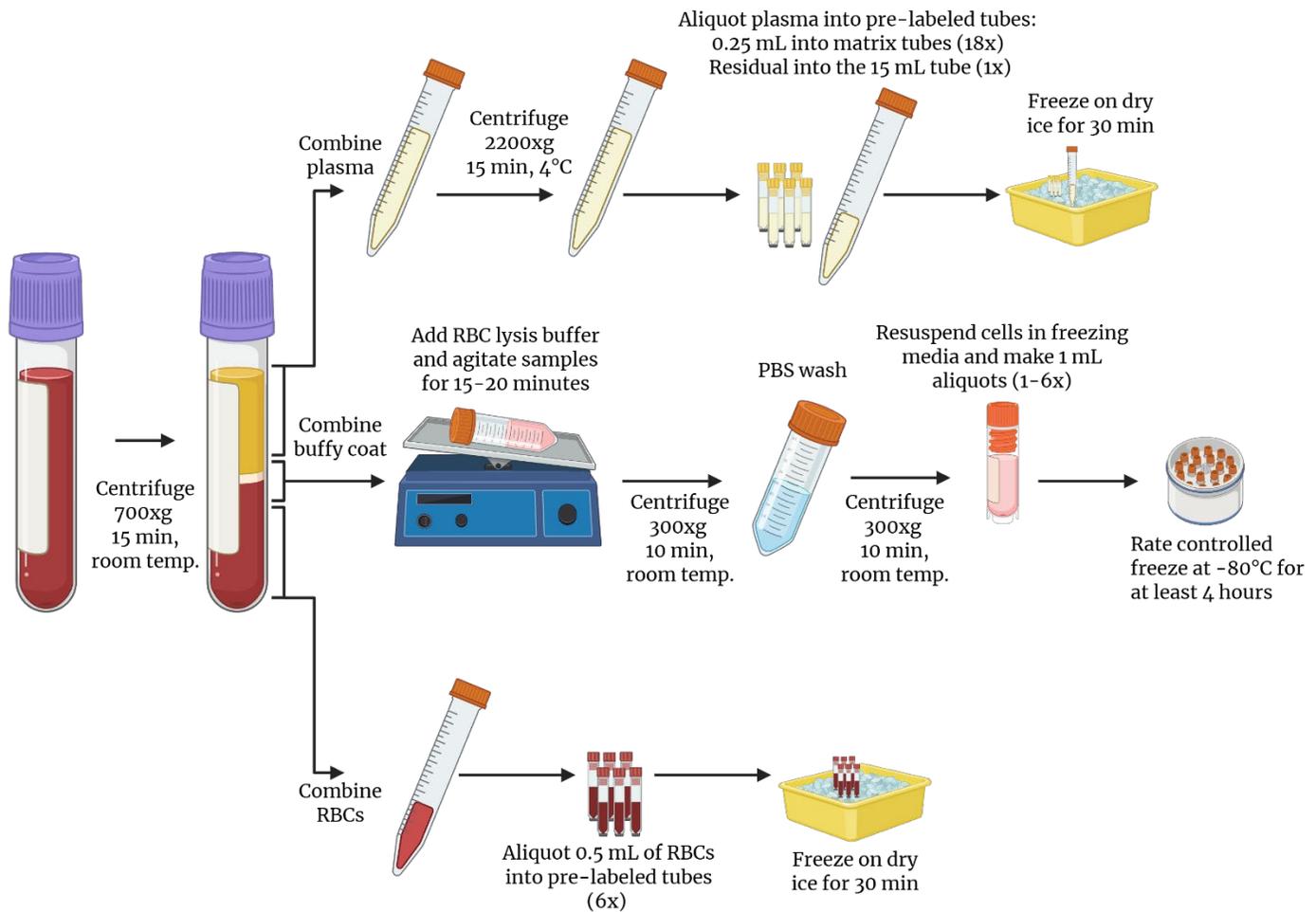


Figure 8. Blood Processing Overview. Created with BioRender.com.

13. Use EDTA vacutainers (lavender top) for isolating plasma, buffy coat, and RBCs.
14. Measure the starting volume of blood from all EDTA vacutainers collected and record on the biospecimen form.
15. Thoroughly mix the EDTA Vacutainers (8-10 gentle inversions). If CyTOF<sup>®</sup>-Ready Samples will be collected, then aliquot 1.1 mL of whole blood from one of the EDTA vacutainers into a 1.5 mL microcentrifuge tube (not provided) for the preparation of duplicate CyTOF<sup>®</sup>-ready samples, found in the [CyTOF<sup>®</sup>-Ready Sample Preparation](#) section.
16. Centrifuge all EDTA Vacutainers at 700xg for 15 minutes at RT in a swing bucket centrifuge.
17. At this point, blood should be clearly separated into three distinct layers. Plasma on top (yellow), buffy coat in the middle (white), and RBCs on the bottom (red).
18. Carefully remove vacutainers from the centrifuge, without disturbing layers.

19. Pull off as much plasma as possible from all tubes without disrupting the lower layers and **combine into a 15mL conical tube.**
  - 19.1. Leave 0.5-1mL of plasma above buffy coat to avoid disturbing the lower layers.
  - 19.2. Plasma may be collected and transferred using a variety of options including serological pipets (10 mL, 5 mL, 2 mL) or sterile transfer pipets.
20. Perform an additional spin of plasma in the swinging bucket centrifuge. Centrifuge at 2200xg, 4°C for 15 minutes to pellet the remaining cell debris.
21. Using a 3 mL transfer pipet, or p1000 wide-bore pipette tips, aspirate the remaining 0.5-1 mL plasma above buffy coat and an additional 1-2 mL of the buffy coat and RBC layers. **Combine buffy coat layers from all vacutainers into a 50 mL conical tube.**
22. Set the buffy coat conical tube aside for use in step 25 for processing the buffy coat.
23. From the EDTA Vacutainers, pool the remaining RBCs together into one vacutainer, mix, then aliquot into labeled matrix tubes.
  - 23.1. Aliquot 0.5 mL RBCs per tube into 6 matrix tubes.
  - 23.2. If there is a small volume of RBCs, decrease the number of aliquots.
  - 23.3. Cap the RBC tubes with red matrix caps and place upright on dry ice for at least 30 minutes until the samples are frozen.
  - 23.4. Once frozen, transfer matrix tubes back to the small biospecimen bag provided with the kit and keep on dry ice.
24. Once the additional 15-minute plasma spin is finished, remove multiple aliquots from the top of air/liquid interface into 18 labeled matrix tubes.
  - 24.1. Aliquot 0.25 mL of plasma per tube.
  - 24.2. Cap the plasma tubes with colorless matrix caps.
  - 24.3. Transfer any remaining plasma to the pre-labeled 15 mL conical.
  - 24.4. Place the plasma tubes upright, on dry ice for at least 30 minutes until the samples are frozen.
  - 24.5. Once frozen, transfer matrix tubes back to the small biospecimen bag provided with the kit and keep on dry ice with the 15 mL conical of plasma.
  - 24.6. Transfer the 15 mL conical of excess plasma to the absorbent sleeve, place it in the medium biospecimen bag and store at -80°C.
    - 24.6.1. Place the medium biospecimen bag back into the large Ziploc bag the kit arrived in.
  - 24.7. If CyTOF<sup>®</sup>-ready samples are **NOT** being collected, transfer the small biospecimen bag with the RBCs and plasma matrix tubes to large Ziploc bag the kit arrived in.
25. Add 2 mL of RBC lysis buffer per 4 mL vacutainer to the buffy coat tube, cap, mix by inversion, and place on a nutating rotator until red cells lyse (the contents of the tube will be a clear dark red color) (about 15-20 mins).

- 25.1. Ex: If two 4 mL vacutainers were collected, add 4 mL of lysis buffer to the buffy coat tube.
26. Centrifuge the buffy coat cells for 10 minutes at room temperature at 300xg.
27. Discard the supernatant slowly by decanting, being careful not to dislodge the pellet.
28. Re-cap the conical tube and dislodge the pellet by gently running the tube along BSC grate.
29. Add 10mL 1X room temp PBS to the buffy coat conical to wash the cells.
  - 29.1. Cap the tube and mix gently by inverting 3-5 times.
30. Centrifuge the buffy coat cells for 10 minutes at room temperature at 300xg.
31. Discard the supernatant slowly by decanting, being careful not to dislodge the pellet.
32. Re-cap the conical tube and dislodge the pellet by gently running the tube along BSC grate.
33. Resuspend the cells gently in 1 mL freezing media for cryopreservation per 4mL vacutainer collected.
  - 33.1. Ex: If two 4 mL vacutainers of blood were collected, resuspend the pooled buffy coat in a total of 2 mL of freezing media.
34. Transfer 1mL of freezing media-cell suspension to each labeled 1.2mL cryogenic vial.
35. Place buffy coat aliquots in a controlled-rate freezing container at -80°C for a minimum of 4 hours and no longer than two weeks.
  - 35.1. Once frozen, aliquots can be transferred to a box in a -80°C freezer for temporary storage (<2 weeks total at -80°C) or kept in the vapor phase of LN2 for long-term storage.
36. Inventory the buffy coat, plasma, and RBC samples in the kit biospecimen form, including visit and participant information, number of aliquots, and any processing or QC notes.
37. If not collecting CyTOF®-Ready Samples, record the kit IDs, participant IDs, and notes from the collection form into the online DS-4C REDCap form. Retain a copy of each collection form for return with their respective biospecimens.

#### *CyTOF®-Ready Sample Preparation*

38. Transfer 0.5 mL of the whole blood from the 1.5 mL tube aliquot into each of the two 15 mL conical tubes of 37°C 1X LFB.
39. Start a 15-minute timer. Invert the conical tubes 5-10 times, making sure the blood is distributed in the LFB and not gathered in the bottom tip of the conical. If this step is skipped, there will be uneven and incomplete lysis.
40. Immediately incubate for the remaining 15 minutes at 37°C in the bead bath or water bath. This step is time sensitive.

41. After the 15-minute incubation is complete, centrifuge at 400xg for 5 minutes at room temperature.
42. Decant supernatant, re-cap the tubes, and run them gently along the BSC grate 5-10 times.
43. Resuspend each cell pellet in 10mL of 1X cold PBS.
44. Centrifuge at 400xg for 5 minutes at 4°C.
45. Decant supernatant, re-cap the tubes, and run them gently along the BSC grate 5-10 times.
46. Thoroughly resuspend each cell pellet in 0.5 mL of Maxpar<sup>®</sup> buffer. Transfer the cell suspension from each conical into a new 2.0 mL microcentrifuge tube labeled with the kit barcodes.
47. Centrifuge at 500xg for 5 minutes at 4°C.
48. Aspirate most of the supernatant (leaving 50 – 100 uL), place the tubes in the small biospecimen bag containing the RBC and plasma matrix tubes. Store the Place store the small biospecimen bag in the large Ziploc bag with the other samples at -80°C until samples are ready for shipment.
49. Inventory the CyTOF<sup>®</sup>-ready samples on the kit biospecimen form, including visit and participant information, number of replicates, and any processing notes.
50. After processing the samples, record the kit IDs, participant IDs, and notes from the collection form into the online DS-4C REDCap form. Retain a copy of each collection form for return with their respective biospecimens.

## Sample Storage Review

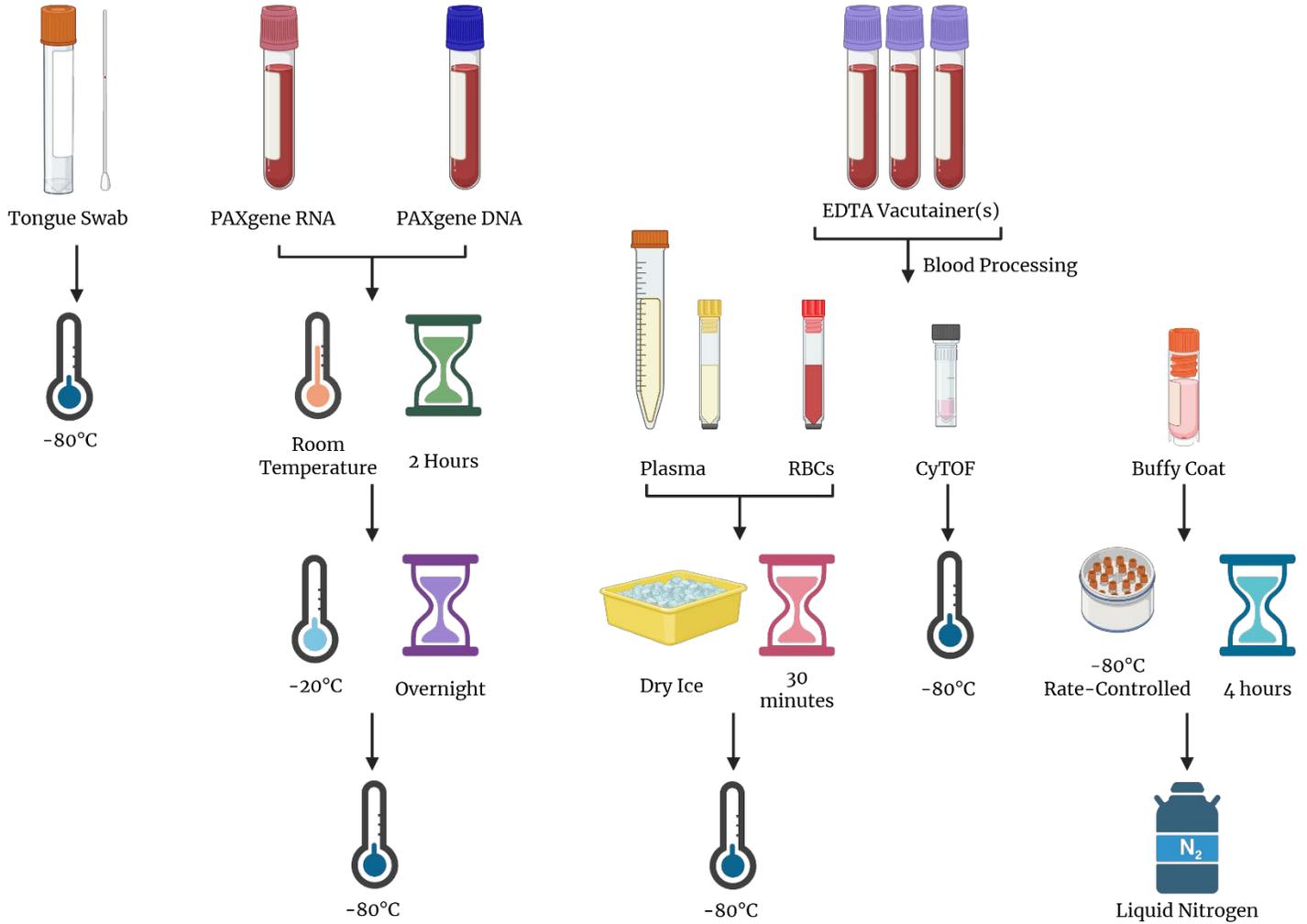


Figure 9. Sample Storage Overview. Created with BioRender.com.

1. The tongue swab, PAXgene® DNA, PAXgene® RNA, plasma, RBCs, and CyTOF®-ready samples should be stored at -80°C.
2. The tongue swab, PAXgene® DNA, PAXgene® RNA, and 15 mL conical of plasma should be placed into the slots of the absorbent sleeve provided with the kit and placed in the medium biospecimen bag.
3. The plasma and RBC matrix aliquots and CyTOF®-ready samples should be placed in the small biospecimen bag.
4. Both biospecimen bags should be placed within the large Ziploc bag and store at -80°C until ready for shipment.
  - 4.1. Ensure that biospecimens from different kits are not mixed in the same bag. Each large kit bag should only contain biospecimens from that kit.
5. Cryopreserved buffy coat cells must remain in vapor phase of liquid nitrogen until ready for shipment.

5.1. Immediately before shipment, the cryogenic vials of buffy coat cells should be transferred to the small biospecimen bag, which is then placed in the large Ziploc bag.

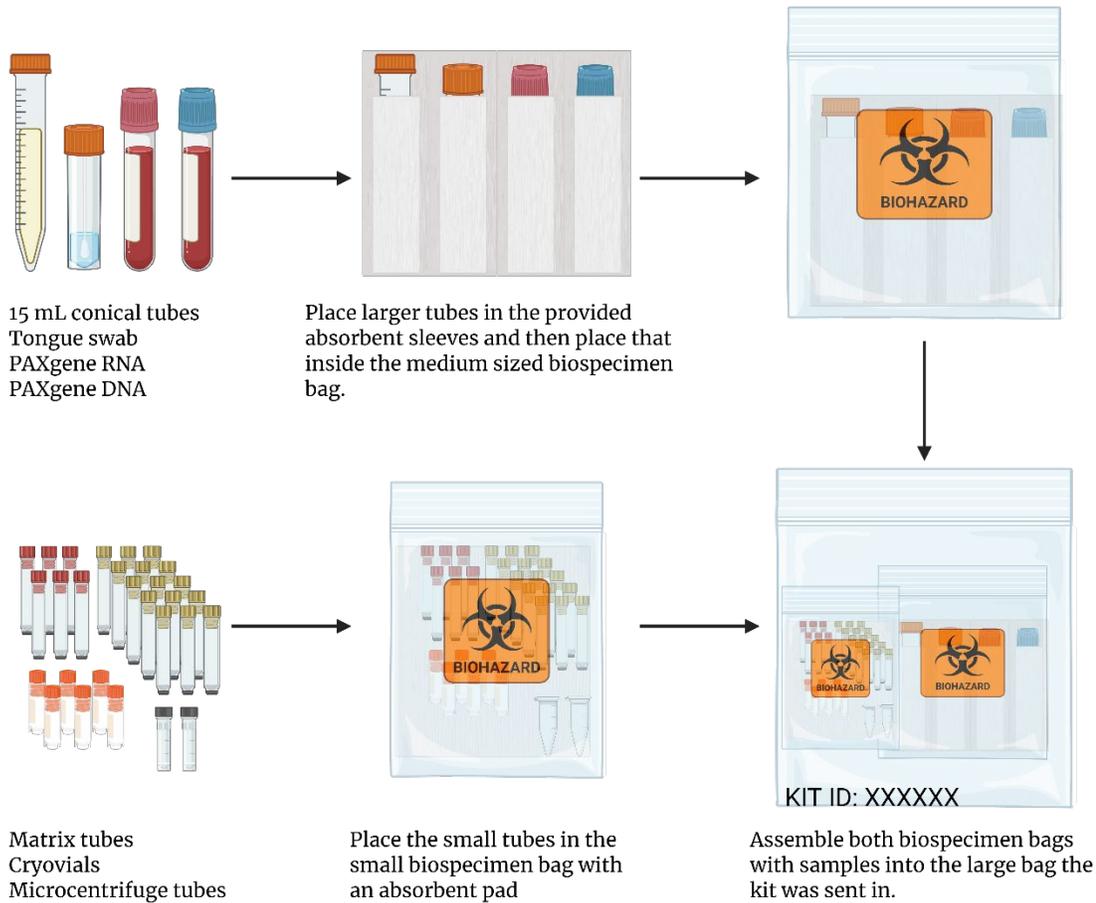


Figure 10. Shipping Assembly Overview. Created with BioRender.com.

## Adult Kit Instructions for Use

### Materials

#### Materials Supplied in Kits

- 1x Biospecimen Bag, small (pre-labeled)
- 1x Biospecimen Bag, medium (pre-labeled)
- 1x Biospecimen Bag, medium
- 1x Conical Tubes, 15 mL (pre-labeled)
- 6x Cryogenic Vials, 1.2 mL (pre-labeled)
- 1x DS-Biorepository Adult Kit Biospecimen Form
- 1x Dry Ice Shipping Label
- 1x Kit Contents Manifest
- 3x K<sub>2</sub> EDTA Vacutainers, 10 mL
- 1x Liquid Medical Waste Fluid Solidifier
- 18x Matrix Tubes with Colorless Caps, 0.5 mL (pre-labeled)
- 6x Matrix Tubes with Red Caps, 0.5 mL (pre-labeled)

- 2x Microcentrifuge Tubes, 2.0 mL (pre-labeled)
- 1x Omnigene Tongue Swab (pre-labeled)
- 1x PAXgene® Blood RNA Vacutainer, 2.5 mL (pre-labeled)
- 1x PAXgene® Blood DNA Vacutainer, 8.5 mL (pre-labeled)
- 1x Therapak™ Aquipak™ Absorbent Sleeve
- 1x UN3373 Shipping Label

### *Supplemental Materials (Supplement Kit)*

- 10x Phosphate Buffered Saline (PBS)
- BD Phosflow™ Lyse/Fix Buffer
- DMSO
- Fetal Bovine Serum (FBS)
- Maxpar® buffer
- RBC Lysis Buffer

### *Equipment (Not Provided)*

- Bead Bath or Water Bath Set to 37°C
- Class II A2 Biological Safety Cabinet
- Corning CoolCell Controlled-Rate Freezing Container or Equivalent
- -80°C Freezer
- Honeywell Xenon Ultra 1960g Barcode Sanner or Equivalent
- Liquid Nitrogen Dewar
- Liquid Nitrogen Safe Freezer Boxes
- Nutating Rotator
- P1000 Pipet
- Refrigerated Adjustable Speed Microcentrifuge (Eppendorf 5417r or Equivalent)
- Refrigerated Swinging Bucket Centrifuge with 15- mL Tube Adaptors (Allegra X-30r or Equivalent)
- Swinging Bucket Centrifuge with 15- and 50-mL Tube Adaptors (Allegra X-30 or Equivalent)
- Speed-Controlled Serological Pipet
- Timer
- Tube Racks

### *Consumables (Not Provided)*

- 1000 uL Barrier Pipet Tips, Sterile
- Conical Tubes, 50 mL
- Conical Tubes, 15 mL
- Dry Ice
- Liquid Nitrogen
- Microcentrifuge Tubes, 1.5 mL
- Serological Pipets, 10 mL
- Transfer Pipets, 3 mL (or 1000 uL Wide-Bore Barrier Tips, Sterile)

## Specimen Collection

### *Notes For Lab*

#### **Before Visit:**

- Check the expiration date on the tongue swab and vacutainers to ensure they have not expired.
- Bring the kit biospecimen form to record collection details and note any issues during the sample collection.
- If a replacement tube is needed at any time, use a tube from the [replacement kit](#). Fill out the proper replacement kit form (provided in the replacement kit) and attach it to the biospecimen form.
- **The following materials for use in sample return shipments:**
  - 1x Biospecimen Bag, small (pre-labeled)
  - 1x Biospecimen Bag, medium (pre-labeled)
  - 1x DS-Biorepository Adult Kit Biospecimen Form
  - 1x Dry Ice Shipping Label
  - 1x Therapak™ Aquipak™ Absorbent Sleeve
  - 1x UN3373 Shipping Label
  - Ziploc bag, large (bag containing all kit contents)

### *Notes For Clinical Coordinators*

#### **During Visit:**

- Meet with participant, obtain updated consent/assent if necessary.

### *Microbiome Tongue Swab Collection*

#### Warning and Precautions

- If the participant presents with oral lesions or bleeding, they should consult their healthcare professional before using this collection kit.
- This kit contains a liquid to stabilize the sample.
- Wash with water if stabilizing liquid comes in contact with eyes or skin. Do NOT ingest.
- Do NOT lay the sponge tip down or touch any other surface once removed from the packaging.
- Ensure the participant has not consumed food or drink, smoked, or chewed gum for 30 minutes before giving their sample.
- Small items may pose a choking hazard.

### *Microbiome Tongue Swab Collection*

1. If the participant has eaten, drunk, smoked or chewed gum in the last 30 minutes, delay collection until 30 minutes has passed.
2. Complete the tongue swab section of the kit biospecimen form.
  - 2.1. Record time of last eating.
  - 2.2. Record time of last drink.
3. Collect microbiome samples using the tongue swab (OMR-120). For consistency in collection, the coordinator should administer the tongue swab.
  - 3.1. Wear gloves when handling the swab.
  - 3.2. Swab tongue only for 30 seconds by rolling swab front to back along the tongue. Note that only tongue is swabbed; avoid the cheeks.

- 3.3. Place swab in collection tube and carefully break off handle. Be sure not to touch the outside of the tube or any other surface with the swab.
- 3.4. Replace the cap on swab.

4. Record the swab collection on the provided kit biospecimen form.

### *Blood Specimen Collection*

5. Let the phlebotomist know of any special instructions for the participant. i.e. nervous about BD, hard stick.
  - 5.1. If the participant is particularly small/slender, check with the phlebotomist that no more than 2.2 mLs/kg are pulled. The participant's weight collected during the medical exam may be used to determine the safe amount of blood to be drawn.
  - 5.2. Remove the necessary EDTA tubes according to weight.
6. Instruct the phlebotomist to perform venipuncture in the following order. Once filled, the vacutainer should be gently inverted 8-10 times to ensure proper mixing of stabilizer and blood.
  - 6.1. **First**, EDTA vacutainers (purple tops)
  - 6.2. **Second**, PAXgene<sup>®</sup> RNA vacutainer (orange top)
  - 6.3. **Third** PAXgene<sup>®</sup> DNA vacutainer (blue top)

If a sample is missed due to difficulties with drawing blood, ask the participant and their study partner, if present, if they would be willing to return at a later date for collection of the missing sample. If the participant is unwilling or unable to return for a redraw, offer a Tasso collection as a last resort.

7. While the blood draw is being completed, fill out specimen collection form with times of blood draw, which tubes have been collected, any draw order or other blood draw issues (e.g. pulled with blood with syringe).
8. If additional sticks are necessary to complete the draw, the participant will be asked if this is ok. (No more than 3 attempts.)
9. Transfer oral and blood specimens to the processing lab.
  - 9.1. The lab team should be available for immediate processing of samples.

## **Sample processing**

### *Equipment Preparation*

1. Set one swinging bucket centrifuge to 4°C and the other to room temp.
2. Start the biological safety cabinet (BSC) and allow it to run for 10 minutes before use.

3. Place the unlabeled medium biospecimen bag in the BSC for waste collection. Pour a small amount of fluid solidifier into the bottom of the bag.

3.1. During sample collection, if there is not enough solidifier to soak up the waste, add more to the biohazard bag.

4. Ensure the Corning CoolCell freezing container core (black ring) is at room temperature and set into the bottom of center cavity.

### *Reagent Preparation*

5. Prepare all buffers in advance in the BSC.

5.1. 1x PBS

5.1.1. 5 mL of 10x PBS with 45 mL of Sterile ultrapure water.

5.2. Prepare 2 mL of freezing media (90% FBS, 10% DMSO) for every 10 mL vacutainer filled.

5.2.1. Ex: 1.8 mL of FBS with 0.2 mL of DMSO.

5.2.2. Keep at 4°C until used.

6. Bring 1X PBS to the proper temperature.

6.1. 20 mL of PBS at 4°C for CyTOF®-ready samples

6.2. 30 mL of PBS at room temp for buffy coat processing

7. Prepare two 15 mL conicals of 1x lyse/fix buffer (LFB) per donor for duplicate CyTOF® samples.

7.1. Each 0.5 mL volume of whole blood processed for CyTOF® requires 10 mL 1X lyse/fix buffer.

7.2. Mix 2mL of 5x LFB with 8mL of sterile ultrapure water in each 15mL conical.

7.3. Warm the 1X LFB by placing it in a 37°C bead bath for 15-30 minutes before processing.

### *DNA, RNA, and Oral Specimen Processing*

8. Place the tongue swab upright at -80°C for a minimum of two hours, until it is frozen.

9. Once frozen, place the tongue swabs in an absorbent sleeve, transfer to the pre-labeled medium biospecimen bag and store at -80°C until ready for shipment.

9.1. Place the medium biospecimen bag into the large Ziploc bag the kit arrived in. This will help keep the samples consolidated for return shipments.

10. Allow PAXgene® RNA and PAXgene® DNA vacutainers to sit upright at room temperature for 2 hours, transfer to -20°C overnight, then transfer to -80°C on the next business day.

10.1. When transferring the tubes to -80°C place them in the absorbent sleeves along with the tongue swab samples and store them in the medium biospecimen bag until ready for shipment.

10.2. Place the medium biospecimen bag back into the large Ziploc bag the kit arrived in. This will help keep the samples consolidated for return shipments.

11. Record the collection vacutainers on the kit biospecimen form, including participant and visit information, date, and any collection notes.

12. Pull the proper number of pre-labeled tubes for biospecimen processing:

Tubes should never be mixed or used with those from another kit, even if they are of the same type. Discard any tubes not used in this collection.

- 18x 0.5 ml Matrix Tubes with Colorless Caps for Plasma
- 1x 15 ml Conical Tube for Extra Plasma
- 6x 0.5 ml Matrix Tubes with Red Caps for RBCS
- 6x 1.2 ml Cryovials for Buffy Coat
- 2x 2.0 mL Microcentrifuge Tubes for CyTOF®-Ready Samples

### *Plasma, Buffy Coat, and RBC isolation*

13. Use EDTA vacutainers (lavender top) for isolating plasma, buffy coat, and RBCs.

14. Measure the starting volume of blood from all EDTA vacutainers collected and record on the biospecimen form.

15. Thoroughly mix the EDTA Vacutainers (8-10 gentle inversions). If CyTOF®-Ready Samples will be collected, then aliquot 1.1 mL of whole blood from one of the EDTA vacutainers into a 1.5 mL microcentrifuge tube (not provided) for the preparation of duplicate CyTOF®-ready samples, found in the [CyTOF®-Ready Sample Preparation](#) section.

16. Centrifuge all EDTA Vacutainers at 700xg for 15 minutes at RT in a swing bucket centrifuge.

17. At this point, blood should be clearly separated into three distinct layers. Plasma on top (yellow), buffy coat in the middle (white), and RBCs on the bottom (red).

18. Carefully remove vacutainers from the centrifuge, without disturbing layers.

19. Pull off as much plasma as possible from all tubes without disrupting the lower layers and **combine into a 15mL conical tube.**

19.1. Leave 0.5-1mL of plasma above buffy coat to avoid disturbing the lower layers.

19.2. Plasma may be collected and transferred using a variety of options including serological pipets (10 mL, 5 mL, 2 mL) or sterile transfer pipets.

20. Perform an additional spin of plasma in the swinging bucket centrifuge. Centrifuge at 2200xg, 4°C for 15 minutes to pellet the remaining cell debris.

21. Using a 3 mL transfer pipet, aspirate the remaining 0.5-1 mL plasma above buffy coat and an additional 1-2 mL of the buffy coat and RBC layers. **Combine buffy coat layers from all vacutainers into a 50 mL conical tube.**

22. Set the buffy coat conical tube aside for use in step 25 for processing the buffy coat.

23. From the EDTA Vacutainers, pool the remaining RBCs together into one vacutainer, mix, then aliquot into labeled matrix tubes.
  - 23.1. Aliquot 0.5 mL RBCs per tube into 6 matrix tubes.
  - 23.2. If there is a small volume of RBCs, decrease the number of aliquots.
  - 23.3. Cap the RBC tubes with red matrix caps and place upright on dry ice for at least 30 minutes until the samples are frozen.
  - 23.4. Once frozen, transfer matrix tubes back to the small biospecimen bag provided with the kit and keep on dry ice.
24. Once the additional 15-minute plasma spin is finished, remove multiple aliquots from the top of air/liquid interface into 18 labeled matrix tubes.
  - 24.1. Aliquot 0.25 mL of plasma per tube.
  - 24.2. Cap the plasma tubes with colorless matrix caps.
  - 24.3. Transfer any remaining plasma to the pre-labeled 15 mL conical.
  - 24.4. Place the plasma tubes upright, on dry ice for at least 30 minutes until the samples are frozen.
  - 24.5. Once frozen, transfer matrix tubes back to the small biospecimen bag provided with the kit and keep on dry ice with the 15 mL conical of plasma.
  - 24.6. Transfer the 15 mL conical of excess plasma to the absorbent sleeve, place it in the medium biospecimen bag and store at -80°C.
    - 24.6.1. Place the medium biospecimen bag back into the large Ziploc bag the kit arrived in.
  - 24.7. If CyTOF®-ready samples are **NOT** being collected, transfer the small biospecimen bag with the RBCs and plasma matrix tubes to large Ziploc bag the kit arrived in.
25. Add 4 mL of RBC lysis buffer per 10 mL vacutainer to the buffy coat tube, cap, mix by inversion, and place on a nutating rotator until red cells lyse (the contents of the tube will be a clear dark red color) (about 15-20 mins).
  - 25.1. Ex: If two 10 mL vacutainers were collected, add 8 mL of lysis buffer to the buffy coat tube.
26. Centrifuge the buffy coat cells for 10 minutes at room temperature at 300xg.
27. Discard the supernatant slowly by decanting, being careful not to dislodge the pellet.
28. Re-cap the conical tube and dislodge the pellet by gently running the tube along BSC grate.
29. Add 20mL 1X room temp PBS to the buffy coat conical to wash the cells.
  - 29.1. Cap the tube and mix gently by inverting 3-5 times.
30. Centrifuge the buffy coat cells for 10 minutes at room temperature at 300xg.
31. Discard the supernatant slowly by decanting, being careful not to dislodge the pellet.

32. Re-cap the conical tube and dislodge the pellet by gently running the tube along BSC grate.
33. Resuspend the cells gently in 2 mL freezing media for cryopreservation per 10mL vacutainer collected.
  - 33.1. Ex: If two 10 mL vacutinners of blood were collected, resuspend the pooled buffy coat in a total of 4 mL of freezing media.
34. Transfer 1mL of freezing media-cell suspension to each labeled 1.2mL cryogenic vial.
35. Place buffy coat aliquots in a controlled-rate freezing container at -80°C for a minimum of 4 hours and no longer than two weeks.
  - 35.1. Once frozen, aliquots can be transferred to a box in a -80°C freezer for temporary storage (<2 weeks total at -80°C) or kept in the vapor phase of LN2 for long-term storage.
36. Inventory the buffy coat, plasma, and RBC samples in the kit biospecimen form, including visit and participant information, number of aliquots, and any processing or QC notes.
37. If not collected CyTOF<sup>®</sup>-Ready Samples, record the kit IDs, participant IDs, and notes from the collection form into the online DS-4C REDCap form. Retain a copy of each collection form for return with their respective biospecimens.

#### *CyTOF<sup>®</sup>-Ready Sample Preparation*

38. Transfer 0.5 mL of the whole blood from the 1.5 mL tube aliquot into each of the two 15 mL conicals of 37°C 1X LFB.
39. Start a 15-minute timer. Invert the conical tubes 5-10 times, making sure the blood is distributed in the LFB and not gathered in the bottom tip of the conical. If this step is skipped, there will be uneven and incomplete lysis.
40. Immediately incubate for the remaining 15 minutes at 37°C in the bead bath or water bath. This step is time sensitive.
41. After the 15-minute incubation is complete, centrifuge at 400xg for 5 minutes at room temperature.
42. Decant supernatant, re-cap the tubes, and run them gently along the BSC grate 5-10 times.
43. Resuspend each cell pellet in 10mL of 1X cold PBS.
44. Centrifuge at 400xg for 5 minutes at 4°C.
45. Decant supernatant, re-cap the tubes, and run them gently along the BSC grate 5-10 times.
46. Thoroughly resuspend each cell pellet in 0.5 mL of Maxpar<sup>®</sup> buffer. Transfer the cell suspension from each conical into a new 2.0 mL microcentrifuge tube labeled with the kit barcodes.
47. Centrifuge at 500xg for 5 minutes at 4°C.

48. Aspirate most of the supernatant (leaving 50 – 100 uL), place the tubes in the small biospecimen bag containing the RBC and plasma matrix tubes. Store the Place store the small biospecimen bag in the large Ziploc bag with the other samples at -80°C until samples are ready for shipment.
49. Inventory the CyTOF®-ready samples on the kit biospecimen form, including visit and participant information, number of replicates, and any processing notes.
50. After processing the samples, record the kit IDs, participant IDs, and notes from the collection form into the online DS-4C REDCap form. Retain a copy of each collection form for return with their respective biospecimens.

## Sample Storage Review

1. The tongue swab, PAXgene® DNA, PAXgene® RNA, plasma, RBCs, and CyTOF®-ready samples should be stored at -80°C.
2. The tongue swab, PAXgene® DNA, PAXgene® RNA, and 15 mL conical of plasma should be placed into the slots of the absorbent sleeve provided with the kit and transferred to the medium biospecimen bag.
3. The plasma and RBC matrix aliquots and CyTOF®-ready samples should be placed in the small biospecimen bag.
4. Both biospecimen bags should be placed within the large Ziploc bag and store at -80°C until ready for shipment.
  - 4.1. Ensure that biospecimens from different kits are not mixed in the same bag. Each large kit bag should only contain biospecimens from that kit.
5. Cryopreserved buffy coat cells must remain in vapor phase of liquid nitrogen until ready for shipment.
  - 5.1. Immediately before shipment, the cryogenic vials of buffy coat cells should be transferred to the small biospecimen bag, which is then placed in the large Ziploc bag.

## Saliva Kit Instructions for Use

### Materials

#### *Materials supplied in Kits*

- 1x Biospecimen Specimen Bag, medium (pre-labeled)
- 1x Dry Ice Shipping Label
- 1x DS-Biorepository Saliva Kit Specimen Form
- 1x Kit Contents Manifest
- 1x ORAgene™ Saliva Kit (pre-labeled)
- 1x Therapak™ Aquipak™ Absorbent Sleeve
- 1x UN3373 Shipping Label

#### *Equipment (Not Provided)*

- -80°C Freezer

## Specimen Collection

The saliva collection kits may be used with or without an assisting sponge. If the participant is able to spit into the collection tube without the sponge, they may do so by following the manufacturer's instructions provided with the kit. Instructions for sponge assisted saliva collection are detailed below. Regardless of the method, 2 mL of saliva must be collected. The appropriate amount of saliva is indicated by the fill line on the tube.

### *Assisted Saliva Collection*

#### **Warning and Precautions**

- If the participant presents with oral lesions or bleeding, they should consult their healthcare professional before using this collection kit.
- This kit contains a liquid to stabilize the sample.
- Wash with water if stabilizing liquid comes in contact with eyes or skin. Do NOT ingest.
- Ensure the participant has not consumed food or drink, smoked, or chewed gum for 30 minutes before giving their sample.
- Small items may pose a choking hazard.

1. Check the sponge for damage before use. Two sponges are provided if one shows any signs of damage.
2. Place one sponge in the cheek pouch and gently move it along the gums and inner cheek for 30 seconds to absorb as much saliva as possible.
3. After the sponge is saturated, insert it into the V-shaped notch of the collection tube.
4. Wringing the sponge out by twisting and pushing it against the inner wall of the notch, filling the tube with saliva.
5. Using the same sponge, repeat the process until the liquid, not the bubbles, reaches the fill line.
6. Check the sponge for damage before each repeated use. If the sponge shows signs of wear and tear, use the second sponge.
7. Tap the tube bottom against a hard surface to help remove bubbles.
8. Hold the base of the tube upright in one hand and close the lid firmly with the other hand.
9. A loud click should be heard, and the liquid in the lid will be released into the tube to mix with the saliva.
10. Unscrew the funnel from the tube.
11. Use the small cap to seal the tube tightly.
12. Shake the capped tube for five seconds.
13. Place the saliva tube upright, at -80°C, for at least 2 hours, until samples are frozen.
  - 13.1. Saliva samples may be frozen and stored at -80°C or -20°C.

14. Once frozen, place the saliva sample in an absorbent sleeve, transfer to the pre-labeled medium biospecimen bag and store at -80°C, or -20°C, until ready for shipment.

14.1. Place the medium biospecimen bag into the large Ziploc bag the kit arrived in.

15. Record the kit IDs, participant IDs, and notes from the collection form into the online DS-4C REDCap form. Retain a copy of the collection form for return with the biospecimen.

## Shipping Instructions

### Notes Before Shipping

- Before shipping, check the DS-Biorepository Central Lab holiday schedule to ensure that samples aren't shipped during a holiday.
- Ship samples **Monday** through **Wednesday** to ensure receipt by **Friday**. Avoid shipping over the following federal holidays:

#### Federal Holidays 2025-2030

- **New Year's Day** - Wed, Jan 1, 2025; Thu, Jan 1, 2026; Fri, Jan 1, 2027; Fri, Dec 31, 2028 (obs.); Mon, Jan 1, 2029; Tue, Jan 1, 2030
- **Memorial Day** - Mon, May 26, 2025; Mon, May 25, 2026; Mon, May 31, 2027; Mon, May 29, 2028; Mon, May 28, 2029; Mon, May 27, 2030
- **Independence Day** - Fri, Jul 4, 2025; Fri Jul 3, 2026 (obs.); Mon, Jul 5, 2027 (obs.); Tue, Jul 4, 2028; Wed, Jul 4, 2029; Thu, Jul 4, 2030
- **Labor Day** – Mon, Sep 1, 2025; Mon, Sep 7, 2026; Mon, Sep 6, 2027; Mon, Sep 4, 2028; Mon Sep 3, 2029; Mon, Sep 2, 2030
- **Thanksgiving Day** - Thu, Nov 27, 2025; Thu, Nov 26, 2026; Thu, Nov 25, 2027; Thu, Nov 23, 2028; Thu, Nov 22, 2029; Thu, Nov 28, 2030
- **Christmas Day** –Thu, Dec 25, 2025; Fri, Dec 25, 2026; Fri, Dec 24, 2027 (obs.); Mon, Dec 25, 2028; Tue, Dec 25, 2029; Wed, Dec 25, 2030

### Sample Return Shipping

This describes the return shipment of biospecimens from the collection sites to the central lab.

#### U.S. Sample Batch–Shipping Instructions

Biospecimens collected from the Tasso, Child, and Adult Kits are intended to be batch-shipped on dry ice once one of the following conditions is met: specimens from **4 participants** have been collected, **or 3 months** have passed since the previous shipment from a site.

These biospecimens will be returned to the INCLUDE DS-Biorepository central lab on dry ice to ensure samples remain frozen.

**Due to logistics, collection sites must provide their own dry ice and shipping containers.**

#### Materials

- Biospecimens

- Up to 4x Biospecimen Bags, Small (Provided with Each Kit)
- Up to 4x Biospecimen Kit Forms
- 1x Dry Ice, 20 lbs (**Not Provided**)
- 1x Dry Ice Label (Provided)
- Up to 4x Ziploc Bag, Large (Provided)
- Up to 4x Therapak™ Aquipak™ Absorbent Sleeves (Provided)
- 1x FedEx Return Labels (Provided with Initial Kit Delivery)
- 1x UN3373 Labels (Provided)
- 1x Insulated Shipping Box with Outer Cardboard Box, Suitable for Dry Ice (**Not Provided**)
  - Recommended: Insulated Foat Shipping Kit, ULINE, Cat. # S13392 (12 x 12 x 11.5 Inches)
- Up to 4x Replacement Kit Forms (If Needed During Collection)

*Procedure:*

1. Inform the central lab of the upcoming shipment via the kit request and submission portal or by email if the portal is inaccessible, [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu).

- 1.1. Record the Kit IDs included in the anticipated shipment and confirm the number of each biospecimen to be returned per participant. Refer to [Collection Kit Quick Reference Guides](#) in the appendix for an example of a potential sample roster from each kit.
- 1.2. Record the FedEx tracking number on the biospecimen forms in each kit and enter tracking information into the shipping portal.

Note that when kits are shipped in the U.S. pre-filled return shipping labels will be provided and should be kept until shipments are returned. However, if shipping labels are lost or damaged, contact the DS-Biorepository central lab via email to request a replacement.

2. Acquire dry ice pellets (**15-20 lbs**) and pour about 1/3 of them into the Styrofoam shipping container.

- 2.1. A box of approximately 12x12x11.5 inches is recommended for returning samples from four collection kits. Ensure the package will accommodate enough dry ice to keep the samples frozen during travel.

3. Ensure all biospecimens from one kit have been consolidated into the large Ziploc bag the kit was shipped in.

- 3.1. Keep samples on dry ice to ensure they don't thaw.
- 3.2. Ensure large loose tubes, such as saliva, tongue swab, PAXgene® DNA, PAXgene® RNA, and 15 mL conical of plasma were placed in the absorbent sleeves provided with the collection kits.
  - 3.2.1. These should be placed inside the medium biospecimen bag.
- 3.3. **Transfer Buffy coat** samples into **the small biospecimen bag** containing the CyTOF®-ready samples, RBC matrix tubes, and plasma matrix tubes.
- 3.4. Return all samples into the larger, large Ziploc bag and seal.

4. Place a maximum of four large bags of biospecimens (4 kits) in the insulated shipping box and bury them under the dry ice and cover them with the remaining dry ice, until the container is full.

4.1. Do not tape the Styrofoam box shut. As dry ice sublimates, the CO<sub>2</sub> gas will increase the internal pressure of the package and can injure those handling the package.

5. Place the Styrofoam container inside a rigid cardboard box.

6. Place copies of the completed biospecimen kit forms for each kit on top of the Styrofoam box and tape the outer cardboard box closed with packing tape.

6.1. Ensure any replacement kit forms used are attached to their corresponding biospecimen kit forms.

7. Attach the return shipping, UN3373, and dry ice labels to the box.

7.1. Ensure the dry ice sticker has been filled out with shipping details and dry ice weight.

8. Attach FedEx shipping label from step 1 to the outside of the box and drop the package off at the nearest location that accepts shipments containing dry ice.

## HTP-LAN Sample Batch–Shipping Instructions

Biospecimens collected from the Tasso, Child, and Adult kits are intended to be batch-shipped on dry ice once one of the following conditions is met: specimens from **10 participants** have been collected, **or 3 months** have passed since the previous shipment from a site.

Sites should begin coordinating their shipments at least 1-2 weeks before they intend to ship the samples. Scheduling shipments through World Courier may take several days.

These biospecimens will be returned to the INCLUDE DS-Biorepository central lab on dry ice to ensure samples remain frozen.

### Materials

- Biospecimens
- Up to 10x Biospecimen Bags, Small (Provided)
- Up to 10x Biospecimen Kit Forms (Provided)
- 1x Dry Ice for Combing Samples (**Not Provided**)
- 1x Dry Ice for Shipping (Provided by World Courier)
- 1x Dry Ice Label (Provided)
- 1x Insulated Shipping Box Suitable for Dry Ice (Provided by World Courier)
- Packing Tape
- 1x Rigid Cardboard Box (Provided by World Courier)
- Up to 10x Replacement Kit Forms (If Needed During Collection)
- 1x Shipping Labels (Provided by World Courier)
- Up to 10x Therapak™ AQUI-Pak™ Absorbent Sleeves (Provided)
- 1x UN3373 Labels (Provided)
- 1x World Courier Customs Invoice Form (Provided)
- 1x World Courier Order Form (Provided)
- 1x World Courier Quote Questionnaire (Provided)
- Up to 10x Ziploc Bag, large (Provided)

**Procedure:**

1. Email the DS-Biorepository, [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu), to notify the central lab of your intent to ship and to request the following shipment forms (also available online):
  - 1.1. World Courier Customs Invoice Form
  - 1.2. World Courier Order Form
  - 1.3. World Courier Quote Questionnaire
2. Request an updated quote from World Courier.
  - 2.1. Fill out the Quote questionnaire and return it to [usquotes@worldcourier.com](mailto:usquotes@worldcourier.com). Be sure to CC [Roxanna.martinez@worldcourier.com](mailto:Roxanna.martinez@worldcourier.com) and the DS-biorepository.
  - 2.2. Quotes are only good for 30 days and a new quote will need to be issued with each shipment.
3. Submit the shipment request to World Courier.
  - 3.1. Email the completed quote, the order form, and the customs invoice to [NewOrder@worldcourier.com](mailto:NewOrder@worldcourier.com) and CC [roxanna.martinez@worldcourier.com](mailto:roxanna.martinez@worldcourier.com) and [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu).
  - 3.2. A job number will be assigned to the shipment and World Courier will provide an “OK to Send” notification to the collection site.
  - 3.3. **This process typically takes 3-5 business days.**
4. Once given the “OK to Send” status, World Courier will work with the collection site to schedule a pickup over the next 2-3 weeks.
  - 4.1. Shipments should be scheduled for Monday or Tuesday to avoid weekend deliveries.
5. **The day before or day of shipment** combine biospecimen from a single kit together into one large and place at -80°C until World Courier is ready to package the samples for shipment.
  - 5.1. Record the Kit IDs included in the anticipated shipment and confirm the number of each biospecimen to be returned per participant.
  - 5.2. Keep samples on dry ice to ensure they don't thaw.
  - 5.3. Ensure large loose tubes, such as saliva, tongue swab, PAXgene® DNA, PAXgene® RNA, and 15 mL conical of plasma were placed in the absorbent sleeves provided with the collection kits.
    - 5.3.1. These should be placed inside the medium biospecimen bag.
    - 5.3.2. It is likely that these steps were already completed after sample collection (see Biospecimen Collection).
  - 5.4. **Transfer buffy coat** samples into **the small biospecimen bag** containing the CyTOF®-ready samples, RBC matrix tubes, and plasma matrix tubes.
    - 5.4.1. Cryopreserved buffy coat should not be stored long-term (>2 weeks) at -80°C. For this reason, unlike the other biospecimens, the buffy coat cannot be pre-assembled in the return kit immediately after biospecimen collection.

- 5.5. Return all samples into the large Ziploc bag the kit came in, seal it, and keep at -80°C until shipment.
6. On the day of shipment, assemble sample shipments in the shipping materials provided to sites.
  - 6.1. World Courier may provide a 2-hour window for shipment pickup.
  - 6.2. During this time, a courier will arrive on site with packing materials and dry ice.
    - 6.2.1. The collection site staff must pack the return samples. World Courier will not handle the samples directly but will wait outside for receipt of packed shipping boxes.
  - 6.3. Bury 10 filled kit bags, in the insulated shipping box under the dry ice provided by World Courier.
  - 6.4. Place the insulated shipping box in the external cardboard shipping box.
  - 6.5. Ensure that copies of the kit biospecimen forms and replacement kit forms, if needed, are included with the shipment. These may be placed between the insulated shipping box and the outer cardboard box.
  - 6.6. Ensure the UN3373, dry ice, and shipping labels are affixed to the outer shipping box. These should be provided by World Courier.
  - 6.7. Close and seal the outer shipping container with packing tape.
7. Transfer the assembled shipment to the courier and provide any updates on tracking information to the DS-Biorepository through email at [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu).

## Courier Details

### National sites

Collection sites in the contiguous United States will be provided with return shipping labels to facilitate sample shipment using **standard overnight shipping with FedEx**.

### International sites

International collection sites and the central lab must coordinate return shipments through World Courier. **Shipping conditions will be overnight. However, international overnight shipping rates and the frequency of these shipments may necessitate alternative strategies if the shipping costs become unmanageable.**

## Quality Control

### Overview

This section of the manual covers the various methods for tracking and evaluating sample quality.

### Sample storage conditions

Sample storage conditions should be monitored through a combination of temperature probes and visual inspections of the freezers. Freezers should be visually inspected **daily** to ensure proper temperature is maintained. Record this using a temperature log.

- -80°C +/- 10 degrees
- -20°C +/- 5 degrees

Liquid nitrogen reserves should be checked **weekly** and refilled to ensure samples are maintained in the liquid nitrogen vapor phase.

If a freezer or liquid nitrogen reservoir falls out of the acceptable storage conditions, staff will move samples, if necessary, record the event for impacted samples, and notify the central laboratory at the time of shipment.

## Shipping conditions

To ensure proper shipping conditions are maintained, upon receipt of samples by the DS-Biorepository, staff will inspect the packages to ensure each of the following:

- Review the date of shipment and tracking history to note any delays during shipment.
- Dry ice is still present in boxes for frozen samples.
- A brief visual inspection of samples to ensure they have not thawed.

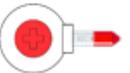
If a shipment fails any of these QC checks, the samples will be stored in their proper conditions, and a note will be made in the laboratory inventory management system and tracked for future sample use. The central lab will also provide retraining with the collection sites as needed.

## Appendix

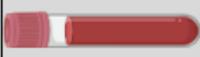
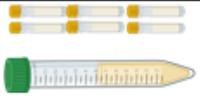
### Collection Kit Quick Reference Guides

The following table provides an overview of the types of biospecimens collected from each of the offered kits, the processing steps for each biospecimen, and terminal storage conditions before shipping to the DS-Biorepository for central lab processing and storage.

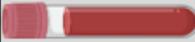
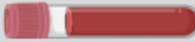
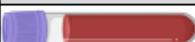
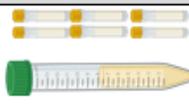
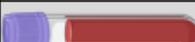
**Tasso Kit**

Biospecimen	Source	Processing steps	Storage tubes	Storage temperature
Plasma	 Tasso EDTA (2x)	Centrifuge— Combine plasma from 2x tasso into 1.5 microcentrifuge tubes — Aliquot plasma into 0.5 mL matrix tubes — Freeze on dry ice	 Matrix tubes (2)	-80°C
RNA	 Tasso EDTA (1x)	Centrifuge— Transfer plasma layer to 1.5 microcentrifuge tubes — Transfer remain blood layer to 2.0 mL microcentrifuge tube with RNAlater — Invert to mix	 2.0 mL Microcentrifuge Tubes (1)	-80°C
DNA (blood)	 Tasso EDTA (1x)	Centrifuge— Transfer plasma layer to 1.5 microcentrifuge tubes — Transfer remain blood layer to clean 2.0 mL microcentrifuge tube	 2.0 mL Microcentrifuge Tubes (1)	-80°C
DNA (microbiome)	 Tongue Swab (1x)	None; Place at -80°C	 Tongue Swab (1)	-80°C

Child Kit

Biospecimen	Source	Processing steps	Storage tubes	Storage temperature
RNA	 PAXgene RNA (1x)	Sit at room temp for 2 hrs— Store at -20°C overnight — Store at -80°C	 PAXgene RNA (1)	-80°C
DNA (blood)	 PAXgene DNA (1x)	Sit at room temp for 2 hrs— Store at -20°C overnight — Store at -80°C	 PAXgene DNA (1)	-80°C
DNA (microbiome)	 Tongue Swab (1x)	None; Place at -80°C	 Tongue Swab (1)	-80°C
Plasma	 EDTA tube (3x)	Centrifuge— Collect plasma— Centrifuge— Aliquot into matrix tubes and 15 mL conical — Freeze on dry ice	 Matrix tubes (18), 15 ml conical (1)	-80°C
Buffy Coat	 EDTA tube (3x)	Centrifuge— Remove plasma — Transfer buffy coat to 50 mL conical and— RBC lysis— Centrifuge— PBS wash— Centrifuge—Resuspend in freezing media— Aliquot into cryogenic vials— Store at -80°C for at least 4 hours— Move to vapor phase LN	 Cryovials (3)	LN
RBCs	 EDTA tube (3x)	Centrifuge— Remove plasma and buffy coat— Aliquot remaining RBCs into matrix tubes — Freeze on dry ice	 Matrix tubes (3)	-80°C
Cytof Ready Samples	 EDTA tube (3x)	Transfer 0.5 mls of blood to Iselfix buffer— Incubate 15 min at 37 C— Centrifuge— Wash in PBS — Centrifuge— Resuspend in Maxpar buffer— Transfer to 1.5 mL tubes— Centrifuge— Remove all but 50-100 ul of superntant	 2.0 mL Microcentrifuge Tubes (2)	-80°C

Adult Kit

Biospecimen	Source	Processing steps	Storage tubes	Storage temperature
RNA	 PAXgene RNA (1x)	Sit at room temp for 2 hrs— Store at -20°C overnight — Store at -80°C	 PAXgene RNA (1)	-80°C
DNA (blood)	 PAXgene DNA (1x)	Sit at room temp for 2 hrs— Store at -20°C overnight — Store at -80°C	 PAXgene DNA (1)	-80°C
DNA (microbiome)	 Tongue Swab (1x)	None; Place at -80°C	 Tongue Swab (1)	-80°C
Plasma	 EDTA tube (3x)	Centrifuge— Collect plasma— Centrifuge— Aliquot into matrix tubes and 15 mL conical — Freeze on dry ice	 Matrix tubes (18), 15 ml conical (1)	-80°C
Buffy Coat	 EDTA tube (3x)	Centrifuge— Remove plasma — Transfer buffy coat to 50 mL conical and— RBC lysis— Centrifuge— PBS wash— Centrifuge—Resuspend in freezing media— Aliquot into cryogenic vials— Store at -80°C for at least 4 hours— Move to vapor phase LN	 Cryovials (6)	LN
RBCs	 EDTA tube (3x)	Centrifuge— Remove plasma and buffy coat— Aliquot remaining RBCs into matrix tubes — Freeze on dry ice	 Matrix tubes (3)	-80°C
Cytof Ready Samples	 EDTA tube (3x)	Transfer 0.5 mls of blood to lysesix buffer— Incubate 15 min at 37 C— Centrifuge— Wash in PBS — Centrifuge— Resuspend in Maxpar buffer— Transfer to 1.5 mL tubes— Centrifuge— Remove all but 50-100 ul of supernatant	 2.0 mL Microcentrifuge Tubes (2)	-80°C

Saliva Kit

Biospecimen	Source	Processing steps	Storage tubes	Storage temperature
Saliva	 Saliva collection tube	None; Place at -80°C	 Saliva Collection Tube	-80°C



Place Kit  
Barcode here

Study Site Lab Processing Log		
Participant ID:		
Sample type	Quantity	This column for DSBR Central Lab use only: Received?
Tongue Swab	1	<input type="checkbox"/> DSBR Comments:
<b>Plasma (up to two aliquots, 0.25mL per aliquot, matrix tubes)</b>		
# of plasma aliquots (0.25 mL):		<input type="checkbox"/>
Time plasma Frozen:	____ : ____ (24hrs)	DSBR Comments:
<b>RNAlater (one aliquot, Tasso 1 remaining sample in 1.3ml RNAlater buffer cryovial)</b>		
# of RNAlater aliquots:		<input type="checkbox"/>
Time RNAlater frozen:	____ : ____ (24hrs)	DSBR Comments:
<b>DNA (one aliquot, Tasso 2 remaining sample in DNA cryovial)</b>		
# DNA Aliquots:		<input type="checkbox"/>
Time DNA Frozen:	____ : ____ (24hrs)	DSBR Comments:
Comments:  <input type="checkbox"/> None	Condition of specimens: <input type="checkbox"/> No issues (intact) <input type="checkbox"/> Insufficient specimen collection <input type="checkbox"/> Container broken/leakage <input type="checkbox"/> Hemolyzed <input type="checkbox"/> Other:	

Participant Specimen Data Entered Into RedCap:

Processing Tech Signature: \_\_\_\_\_

Shipped Date:    /    /       Tracking number: \_\_\_\_\_  
      M M    D D    Y Y

DSBR Rec'd Date: \_\_\_\_\_

Place Kit  
Barcode here

**INCLUDE DS-Biorepository Child Kit Biospecimen Form**

- Attention Collection site -

PLEASE ENSURE ALL SECTIONS ARE COMPLETE AND SIGN FORM BEFORE SHIPMENT  
Please contact [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu) with questions

Participant and Collection Information:	Participant ID: _____		Participant Initials: _____		
	Site ID: _____		Visit #: _____		
	____ / ____ / ____ M M D D Y Y Y Y		Collection Time ____ : ____ (24hrs)		
Collected?	Blood Tubes, Collect in the following order:				Tongue Swab
	1 EDTA	2 EDTA	3 EDTA	4 PAX RNA	5 PAX DNA
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Quality Control issues:			Specimen comments		
EDTA Issues	<input type="checkbox"/> Incorrect tube draw order	<input type="checkbox"/> Underfilled tube	<input type="checkbox"/> None		
PAX RNA Issues	<input type="checkbox"/> Incorrect tube draw order	<input type="checkbox"/> Underfilled tube	<input type="checkbox"/> None		
PAX DNA Issues	<input type="checkbox"/> Incorrect tube draw order	<input type="checkbox"/> Underfilled tube	<input type="checkbox"/> None		
Tongue swab issues	<input type="checkbox"/> Food/Drink Contamination	<input type="checkbox"/> Chewed/damaged	<input type="checkbox"/> None		

To be Completed by Study Site Lab Personnel			
Date and Time of Received in study site lab		____ / ____ / ____ : ____ : ____ (24hrs) M M D D Y Y	
Specimen		Received	Data
EDTA (4 mL)	1	<input type="checkbox"/>	Total Volume (mL): _____
	2	<input type="checkbox"/>	Time in Centrifuge (first spin): ____ : ____ (24hrs)
	3	<input type="checkbox"/>	
PAXRNA (2.5mL)	4	<input type="checkbox"/>	Date at -20°C (day of collection): ____ : ____ (24hrs) Date at -80°C (next business day): ____ / ____ / ____ M M D D Y Y Time at -80°C: ____ : ____ (24hrs)
PAXDNA (2.5mL)	5	<input type="checkbox"/>	Time at -20°C (day of collection): ____ : ____ (24hrs) Date at -80°C (next business day): ____ / ____ / ____ M M D D Y Y Time at -80°C: ____ : ____ (24hrs)
Tongue swab	6	<input type="checkbox"/>	Time at -80°C (day of collection): ____ : ____ (24hrs)

Place Kit  
Barcode here

**Study Site Lab Processing Log**

Participant ID: \_\_\_\_\_

Sample type	Quantity	This column for DSBR Central Lab use only: Received?
PAX RNA	1	<input type="checkbox"/> DSBR Comments:
PAX DNA	1	<input type="checkbox"/> DSBR Comments:
Tongue Swab	1	<input type="checkbox"/> DSBR Comments:
<b>Red Blood Cells (up to six aliquots, 0.5mL per aliquot)</b>		
# of RBC aliquots:		<input type="checkbox"/>
Time RBCs Frozen:	____ : ____ (24hrs)	DSBR Comments:
<b>Plasma (up to eighteen aliquots, 0.25mL per aliquot – remaining plasma in 15ml tube)</b>		
# of plasma aliquots (0.25 mL):		<input type="checkbox"/>
15mL conical (remaining plasma):	_____ mL	<input type="checkbox"/>
Time plasma Frozen:	____ : ____ (24hrs)	DSBR Comments:
<b>Viable Buffy Coat (up to 3 aliquots, 1mL of cells in Freezing Media per aliquot)</b>		
# of VBC aliquots:		<input type="checkbox"/>
Time buffy coat frozen at -80°C:	____ : ____ (24hrs)	DSBR Comments:
Date & time buffy coat in LN2:	____ / ____ / ____ M M D D Y Y ____ : ____ (24hrs)	
<b>CyTOF (up to two cell pellet aliquots)</b>		
# CyTOF-Ready WBC pellets:		<input type="checkbox"/>
Time CyTOF pellets Frozen:	____ : ____ (24hrs)	DSBR Comments:
Comments:  <input type="checkbox"/> None	Condition of specimens: <input type="checkbox"/> No issues (intact) <input type="checkbox"/> Insufficient specimen collection <input type="checkbox"/> Container broken/leakage <input type="checkbox"/> Hemolyzed <input type="checkbox"/> Other: _____	

Participant Specimen Data Entered Into RedCap:  Processing Tech Signature: \_\_\_\_\_

Shipped Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_ Tracking number: \_\_\_\_\_  
M M / D D / Y Y

DSBR Rec'd Date: \_\_\_\_\_

Place Kit  
Barcode here

**INCLUDE DS-Biorepository Adult Kit Biospecimen Form**

- Attention Collection site -

PLEASE ENSURE ALL SECTIONS ARE COMPLETE AND SIGN FORM BEFORE SHIPMENT

Please contact [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu) with questions

Participant and Collection Information:	Participant ID: _____		Participant Initials: _____			
	Site ID: _____		Visit #: _____			
	____ / ____ / ____ M M / D D / Y Y Y Y		Collection Time ____ : ____ (24hrs)			
	Blood Tubes, Collect in the following order:			Tongue Swab		
	1 EDTA	2 EDTA	3 EDTA	4 PAX RNA	5 PAX DNA	Time since last food (hrs): ____
						Time since last drink (hrs): ____
Collected?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Quality Control issues:			Specimen comments			
EDTA Issues	<input type="checkbox"/> Incorrect tube draw order	<input type="checkbox"/> Underfilled tube	<input type="checkbox"/> None			
PAX RNA Issues	<input type="checkbox"/> Incorrect tube draw order	<input type="checkbox"/> Underfilled tube	<input type="checkbox"/> None			
PAX DNA Issues	<input type="checkbox"/> Incorrect tube draw order	<input type="checkbox"/> Underfilled tube	<input type="checkbox"/> None			
Tongue swab issues	<input type="checkbox"/> Food/Drink Contamination	<input type="checkbox"/> Chewed/damaged	<input type="checkbox"/> None			

To be Completed by Study Site Lab Personnel			
Date and Time of Received in study site lab		____ / ____ / ____ : ____ : ____ (24hrs) M M / D D / Y Y	
Specimen	Received	Data	
EDTA (10mL)	1 <input type="checkbox"/>	Total Volume (mL): _____	
	2 <input type="checkbox"/>	Time in Centrifuge (first spin): ____ : ____ (24hrs)	
	3 <input type="checkbox"/>		
PAXRNA (2.5mL)	4 <input type="checkbox"/>	Date at -20°C (day of collection): ____ : ____ (24hrs)	
		Date at -80°C (next business day): ____ / ____ / ____ Time at -80°C: ____ : ____ (24hrs) M M D D Y Y	
PAXDNA (8.5mL)	5 <input type="checkbox"/>	Time at -20°C (day of collection): ____ : ____ (24hrs)	
		Date at -80°C (next business day): ____ / ____ / ____ Time at -80°C: ____ : ____ (24hrs) M M D D Y Y	
Tongue swab	6 <input type="checkbox"/>	Time at -80°C (day of collection): ____ : ____ (24hrs)	

Place Kit  
Barcode here

**Study Site Lab Processing Log**

Participant ID: \_\_\_\_\_

Sample type	Quantity	This column for DSBR Central Lab use only: Received?
PAX RNA	1	<input type="checkbox"/> DSBR Comments:
PAX DNA	1	<input type="checkbox"/> DSBR Comments:
Tongue Swab	1	<input type="checkbox"/> DSBR Comments:
<b>Red Blood Cells (up to six aliquots, 0.5mL per aliquot)</b>		
# of RBC aliquots:		<input type="checkbox"/>
Time RBCs Frozen:	____ : ____ (24hrs)	DSBR Comments:
<b>Plasma (up to eighteen aliquots, 0.25mL per aliquot – remaining plasma in 15ml tube)</b>		
# of plasma aliquots (0.25 mL):		<input type="checkbox"/>
15mL conical (remaining plasma):	_____ mL	<input type="checkbox"/>
Time plasma Frozen:	____ : ____ (24hrs)	DSBR Comments:
<b>Viable Buffy Coat (up to six aliquots, 1mL of cells in Freezing Media per aliquot)</b>		
# of VBC aliquots:		<input type="checkbox"/>
Time buffy coat frozen at -80°C:	____ : ____ (24hrs)	DSBR Comments:
Date & time buffy coat in LN2:	____ / ____ / ____ M M D D Y Y ____ : ____ (24hrs)	
<b>CyTOF (up to two cell pellet aliquots)</b>		
# CyTOF-Ready WBC pellets:		<input type="checkbox"/>
Time CyTOF pellets Frozen:	____ : ____ (24hrs)	DSBR Comments:
Comments:  <input type="checkbox"/> None		Condition of specimens: <input type="checkbox"/> No issues (intact) <input type="checkbox"/> Insufficient specimen collection <input type="checkbox"/> Container broken/leakage <input type="checkbox"/> Hemolyzed <input type="checkbox"/> Other:

Participant Specimen Data Entered Into RedCap:  Processing Tech Signature: \_\_\_\_\_

Shipped Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_ Tracking number: \_\_\_\_\_  
M M / D D / Y Y

DSBR Rec'd Date: \_\_\_\_\_

Place Kit  
Barcode here

**INCLUDE DS-Biorepository Saliva Kit Biospecimen Form**

- Attention Collection site -

PLEASE ENSURE ALL SECTIONS ARE COMPLETE AND SIGN FORM BEFORE SHIPMENT

Please contact [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu) with questions

Participant and Collection Information:	Participant ID: _____	Participant Initials: _____
	Site ID: _____	Visit #: _____
	____ / ____ / ____ M M / D D / Y Y Y Y	Collection Time ____ : ____ (24hrs)
Saliva	Time since last food (hrs): _____ Time since last drink (hrs): _____	
Collected?	<input type="checkbox"/>	
Quality Control issues:		Specimen comments
Saliva Issues	<input type="checkbox"/> Food/Drink Contamination <input type="checkbox"/> Underfilled tube	<input type="checkbox"/> None

To be Completed by Study Site Lab Personnel			
Date and Time of Received in study site lab		____ / ____ / ____ M M / D D / Y Y	____ : ____ (24hrs)
Specimen	Received	Specimen Data	This column for DSBR Central Lab use only: Received?
Saliva	<input type="checkbox"/>	Time at -80°C (day of collection): ____ : ____ (24hrs)	<input type="checkbox"/>
			DSBR Comments:

Participant Specimen Data Entered Into RedCap:

Processing Tech Signature: \_\_\_\_\_

Shipped Date: \_\_\_\_ / \_\_\_\_ / \_\_\_\_    Tracking number: \_\_\_\_\_  
 M M / D D / Y Y

DSBR Rec'd Date: \_\_\_\_\_

Place Kit  
Barcode here

**INCLUDE DS-Biorepository Tube Replacement Form**

- Attention -

PLEASE ENSURE ALL SECTIONS ARE COMPLETE AND SIGN FORM BEFORE SHIPMENT

Please contact [DS-Biorepository@cuanschutz.edu](mailto:DS-Biorepository@cuanschutz.edu) with questions.



Damaged/Lost Tube Information	Replacement Tube ID	Reason for Replacement
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____

Place Kit  
Barcode here

Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____
Damaged/Lost Tube ID:	Replacement Tube ID:	<input type="checkbox"/> Damaged tube <input type="checkbox"/> Expired tube <input type="checkbox"/> Other: _____



Tube Replacement Data Entered Into RedCap:

Tube Replacement Form Attached To Biospecimen Collection Form:

Processing Tech Signature: \_\_\_\_\_