BioFIND

THE FOX INVESTIGATION FOR
NEW DISCOVERY OF BIOMARKERS

Play a Part in Parkinson's Research

BioFIND

(Fox Investigation for New Discovery of Biomarkers)

Research Biomarkers Laboratory Manual

Protocol #: 001
Version #: 2.0
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1 Biorepository Contacts

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2 Laboratory Information

2.1 Hours of Operation

Coriell operates from 9 AM to 5 PM Eastern Time, Monday through Friday.

2.2 Holiday Schedules

Please note that courier services may observe a different set of holidays. Please be sure to verify with your courier's schedule prior to any holiday.

Frozen samples must be shipped Monday – Wednesday only.
Frozen samples must be shipped within two weeks of sample collection; if frozen samples are not shipped immediately please ensure adequate storage at -80°C prior to shipment.

Ambient samples may be shipped Monday – Friday (preferably Monday – Thursday) provided they are received at Coriell within 5 days of collection.

Weekend/holiday delivery must be arranged in advance with Coriell.
*Additional information about shipping restrictions will be provided as necessary.

2.3 Holiday Observations* – United States

Date	Holiday
Thursday, Nov 28, 2014	Thanksgiving
Friday, Nov 29, 2014	Day after Thanksgiving
Thursday, Dec 25, 2014	Christmas Day
Thursday, Jan 1, 2015	New Year's Day
Monday, Jan 19, 2015	Martin Luther King Day
Monday, May 25, 2015	Memorial Day
Saturday, July 4, 2015	Independence Day
Monday, Sept 7, 2015	Labor Day
Thursday, Nov 26, 2015	Thanksgiving Day
Thursday, Nov 27, 2015	Day after Thanksgiving
Wednesday, Dec 25, 2015	Christmas Day

3 Research and Clinical Laboratory Collection Schedule

3.1 Biospecimens to be sent to Research (Coriell) or Clinical (Local) Labs

The processing, storage location and timing of sample collection are in the order in which they appear in the table below.

Baseline/Visit 01:

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes Shipped to Coriell	Tubes Retained at Site	Tubes Shipped to Local Lab
Whole-blood: for PT/PTT analysis	2.7 ml Light blue top Sodium Citrate Tube	1	N/A	0	0	1
Whole-blood: for CBC, platelets analysis	10ml Lavender Top EDTA Tube	1	N/A	0	0	1
Whole-blood: for isolation of plasma/ pellet	2 ml microcentrifuge tubes	6	1 ml plasma aliquots in each 2 ml microcentrifuge tube	3	0-3	0
	10ml Lavender Top EDTA Tube	1	Retain blood pellet in EDTA tube	0	1	0
Whole-blood: for extraction of DNA	8.5 ml Yellow Top ACD Tube	1	N/A	1	0	0
TOTAL V01		***10***		***4***	***1-4***	***2***

Final Visit/Visit 02

Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/ Aliquoting	Tubes Shipped to Coriell	Tubes Retained at Site	Tubes Shipped to Local Lab
Whole-blood: for RNA extraction	2.5 ml PAXgene™ Tube	3	N/A	2	1	0
Whole-blood: for isolation of plasma/pellet	2 ml microcentrifuge tubes	18	1 ml plasma aliquots in each 2 ml microcentrifuge tube	9	0-9	0
	10ml Lavender Top EDTA Tube	3	Retain blood pellet in EDTA tube	2	1	0
CSF	50 ml conical Tube	1	Combine and mix total CSF	N/A		
	15 ml conical tubes	2	Divide and spin total CSF			
	2 ml microcentrifuge tubes	18	1 ml CSF aliquots in each 2 ml microcentrifuge tube	10	0-8	0
	2 ml purple-top microcentrifuge tubes	2	1 ml CSF aliquots in each 2 ml purple-top microcentrifuge tube	0	0	2
Saliva	50 ml conical tube	1	Processed and centrifuged in 15 ml conical tubes. Aliquoted in 2 ml microcentrifuge tubes			
	2X15 ml conical tube	2				
	2 ml microcentrifuge tubes	10		6	0-4	0
Urine	50 ml orange top cup	1	Aliquot in 2X15 ml conical tubes	1	1	0
	4X15 ml conical tubes	4				
TOTAL V02		***65***		***30***	***2-24***	***2***

The following samples will be collected according to the visit schedule noted above.

- Blood for standard clinical safety analysis
- Blood to isolate plasma suitable for proteomic, metabolomic and other analyte studies and pellets of cells for protein analyses.
- Whole blood for appropriate DNA and RNA analysis
- Urine
- Saliva
- Cerebrospinal fluid

If a sample is not obtained at a particular visit, this should be recorded on the appropriate data form and a reason should be provided.

4 Specimen Collection Kits and Supplies

Research specimen collection kits as well as clinical lab supplies (except dry ice and Protease Inhibitor Cocktail) will be provided to you by Coriell with materials needed for blood, urine, saliva and CSF collection, containers for plasma, urine, saliva and CSF aliquots, as well as shipping labels to send materials back to the research laboratory. Tube labels will be provided to you by the CTCC. Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment (see Appendix N).

4.1 Coriell – Specimen Collection Kit Contents

Collection kits contain the following and provide the necessary supplies to collect samples from 1 subject. BioFIND kit components have been carefully selected to suit the needs of this project. Do not replace or supplement any of the tubes or kit components provided by Coriell with your own supplies unless you have received approval from MJFF/Coriell to do so. Note that "supplemental" kits will be provided to sites should you require additional supplies from those contained in the visit specific kits. See Section 9.1 for LP Kit contents.

Baseline Visit/Visit 01 Supplies

Quantity	Baseline/Visit 01 Kit Component
6	Polypropylene microcentrifuge tubes (2 ml)
2	EDTA (lavender top) blood collection tube (10 ml)
2	Air waybill (pre-filled)
2	Shipping instruction sheet
1	Sodium citrate (light blue top) blood collection tube (2.7 ml)
1	ACD Sol A (yellow top) blood collection tube (8.5 ml)
1	Shipping carton & plastic/foam liner (to be supplied in a separate box; for shipment of yellow-top ACD tube)
1	Overpack: Biological Substance Category B (for shipment of yellow-top ACD tube in shipping carton & plastic/foam liner)
1	Bubble wrap pouch
1	Plastic Biohazard bag
1	Tyvek envelope
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker

Final Visit/Visit 02 Supplies

Quantity	Final Visit/Visit 02 Kit Component
46	Polypropylene microcentrifuge tubes, sterile (2 ml)
3	PAXgene™ blood collection tube (2.5 ml)
3	EDTA (lavender top) blood collection tube (10 ml)
2	CSF lab (purple top) microcentrifuge tubes, sterile (2 ml)
2	Plastic Biohazard bag
2	Tyvek envelope
8	Conical centrifuge tube (15 ml)
2	Conical centrifuge tube (50 ml)
1	Microcentrifuge tube box
1	Orange top cup
1	Bubble wrap pouch
1	Shipping box/Styrofoam container
1	Warning label packet with dry ice sticker
1	Air waybill (pre-filled)
1	Shipping instruction sheet
1	Lumbar puncture tray (to be supplied in a separate box)

Supplemental Supplies

Quantity	Supplemental Kit
100	Polypropylene microcentrifuge tubes, sterile (2 ml)
20	EDTA (purple top) blood collection tube (10 ml)
15	PAXgene™ blood collection tube (2.5 ml)
10	CSF lab (purple top) microcentrifuge tubes, sterile (2 ml)
10	Conical centrifuge tube (15 ml)
5	ACD Sol A (yellow top) blood collection tubes (8.5 ml)
5	Sodium citrate (light blue top) blood collection tube (2.7 ml)
5	Conical centrifuge tube (50 ml)
1	Box of individually wrapped pencil point spinal needles, 24 G x 3.5 in. (0.55 mm x 90 mm)
1	Box of Introducer needles, 20 G x 1.25 in. (0.90mm x 32mm)

Each Site Will Need To Provide:

Dry ice
Tourniquet
Alcohol Prep Pad
Gauze Pad
Bandage
Butterfly needles
Microcentrifuge tube rack
Crushed Ice
Gloves
Sharps bin and lid
Micropipette and 1 ml micropipette tips
Pipette filler and 10 ml serological pipettes

4.2 Coriell – Initial Supply

Each site will be initially supplied 4 Visit 01 kits and 4 Visit 02 kits. This means sites will have sufficient kits for four subjects. Sites will also be supplied with one box of supplemental supplies. Subsequent kits should be ordered from Coriell when needed.

4.3 Coriell – Resupply

Each individual site will be responsible for ordering additional kits from Coriell after the initial supply has been sent. QUEUE is Coriell's online database that you will use for ordering collection kits. Once a site is activated by the CTCC to begin enrolling subjects, Coriell will provide the site coordinator/s with a username and password to access the database.

1. Log in to QUEUE with your username and password at: https://queue.coriell.org/q/.
    - Under Contracts link in the upper left-hand corner of the screen, select BioFIND Project Management.
    - A menu will appear; click on the Kit Request link.
2. Use the drop-down menu on the Kit Request page to select where the supplies should be shipped. If the shipping address(es) on the list is not the one you need, contact ninds@coriell.org
3. Enter any special requests/remarks in the Request field.
4. Click the Submit button.

RESUPPLY: Be sure to check your supplies and order additional materials before you run out so you are prepared for both scheduled and unanticipated visits. Please allow **TWO weeks** for kit orders to be processed and delivered.

5 Site Required Equipment

In order to process samples consistently across all sites and ensure the highest quality samples possible, sites must have access to the following equipment:

4°C Refrigerated and Room Temperature Centrifuge
-80°C Freezer (for sample storage)
-20°C Freezer (for Protease Inhibitor Cocktail storage)

6 Blood Collection and Processing Procedures

Blood samples at Visit 2 should be collected in the morning between 8 am – 10 am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (see Appendix P). Record time of last meal (and whether low fat diet followed, if applicable) on the Laboratory Procedures data form.

*****Important Note*****

In order to ensure the highest quality samples are collected, processed and stored, it is essential to follow the specific collection, processing and shipment procedures detailed in the following pages.

SPECIFIC INSTRUCTIONS FOR COLLECTION AND PROCESSING OF EACH SAMPLE ARE DETAILED ON THE FOLLOWING PAGES. See Appendix O for lab flow worksheet that may be used for processing of all lab samples.

6.1 Labeling Samples

In order to ensure the label adheres properly and remains on the tube, please follow these instructions (see Appendix N diagram):

1. Place labels on **ALL** collection and aliquot tubes **BEFORE** any cooling of tubes, sample collection or sample processing/freezing. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.

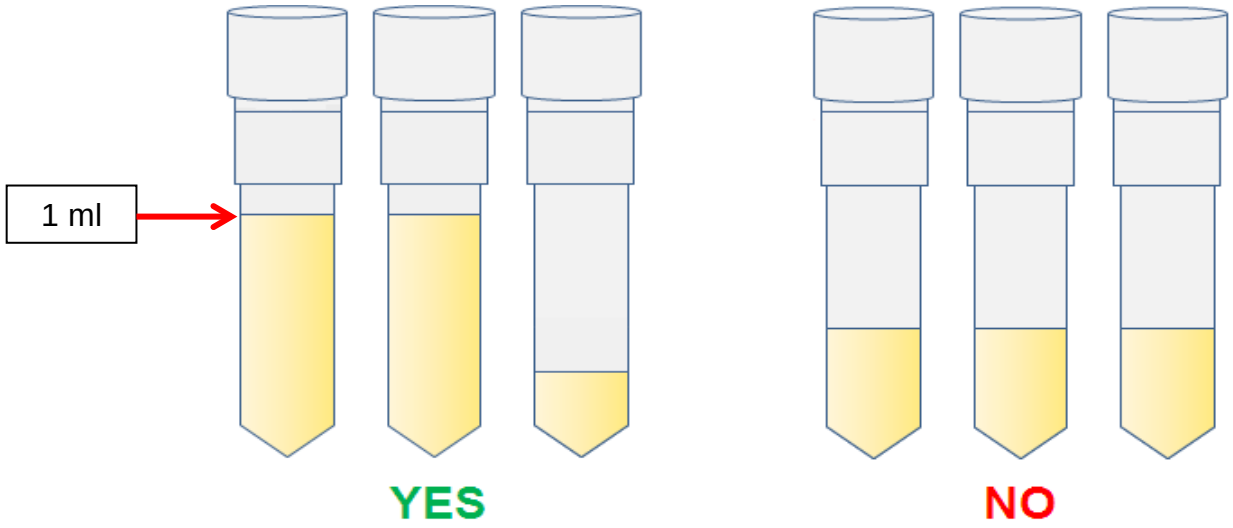
2. Labels appropriate for local laboratory processing should be prepared and attached to the tubes that will be sent to the local labs.

3. Place label **horizontal** on the tube (wrapped around sideways if tube is upright) and **just below the ridges** of the aliquot tubes (see attached labeling diagram). There is enough space on the aliquot tube for the label to be placed without overlapping the ridges.

4. Take a moment to ensure the label is **completely adhered** to each tube. It may help to roll the tube between your fingers after applying the label.

6.2 Filling Aliquot Tubes (Plasma, saliva and CSF)

In order to ensure that Coriell receives a sufficient amount of the sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should ideally be filled to 1.0 milliliters (see picture below) with the respective biologic material after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of that sample. If there is biologic material remaining that will not fill a subsequent aliquot tube to 1.0 ml, that remaining amount should still be included and shipped to Coriell. Essentially, as much material as possible should be shipped to Coriell, ensuring maximum amount in as many aliquot tubes as will allow after processing the sample. You do not have to fill all microcentrifuge tubes provided; you should attempt to fill as many tubes as possible with 1.0 ml of sample. For example, if 3.5 ml of sample is obtained, you should fill three microcentrifuge tubes each with 1.0 ml, and one additional microcentrifuge tube with the remaining 0.5 ml.



6.3 Whole Blood Collection for PT/PTT Analysis: Sodium citrate Light-blue Top Tube (for shipment to local lab) – BASELINE VISIT/VISIT 01

1. Label the light-blue top sodium citrate tube according to your own institutional format; this tube should **not** be labeled with a pre-printed BioFIND label supplied by CTCC. Label the tube (per local lab requirement) prior to blood draw; no processing is required for this tube, it will be sent as-is to the **local lab** for analysis.

2. CRITICAL STEP: **Store empty sodium citrate Light-blue Top Tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use.**

3. Using a blood collection set and a holder, collect blood into the **2.7 ml sodium citrate tube** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 2.7 ml of blood into the tube.

5. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the sodium citrate tube 3-4 times.**

6.4 Whole Blood Collection for CBC, platelet Analysis: EDTA Lavender Top Tube (for shipment to local lab)-BASELINE VISIT/VISIT 01

1. Label the lavender-top EDTA tube according to your own institutional format; this tube should **not** be labeled with a pre-printed BioFIND label supplied by CTCC. Label the tube (per local lab requirement) prior to blood draw; no processing is required for this tube, it will be sent as-is to the **local lab** for analysis.

2. CRITICAL STEP: **Store empty EDTA Lavender Top Tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use.**

3. Using a blood collection set and a holder, collect blood into the **10 ml EDTA tube** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

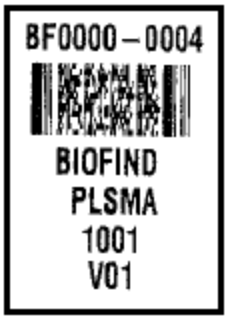
4. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

5. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

6.5 Whole Blood Collection for Isolation of Plasma/Pellet: EDTA Lavender Top (for processing of plasma aliquots and retention of blood pellet) – BASELINE VISIT/VISIT 01

1. Place "**PELLET**" label on the 10ml EDTA tube prior to blood draw (per Section 6.1); this tube containing the pelleted blood sample will be retained and frozen following separation and aliquoting of the plasma layer. **The Visit 01 EDTA tube containing the blood pellet will be retained at the collection site. Plasma aliquots are to be shipped to Coriell as well as retained at the collection site according to the specifications set in Section 3.1.**

2. Place pre-printed "**PLSMA**" labels on the 2 ml microcentrifuge tubes.



3. CRITICAL STEP: **Store EDTA 10 ml Lavender Top Tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use. Place 2 ml microcentrifuge aliquot tubes on ice prior to procedure, but after labeling, so they are pre-cooled.**

4. Using a blood collection set and a holder, collect blood into the **10 ml EDTA tube** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

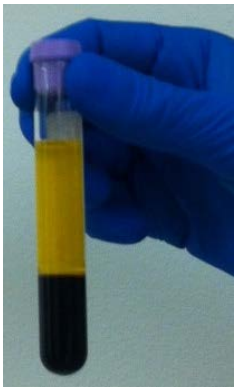
5. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

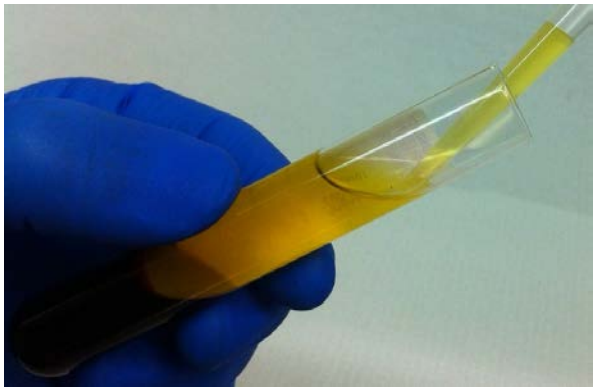
6. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

7. Within 30 minutes of blood collection, centrifuge balanced tubes at **4°C** for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.**

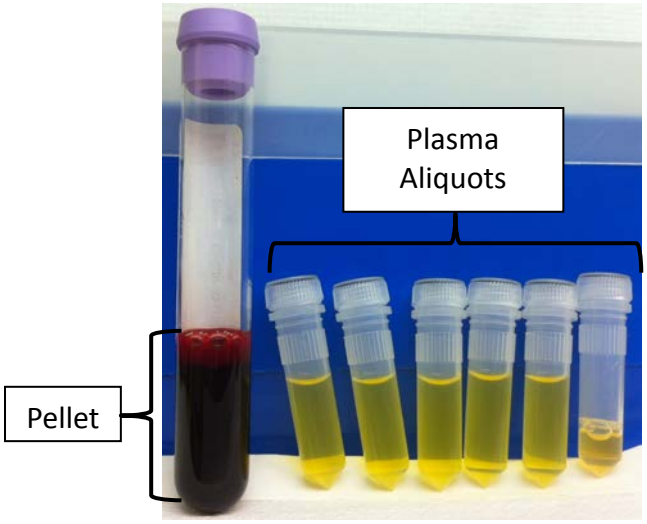
- Equivalent rpm for spin at 1500 x g = _____
- While centrifuging record the time of centrifuge start on the Laboratory Procedures data form.

8. Using a **micropipette**, transfer 1.0 ml of blood plasma (top layer) into each labeled, pre-cooled, aliquot tube. The EDTA tube should yield, on average, 4.5 ml of blood plasma for a total of 4-6 aliquot tubes per subject. Take caution not to disturb the pellet at the bottom of the tube by tilting the tube and placing the pipette tip along the lower side of the glass wall without touching the pellet so that plasma is not contaminated by pellet material (see below).





9. Retain the used EDTA tube containing the pelleted blood sample; freeze and store EDTA tube at **-80°C Freezer**.

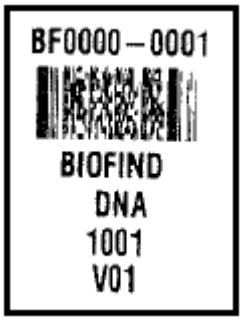


10. Freeze all samples (blood pellet and plasma aliquots) immediately following processing by transferring to **-80°C Freezer**. If samples cannot be immediately transferred to **-80°C Freezer**, prepare a sufficient amount of dry ice for immediate freezing. Store all samples at **-80°C Freezer** until you ship on dry ice (3 plasma aliquots are to be shipped to Coriell, 0-3 plasma aliquots and the blood pellet will remain at the collection site). Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the eClinical database.

6.6 Whole Blood Collection for Extraction of DNA: Yellow Top ACD Tube (for shipment to Coriell) – BASELINE VISIT/VISIT 01

Yellow-top ACD blood sample must be received at Coriell within 5 days of being collected. Samples not received within 5 days of collection must be re-drawn at the site.

1. Place pre-printed "**DNA**" label on the ACD tube prior to blood draw (per Section 6.1); no processing is required for this tube, it will be sent as-is to **Coriell**.



2. Using a blood collection set and a holder, collect blood into the ACD tube using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

3. Allow at least 10 seconds for a complete blood draw to take place. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 8.5 ml of blood into the tube.

4. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the ACD tube 8 – 10 times**

5. Seal the 1 ACD tube in the ambient shipment kit and complete the BioFIND Sample Record and Summary Shipment Notification Form.

6. Ship the sample back to Coriell at **room temperature** according to kit instructions within 12 hours of collection. If sample cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. **Sample must be received at Coriell within 5 days of being collected.**

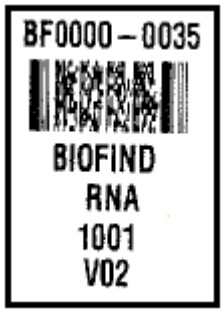
7. Complete the DNA Sample source worksheet and ensure timely entry of data into eClinical database.

6.7 Whole Blood Collection for Extraction of RNA: PAXgene™ Tubes – FINAL VISIT/VISIT 02

See training videos for blood collection (http://www.preanalytix.com/videos/rna-tube-collection-video/) and freezing (http://www.preanalytix.com/videos/rna-tube-freezing-video/).

***NOTE: The preanalytix training video recommends drawing the PAXgene blood tubes last in the draw order however for the purpose of this study, the PAXgene blood tubes should be drawn first.**

1. Place "**RNA**" label on the PAXgene RNA tubes prior to blood draw (per Section 6.1); no processing is required for these tubes, **two tubes are to be shipped as-is to Coriell, one tube is to be retained at the collection site.**



2. CRITICAL STEP: **Store PAXgene™ Blood RNA Tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use.**

3. CRITICAL STEP: The **PAXgene™ Blood RNA Tubes should be the first tubes drawn** in the phlebotomy procedure.

4. Using a blood collection set and a holder, collect blood into the **first of the three PAXgene™ Blood RNA Tubes** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place donor's arm in a downward position.
   b. Hold tube in a vertical position, below the donor's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The PAXgene™ Blood RNA Tube with its vacuum is designed to draw 2.5 ml of blood into the tube. Record time of draw on Laboratory Procedures data form.

8. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube 8 – 10 times**.

9. **REPEAT STEPS 4 TO 6** for the second and third PAXgene™ Blood RNA Tubes to be collected.

10. CRITICAL STEP: Incubate the PAXgene™ Blood RNA Tubes **UPRIGHT at room temperature (18°C to 25°C) for 24 hours**. Record time and date of draw on Laboratory Procedures data form.

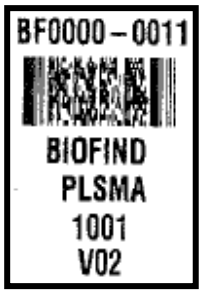
- *If blood is drawn on a Friday and you are unable to return on Saturday to place tubes in the freezer, transfer the tubes as late as possible before leaving on Friday.* ***Samples must sit at room temperature for a minimum of 2 hours.***

11. After **24 hours** at room temperature, transfer the three PAXgene tubes to **-80°C (minus eighty) freezer.** Keep the **PAXgene™ Blood RNA Tubes at -80 °C** until you ship on dry ice (two tubes are to be shipped to Coriell, the third will remain at the collection site). Complete remainder of the Laboratory Procedures data form.

6.8 Whole Blood Collection for Isolation of Plasma/Pellet: EDTA Lavender Top (for processing of plasma aliquots and retention of blood pellet) – FINAL VISIT/VISIT 02

1. Place "**PELLET**" label on all three 10 ml EDTA tubes prior to blood draw (per Section 6.1); these tubes containing the pelleted blood samples will be retained and frozen following separation and aliquoting of the plasma layer. **Two of the Visit 02 EDTA tubes containing the blood pellets will be shipped to Coriell; the third tube will be retained at the collection site. Plasma aliquots are to be shipped to Coriell as well as retained at the collection site according to the specifications set in Section 3.1.**

2. Place pre-printed "**PLSMA**" labels on the 2 ml microcentrifuge tubes.



3. CRITICAL STEP: **Store EDTA 10 ml Lavender Top Tubes at room temperature 64^{0}F - 77^{0}F (18°C to 25°C) before use. Place 2 ml microcentrifuge aliquot tubes on ice prior to procedure, but after labeling, so they are pre-cooled.**

4. Using a blood collection set and a holder, collect blood into the **10 ml EDTA tube** using your institution's recommended procedure for standard venipuncture technique.

The following techniques shall be used to prevent possible backflow:
a. Place donor's arm in a downward position.
b. Hold tube in a vertical position, below the donor's arm during blood collection.
c. Release tourniquet as soon as blood starts to flow into tube.
d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

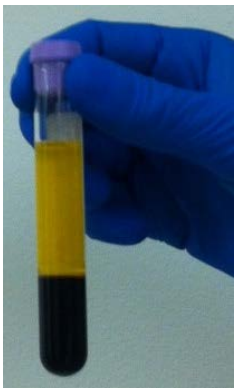
5. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.

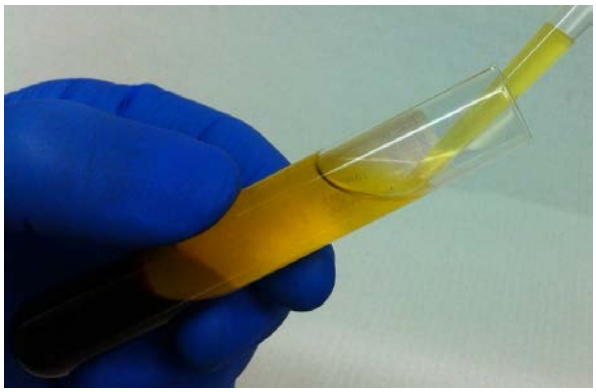
6. CRITICAL STEP: **Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8 – 10 times.**

7. Within 30 minutes of blood collection, centrifuge balanced tubes at **4°C** for 15 minutes at 1500 x g. **It is critical that the tubes be centrifuged at the appropriate speed to ensure proper plasma separation.**

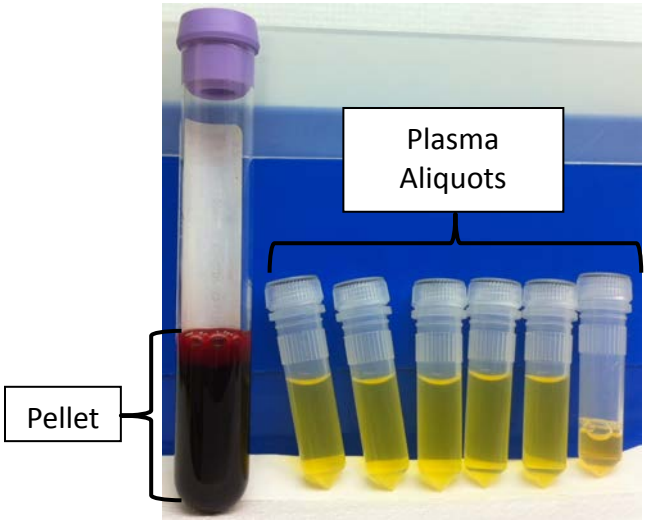
- Equivalent rpm for spin at 1500 x g = _____
- While centrifuging record the time of centrifuge start on the Laboratory Procedures data form.

8. Using a **micropipette**, transfer 1.0 ml of blood plasma (top layer) into each labeled, pre-cooled, aliquot tube. The EDTA tube should yield, on average, 4.5 ml of blood plasma for a total of 4-6 aliquot tubes per subject. Take caution not to disturb the pellet at the bottom of the tube by tilting the tube and placing the pipette tip along the lower side of the glass wall without touching the pellet so that plasma is not contaminated by pellet material (see below).





9. Retain the used EDTA tubes containing the pelleted blood samples; freeze and store EDTA tubes at **-80°C Freezer**.



10. Freeze all samples (blood pellet and plasma aliquots) immediately following processing by transferring to **-80°C Freezer**. If samples cannot be immediately transferred to **-80°C Freezer**, prepare a sufficient amount of dry ice for immediate freezing. Store all samples at **-80°C Freezer** until you ship on dry ice (9 plasma aliquots and 2 blood pellets are to be shipped to Coriell, 0-9 plasma aliquots and one blood pellet will remain at the collection site). Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the eClinical database.

7 Urine collection

1. Label collection cup and the 15 ml conical tubes prior to urine collection. Labels are pre-printed by the CTCC. Label all tubes horizontally.

2. Ask the study subject to collect urine in the collection cup, preferably midstream. Urine collection should be as sterile as possible.

3. Pour 10 ml of urine in two 15 conical tube and cap the tubes (see appendix J)

4. Within 30 minutes of collection, centrifuge at 2,500 g for 15 minutes 4°C.

    1) Equivalent rpm for spin at 2,500 x g = _____

    2) While centrifuging, record the time of centrifuge start on the Saliva and Urine Samples data form.

5. Using transfer pipette, transfer the urine into 2 fresh 15 ml conical tubes without disturbing the sediment at the bottom of the tube. Discard the original tubes.

6. After transfer to the new conical tubes, place the labeled tubes upright in dry ice and allow for complete freeze of the urine sample.

7. Once the urine sample is frozen, store upright at -80°C until shipment.

8. Ship one 15 ml conical tube to the Repository while the second 15 ml conical tube remains at the collection site.

8 Saliva Collection

Saliva should be collected in the morning, preferably fasted. Subjects should refrain from eating, drinking or using oral hygiene products for at least 1 hour prior to collection. Note that sites will receive 100X Protease Inhibitor Cocktail (PIC; Sigma-Aldrich P8340-1ML) directly from the vendor and should store the PIC at -20^{o}C. Note that the CTCC Project Manager should be notified if/when additional stock PIC is needed.

8.1 Required equipment

1. -20^{o}C freezer for protease inhibitor cocktail (PIC) storage
2. Serological pipette filler
3. 10 ml serological pipette
4. p1000 micropipette and tips

8.2 Preparation of PIC (Appendix K)

The Repository will provide ddH_2O (sterile water) needed to prepare 100X PIC as a 10X reagent to add to saliva samples to prevent digestive enzymes in saliva from breaking down candidate biomarker proteins. Aliquoting of the 100X PIC should be done prior to subject visit to minimize work during subject visit. Aliquot the 100X PIC as follows:

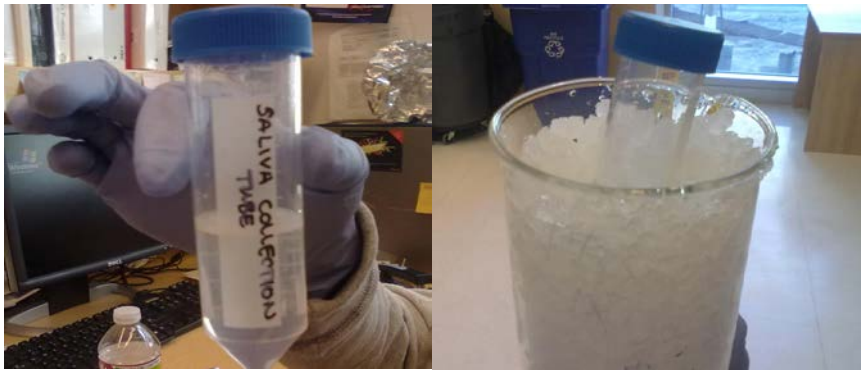
1. Thaw 100X PIC at room temperature prior to aliquoting (avoid freeze thaw cycles)
2. Using permanent marker label 10 X 1.5 ml centrifuge tubes – 100X PIC and date
3. Aliquot 100 µl of 100X PIC into each labeled 1.5 ml centrifuge tube.
4. Store 100x PIC aliquots at -20^{o}C and minimize exposure to light.

8.3 Saliva collection at The Day of Visit (Appendix L)

On the day of the subject visit remove a single aliquot of 100X PIC and thaw at room temperature. Prepare the 10X PIC by diluting it 1:10, add 900 µl sterile ddH_20 to 100 µl of 100X PIC. Invert 8-10 times to mix and keep it at room temperature until saliva sample processing.

1. Label (write subject ID) and pre-chill 50 ml conical tube. Label (pre-printed labels) ALL 15 ml conical and 2 ml micro centrifuge tubes and place on ice.

2. Place the labeled 50 ml conical tube in ice in a plastic/glass container.



3. Prepare subject for collection

    i. Instruct subject to thoroughly rinse their mouth for 1 minute

    ii. Escort subject to an empty, quiet room and let them rest for 5 minutes

    iii. Do not use induction techniques such as sugar. Instruct subject not to spit saliva into the 50 ml conical collection tube, but rather have them lean forward and hold their head down over the collection tube. The saliva will slowly accumulate in their mouth, and will come out of the mouth by itself. *Subject may swallow during collection while maintaining a leaning forward position.*

    iv. Record answers to each relevant item on the Saliva and Urine Samples data form as you progress through the collection and processing procedures.

    v. Collect saliva for 20 minutes or until 5 ml is obtained, whichever comes first.

4. Saliva sample processing

    i. Using serological pipette, transfer saliva to 15 ml conical tube

    ii. Add appropriate amount of 10X PIC using the table below:

Volume of saliva transferred to 15 ml conical	Volume of 10X PIC to add
0.5 ml	100 µl (0.1 ml) – minimum volume possible with p1000
1.0 ml	100 µl (0.1 ml)
1.5 ml	150 µl (0.15 ml)
2.0 ml	200 µl (0.2 ml)
5.0 ml	500 µl (0.5 ml)
10.0 ml	1000 µl (1 ml)

iii. Mix the sample by vortexing or vigorously shaking/inverting the sample and protease inhibitor cocktail for 20 – 30 seconds. This step is variable as the consistency of the saliva will vary among subjects. The more viscous the sample, the longer samples will need to be mixed to create a homogenous sample.

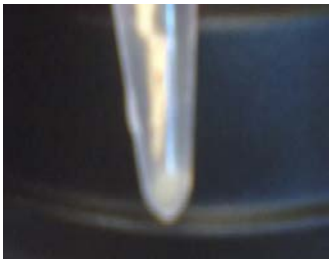
Within 30 minutes of collection, centrifuge samples at 2,000 g for 15 minutes at 4°C. It is critical that the tubes be centrifuged at the appropriate speed and time to remove cellular debris.

a. Equivalent rpm for spin at 2000 x g = _____

b. While centrifuging, record the time of centrifuge start on the Saliva and Urine Samples data form.

iv. CRITICAL STEP: Place the labeled 2 ml centrifuge tubes on ice

v. **Using a** micropipette**, transfer 0.5 ml of centrifuged saliva into each labeled, pre-cooled, aliquot tube.** Take caution not to disturb the white pellet at the bottom of the tube by tilting the tube and placing the pipette tip along the lower side of the glass wall without touching the pellet so that saliva is not contaminated by pellet material (see below). This will yield, on average, 2-10 aliquot tubes per subject.



vi. Freeze samples immediately following processing by transferring to -80°C freezer. If samples cannot be immediately transferred to -80°C freezer, prepare a sufficient amount of dry ice for immediate freezing. Store all samples in -80°C freezer until you ship on dry ice (up to 6 saliva aliquots are to be shipped to Coriell although there may be fewer aliquots available if less than 3 ml saliva is collected; 0-4 saliva aliquots will remain at the collection site).

9 Lumbar Puncture

CSF should be collected in the morning between 8 am – 10 am, preferably fasted. If fasting is not feasible, the low fat diet should be followed (see Appendix P). Record time of last meal (and whether low fat diet followed, if applicable) on the Lumbar Puncture data form.

9.1 Lumbar Puncture Supplies

The lumbar puncture tray contains the following items which will be used to perform lumbar puncture. Check the dates of expiration: these reflect the expiration date of the lidocaine.
Supplies for collection of CSF are sent to sites in a separate kit from Coriell.

Lumbar Puncture Tray – FINAL VISIT/VISIT 02

Quantity	Kit Component
1	Sprotte Spinal Needle, 24G x 90 mm
1	Introducer needle, 1mm x 30mm
1	22G x 1.5 in. Needle
4	Plastic Syringes (5 ml, Luer Lock)
1	Plastic Syringe (3 ml, Luer Lock) with 25G x 5/8 in. Needle (Attached)
1	Needle stick pad
1	Adhesive bandage
1	Fenestrated Drape
2	Towels
6	Gauze Sponges
3	Sponge Applicators
1	Lidocaine HCl (1%), 5 ml
1	Povidone-Iodine Solution, 0.75 oz

1. LP Kits - Initial and Resupply

Each site will be initially supplied 4 LP trays. Subsequent trays should be ordered from Coriell when needed (see Section 4.3 for instructions to access Queue).

9.2 Setting up for the LP

On an overbed table, remove the contents of the LP kit from outer plastic packaging, leaving the contents wrapped in their sterile drape. Leave everything wrapped until the person performing the LP is seated, and begins examining the subject.

Feel the outside of the LP kit (still wrapped up) to determine which end contains the spongy swabs. Turn this end toward the person performing the LP and begin unwrapping the kit.

Touch only the outside of the paper wrapper**.** When you grab an edge to unfold it, touch only the folded under portions of the outside of the wrapper. Also, don't let the outside of the wrapper touch any part of the inside. If you touch any part of the inside of the paper wrapper, or if any non-sterile object or outside of the wrapper touches any part of the inside of the wrapper, throw the kit away and start over. If you are in doubt as to whether something touched the inside of the paper wrapper, throw the kit away and start over.

Maintaining the sterile field

Keep in mind that there is usually a lot of staff in the room during an LP, and a big part of assisting with the LP is keeping the field sterile, and keeping people away from it, and reminding people to be careful around it. If anybody touches the inside of the paper wrapper or any part of the contents of the kit, throw the kit away and start over. If you are in doubt as to whether someone touched the kit, throw it away and start over. Also, you are the monitor for whether the person performing the LP has broken sterility – usually by touching something not sterile with a sterile gloved hand. Feel free to be the boss of people if need be. Be assertive.

9.3 Tips for clinicians performing lumbar puncture

<u>Optimizing patient comfort and minimizing risk of adverse events.</u>

1. Talk the patient through the procedure - no surprises.

2. Use of a Sprotte 24G x 90 mm atraumatic spinal needle and careful technique are optimal for reducing post-LP headache risk. A pencil point spinal needle such as Spinocan, 24G x 90 mm is used.

3. Use adequate local anesthesia. Use the 22G x 1/2" needle and inject lidocaine to raise a skin wheal. Then inject lidocaine using the pattern of a square - first the center and then to all 4 corners. If the subject is thin, do not insert the deep infiltration needle OR the spinal introducer all the way. Use only about 2/3 of their length (to prevent entering the subarachnoid space with anything other than the 24G pencil point spinal needle).

4. Increasing fluid intake immediately after LP is helpful.

5. Be sure to give post-LP care instructions verbally to subject (see below).

9.4 Post-LP Care Instructions

1. Advise the subject to refrain from exertion (e.g., exercise, housework, gardening, lifting, sexual activity or any other strenuous activities) for 24 hours after the LP.

2. Advise the subject to continue with increased fluid intake.

Mild to Moderate headache after a lumbar puncture:

- Mild to moderate headache following lumbar puncture usually resolves within 3-4 days.
- Treatment of Mild to Moderate headache:
  - ➪ Limit physical activity as much as possible.
  - ➪ Oral fluids and caffeine are helpful. Drinking a can of Mountain Dew soft drink (for example) is preferable to coffee (which has some diuretic activity).
  - ➪ Tylenol should be used for symptomatic relief. If a subject cannot tolerate Tylenol, ibuprofen should be used. Avoid aspirin. If these do not relieve the headache, Tylenol with codeine or equivalent could be considered.

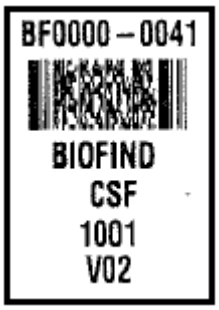
Severe headache after a lumbar puncture:

- If the headache becomes severe, posturally sensitive (relieved by supine posture), or is accompanied by nausea, vomiting, tinnitus and/or visual disturbances, the subject should contact the site study staff for further instruction per standard clinical care.

9.5 Detailed Lumbar Puncture Procedure

CSF is processed at Room Temperature [64^{0}F - 77^{0}F (18°C to 25°C)]. Also, a portion of the CSF must be sent to your clinical lab and analyzed within 4 hours of collection.

1. Place "CSF" label on the 2 ml microcentrifuge aliquot tubes (per section 6.1). Prepare at least 20 aliquot tubes (including 2 purple-top tubes for local lab analysis) based on the collection of 15-20 mls of CSF. Unlike the plasma aliquot tubes, the CSF aliquot tubes should remain at room temperature, not pre-cooled.



2. Perform lumbar puncture using the atraumatic technique.

3. Collect CSF into syringes (if a noticeably bloody tap, discard first 1-2 mls). After the LP has begun and fluid is being collected, take the first 2 mls of CSF from the first syringe and place in the CSF labs tubes (1 ml in each purple-top microcentrifuge tube), and send it to the local lab for routine diagnostic tests. Do not freeze this sample.

   ❖ Send at room temperature to local clinical lab for basic CSF analyses.
   NOTE: Sample must be analyzed within 4 hours of collection.

   1. **Cell count (erythrocytes first)**
   2. **Total protein**
   3. **Glucose**

4. Collect an additional 15-20 mls of CSF and transfer to 50 ml conical polypropylene tube; mix gently by inverting 3-4 times. Record time of draw (i.e., once collection is complete) on the Lumbar Puncture data form.

5. Within 15 minutes of collection, transfer the CSF from the 50 ml conical tube to the two 15 ml conical tubes ensuring that there is equal volume in each 15 ml conical tube. Spin the CSF sample at 2000 x g for 10 minutes at Room Temperature [64^0F - 77^0F (18°C to 25°C)].

- Equivalent rpm for spin at 2000 x g = _____
- While centrifuging record the time of centrifuge start on the Laboratory Procedures data form.

6. Using a micropipette, transfer 1.0 ml of supernatant directly into the polypropylene CSF collection aliquot tubes (clear-top, 2 ml microcentrifuge tubes). This will yield, on average, 15-20 aliquot tubes per subject.

7. Freeze samples immediately following processing by transferring to -80°C Freezer. If samples cannot be immediately transferred to -80°C Freezer, prepare a sufficient amount of dry ice for immediate freezing. Store all samples at -80°C Freezer until you ship on dry ice (10 CSF aliquots are to be shipped to Coriell, 0-8 CSF aliquots will remain at the collection site). Complete the remainder of the Laboratory Procedures data form and ensure timely entry of data into the eClinical database.

10 Packaging and Shipping Instructions: Biospecimen Shipments to Coriell

*****Important Note*****

Ambient shipments (yellow-top ACD tubes) must be received at Coriell within five days of collection. Up to three yellow-top ACD tubes can be shipped in one ambient shipping container.

For frozen shipments, include no more than two packing envelopes per shipping container in order to maintain space for a sufficient amount of dry ice to keep samples frozen up to 24 hours.

For V01 frozen shipments, include no more than two subject visit sample sets (plasma aliquots from two subjects) per shipping container. Include no more than one set of samples per packing envelope.

For V02 shipments, include only one subject visit sample set (PAXgene tubes, blood pellets, plasma, saliva, urine and CSF aliquots) per shipping container. V02 shipments require two packing envelopes per subject sample set.

10.1 Packing and Shipping: BASELINE VISIT/ VISIT 01

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT)**
- **Frozen 1ml aliquots of plasma (FROZEN SHIPMENT)**

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY! **Include no more than ONE set of samples per packing envelope.** **Include no more than TWO packing envelopes per shipping container.**
AMBIENT SAMPLES MAY BE SHIPPED MONDAY-FRIDAY (PREFERABLY MONDAY-THURSDAY) PROVIDED THEY ARE RECEIVED AT CORIELL WITHIN 5 DAYS OF COLLECTION

Sample Packaging and Shipment Instructions

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Coriell of shipment by emailing **ninds@coriell.org** (preferred) or faxing (856) 966-5067 a copy of the completed Sample Record Summary and Shipment Notification Form.

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT)**

3. Insert yellow top ACD tube in the foam-lined plastic box and close securely.

4. Place the plastic container into the clear plastic bag and seal. Enclose this sealed bag in the cardboard shipping container.

5. Apply the UN3373 label to the outside of the cardboard container.

6. Place the cardboard shipping container and the completed Sample Record Summary and Shipment Notification Form in the FedEx Clinical Pak, making sure the UN3373 label is visible through the Clinical Pak, and seal according to the instructions on the envelope.

7. Complete the "From" portion of the provided FedEx air waybill by filling in your name, address and phone number. FedEx is likely to reject or return your shipment without this information.

8. Apply completed FedEx air waybill to outside of package and arrange for FedEx pick up.

9. Ship the sample to Coriell on the day of collection. If sample cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. Sample must be received at Coriell within 5 days of collection.

- **Frozen 1ml aliquots of plasma (FROZEN SHIPMENT)**

10. Place all frozen 1ml aliquots in the provided clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

11. Place the envelope upright in the provided Styrofoam-lined shipping carton, as shown below:

Up to two envelopes containing samples from one subject each may be shipped in one container if necessary. Please do not include more than two envelopes within the container as this will diminish the space needed for sufficient dry ice. Please do not include more than one subject set of samples per packing envelope.

12. **<u>FILL</u> the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds the envelope(s) and reaches the <u>top</u> of the carton, as shown below:**

13. Place the completed Sample Record Summary and Shipment Notification Form in the package, replace the lid on the Styrofoam carton, and close and seal the outer cardboard shipping carton with packing tape.

IMPORTANT!
Complete the required fields on the FedEx air waybill and Class 9 Dry Ice label, or FedEx may reject or return your package.

14. Complete the FedEx air waybill with the following info-
    a. Section 1, "From": fill in your name, address and phone number
    b. Section 6, "Special Handling and Delivery Signature Options": under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) x the net weight of dry ice in kg.

15. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: Coriell Institute, 403 Haddon Ave, Camden, NJ 08103
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

16. Apply all provided warning labels and the completed FedEx air waybill to outside of package, taking care not to overlap labels.

17. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.

10.2 Packaging and Shipping: Final Visit/Visit 02 (FROZEN SHIPMENT)

- **Frozen 1ml aliquots of plasma and CSF**
- **Frozen blood pellets in EDTA tubes**
- **Frozen PAXgene tubes**
- **Frozen 0.5ml aliquots of saliva**
- **Frozen urine in 15 ml conical tubes**

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY! **Only ONE set of samples may be shipped in a single package.**

Sample Packaging and Shipment Instructions

1. Contact FedEx to confirm service is available and schedule package to be picked up.

2. Notify Coriell of shipment by emailing **ninds@coriell.org** (preferred) or faxing (856-966-5067) a copy of the completed Sample Record Summary and Shipment Notification Form.

3. Place all frozen 1 ml aliquots of plasma and CSF and 0.5 ml aliquots of saliva in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID (four digit number on specimen labels).

4. Place the cryobox in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

5. Insert PAXgene tubes, EDTA tubes (containing blood pellets) and 15 ml conical tubes (containing urine) into the bubble wrap pouch provided.

6. Place bubble-wrapped tubes into the 2nd clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.

7. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:

8. **FILL the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds both envelopes and reaches the top of the carton, as shown below:**

9. Place the completed Sample Record Summary and Shipment Notification Form in the package, replace the lid on the Styrofoam carton, and close and seal the outer cardboard shipping carton with packing tape.

IMPORTANT!
Complete the required fields on the FedEx air waybill and Class 9 Dry Ice label, or FedEx may reject or return your package.

10. Complete the FedEx air waybill with the following info-
    a. Section 1, "From": fill in your name, address and phone number
    b. Section 6, "Special Handling and Delivery Signature Options": under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) x the net weight of dry ice in kg.

11. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following info-
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: Coriell Institute, 403 Haddon Ave, Camden, NJ 08103
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

12. Apply all provided warning labels and the completed FedEx air waybill to outside of package, taking care not to overlap labels.

13. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.

11 Shipping and Tracking Instructions

SHIP ALL FROZEN SAMPLES MONDAY - WEDNESDAY ONLY! AMBIENT SAMPLES MAY BE SHIPPED MONDAY-FRIDAY (PREFERABLY MONDAY-THURSDAY) PROVIDED THEY ARE RECEIVED AT CORIELL WITHIN 5 DAYS OF COLLECTION.
BE AWARE OF HOLIDAYS!!

Frozen samples must be shipped within two weeks of sample collection; if frozen samples are not shipped immediately please ensure adequate storage at -80°C prior to shipment.

Remember to complete the Sample Record Summary and Shipment Notification (Appendix B), include a copy in your shipment AND notify Coriell IN ADVANCE to confirm the shipment.

1. Complete the Sample Record Summary and Shipment Notification form. Only one specimen type per row should be listed. Multiple sample types may be included on this form.
2. Once completed, package the samples in the return box and include a copy of the Sample Record Summary and Shipment Notification form in the package.
3. Provide copy of Shipment Notification to Coriell via email (ninds@coriell.org) or fax (856-966-5067) to give advance notice that you are sending samples. Ensure tracking number is indicated.
4. Ship samples via FedEx Priority Overnight. Frozen samples packed on dry ice should be held in a -80°C freezer until the time of FedEx pickup.

12 Sample Quality Checks and Feedback to Sites

In addition to tracking and reconciliation of samples, the condition and amount of samples received is tracked by Coriell for each sample type. Sites are responsible to ensure the requested amounts of each fluid are collected to the best of their ability and that samples are packed with sufficient amounts of dry ice to avoid thawing in the shipment process. Coriell will complete a Non-Conformance Report (Appendix Q) should there be any issues with a shipment and will provide this feedback to the site. Issues of concern that may impact collection, processing or future analyses of the samples will be addressed by the BioFIND Steering Committee and communicated to sites.

13 Data Queries and Reconciliation

The Laboratory Procedures and Lumbar Puncture source worksheets must be completed on the day that samples are collected since they capture information related to the details of the sample collection and processing. These forms include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses. All data should be recorded on the DNA Sample, Laboratory Procedures, LP, Saliva and Urine Samples worksheets and entered into the eClinical database within 2 days of the visit date.

The Clinical Trials Coordination Center (CTCC) will be collaborating with Coriell to reconcile information captured in the EDC database compared to samples received and logged at Coriell. Information that appears incorrect in the EDC database will be queried through the standard Query Management system. Additional discrepancies that may be unrelated to data entry will be resolved with sites in a separate follow up communication.

Data queries or discrepancies with samples shipped versus received at Coriell may result from:

- Missing samples at Coriell
- Incorrect samples collected and shipped to Coriell
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Sample Record Summary and Shipment Notification Form and logged at Coriell compared to information entered into EDC

14 Appendices

Appendix A: Rate of Centrifugation Worksheet

Appendix B: Sample Record Summary and Shipment Notification Form

Appendix C: Coriell Detailed Domestic Shipping Instructions: Baseline Visit/Visit 01

Appendix D: Coriell Detailed Domestic Shipping Instructions: Final Visit/Visit 02

Appendix E: 2.7 ml Light-blue top sodium citrate for PT/PTT Processing Diagram

Appendix F: 10 ml Lavender top EDTA for CBC, platelet Processing Diagram

Appendix G: 10 ml Lavender top EDTA for Plasma and Pellet Processing Diagram

Appendix H: 8.5 ml Yellow top ACD for DNA Processing Diagram

Appendix I: PAXgene™ RNA Processing Diagram

Appendix J: Urine Collection Diagram

Appendix K: Saliva Collection: Protease Inhibitor Cocktail Preparation

Appendix L: Saliva Collection Diagram

Appendix M: CSF Preparation Diagram

Appendix N: Aliquot Tube Label Diagram

Appendix O: BioFIND Lab Worksheet and Data Record for Visit 01 and Visit 02

Appendix P: BioFIND Low Fat Diet Menu Suggestions

Appendix Q: BioFIND Sample Submission Non-Conformance Report

Appendix R: BioFIND Lab Sample Collection and Processing Summary – Baseline Visit/Visit 01

Appendix S: BioFIND Lab Sample Collection and Processing Summary – Final Visit/Visit 02

Appendix A

Rate of Centrifugation Worksheet

Please complete and return this form (by email or fax) to the BIOFIND Project Manager if you have any questions regarding sample processing. The correct RPM will be sent back to you. Make note of this in your BIOFIND Biologics Manual.

Submitter Information

Name:

Site Number:

Submitter Email:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type:

☐ Fixed Angle Rotor
☐ Swing Bucket Rotor

Radius of Rotation (mm):

Determine centrifuge's radius of rotation (in mm) by measuring distance from center of centrifuge spindle to bottom of device when inserted into rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Comments

Fax this form to:
BIOFIND Project Manager at
585-461-3554
Or
Email to
alice.rudolph@chet.rochester.edu

It is very important to this study that all samples be processed correctly.

Please call with any questions at 585-275-0556

Appendix B

CORIELL INSTITUTE FOR MEDICAL RESEARCH

BioFIND Sample Record Summary and Shipment Notification

Site Name/Number: **Principal Investigator:**

Coordinator: **Telephone:** **Email:**

Please list only ONE subject per Sample Record Summary and Shipment Notification Form

Subject ID Number: **Diagnosis:**

Gender: **Visit Type (Baseline Visit/V01 or Final Visit/V02):**

Date Sample(s) Shipped: **FedEx Tracking Number:**

Instructions: Ship Frozen Shipments Monday – Wednesday ONLY! Ambient Shipments (yellow-top ACD tube) may be shipped Monday – Friday (preferably Monday – Thursday) provided they are received at Coriell within five days of collection. This form must be completed for shipment of all research samples. Notify Coriell (email preferred) in advance of shipment using contact information below. Place a copy in the shipment box and file a copy of the completed form in the study binder. Site will be contacted should there be issues with samples noted upon receipt or shipment did not include this form. **Ensure all frozen shipments are filled with dry ice.**

In the table below, please indicate the date of specimen collection, and the tube ID (e.g. BF0000-0001)

Completed by Submitter/Site			Completed by Repository
Date of Draw	Specimen Type	Tube ID Number (BF#)	Notation of Problems
	DNA		
	Plasma		
	RNA		
	Pellet		
	CSF		

Contact Information: Coriell Institute for Medical Research: Email: ninds@coriell.org **Fax:** 856-966-5067 **Ph:** 856-757-9742

Form 1407-11 Rev A-091514

Appendix B

BioFIND Sample Record Summary and Shipment Notification (continued)

Please list the SAME subject as the previous page

Subject ID Number: **Diagnosis:**

Gender: **Visit Type (Baseline Visit/V01 or Final Visit/V02):**

In the table below, please indicate the date of specimen collection, and the tube ID (e.g. BF0000-0001)

Completed by Submitter/Site			Completed by Repository
Date of Draw	Specimen Type	Tube ID Number (BF#)	Notation of Problems
	Urine		
	Saliva		

Contact Information: Coriell Institute for Medical Research; Email: ninds@coriell.org Fax: 856-966-5067 Ph: 856-757-9742

Form 1407-11 Rev A-091514

Appendix C

NINDS BioFIND SITES: Baseline Visit/Visit 01 Sample Packaging and Shipment to Coriell

BASELINE VISIT/ VISIT 01

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT)**
- **Frozen 1ml aliquots of plasma (FROZEN SHIPMENT)**

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY! **Include no more than ONE set of samples per packing envelope.** **Include no more than TWO packing envelopes per shipping container.**
AMBIENT SAMPLES MAY BE SHIPPED MONDAY-FRIDAY (PREFERABLY MONDAY-THURSDAY) PROVIDED THEY ARE RECEIVED AT CORIELL WITHIN 5 DAYS OF COLLECTION

Sample Packaging and Shipment Instructions

1. Contact FedEx to confirm service is available and schedule package to be picked up.
2. Notify Coriell of shipment by emailing ninds@coriell.org (preferred) or faxing (856) 966-5067 a copy of the completed Sample Record Summary and Shipment Notification Form.

- **DNA Yellow Top ACD Blood Tube (AMBIENT SHIPMENT)**

3. Insert yellow top ACD tube in the foam-lined plastic box and close securely.
4. Place the plastic container into the clear plastic bag and seal. Enclose this sealed bag in the cardboard shipping container.
5. Apply the UN3373 label to the outside of the cardboard container.
6. Place the cardboard shipping container and the completed Sample Record Summary and Shipment Notification Form in the FedEx Clinical Pak, making sure the UN3373 label is visible through the Clinical Pak, and seal according to the instructions on the envelope.
7. Complete the "From" portion of the provided FedEx air waybill by filling in your name, address and phone number. FedEx is likely to reject or return your shipment without this information.
8. Apply completed FedEx air waybill to outside of package and arrange for FedEx pick up.
9. Ship the sample to Coriell on the day of collection. If sample cannot be shipped the same day as collected, hold at room temperature until shipping can be arranged. Sample must be received at Coriell within 5 days of collection.

Appendix C

CORIELL INSTITUTE FOR MEDICAL RESEARCH

NINDS BioFIND Baseline Visit/Visit 01 Shipping Instructions / Domestic

- **Frozen 1ml aliquots of plasma (FROZEN SHIPMENT)**

10. Place all frozen 1ml aliquots in the provided clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.

11. Place the envelope upright in the provided Styrofoam-lined shipping carton, as shown below:

Up to two envelopes containing samples from two subjects may be shipped in one container if necessary. Please do not include more than two envelopes within the container as this will diminish the space needed for sufficient dry ice. Please do not include more than one subject set of samples per packing envelope.

12. **FILL the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds the envelope and reaches the top of the carton, as shown below:**

13. Place the completed Sample Record Summary and Shipment Notification Form in the package, replace the lid on the Styrofoam carton, and close and seal the outer cardboard shipping carton with packing tape.

Appendix C

IMPORTANT!
Complete the required fields on the FedEx air waybill and Class 9 Dry Ice label, or FedEx may reject or return your package.

14. Complete the FedEx air waybill with the following info-
    a. Section 1, "From": fill in your name, address and phone number
    b. Section 6, "Special Handling and Delivery Signature Options": under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) x the net weight of dry ice in kg.

15. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following information:
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: Coriell Institute, 403 Haddon Ave, Camden, NJ 08103
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

16. Apply all provided warning labels and the completed FedEx air waybill to outside of package, taking care not to overlap labels.

17. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.

APPENDIX D

CORIELL INSTITUTE FOR MEDICAL RESEARCH

NINDS BioFIND Final Visit/Visit 02 Shipping Instructions / Domestic

NINDS BioFIND SITES: FINAL VISIT/VISIT 02 PACKAGING AND SHIPMENT TO CORIELL

Final Visit/Visit 02 (FROZEN SHIPMENT)

- **Frozen 1ml aliquots of plasma and CSF**
- **Frozen blood pellets in EDTA tubes**
- **Frozen PAXgene tubes**
- **Frozen 0.5ml aliquots of saliva**
- **Frozen urine in 15 ml conical tubes**

IMPORTANT!
FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY! **Only ONE set of samples may be shipped in a single package.**

Sample Packaging and Shipment Instructions

1. Contact FedEx to confirm service is available and schedule package to be picked up.
2. Notify Coriell of shipment by emailing ninds@coriell.org (preferred) or faxing (856-966-5067) a copy of the completed Sample Record Summary and Shipment Notification Form.
3. Place all frozen aliquots of plasma and CSF and saliva in the provided cardboard cryobox. Label the outside of the cryobox with the subject ID (four digit number on specimen labels).
4. Place the cryobox in the clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the white Tyvek biohazard envelope and seal according to the instructions on the envelope.
5. Insert PAXgene tubes, Urine tubes and EDTA tubes (containing blood pellets) into the bubble wrap pouch provided.
6. Place bubble-wrapped tubes into the 2nd clear plastic biohazard bag (do NOT remove the absorbent material found in the bag) and seal according to the instructions on the bag. Insert this into the 2nd white Tyvek biohazard envelope and seal according to the instructions on the envelope.
7. Place both envelopes upright and side-by-side in the provided Styrofoam-lined shipping carton, as shown below:

APPENDIX D

CORIELL INSTITUTE FOR MEDICAL RESEARCH

NINDS BioFIND Final Visit/Visit 02 Shipping Instructions / Domestic

8. **FILL the remaining space in the shipping carton with approximately 10 lbs of dry ice, ensuring ice surrounds both envelopes and reaches the top of the carton, as shown below:**

9. Place the completed Sample Record Summary and Shipment Notification Form in the package, replace the lid on the Styrofoam carton, and close and seal the outer cardboard shipping carton with packing tape.

IMPORTANT!
Complete the required fields on the FedEx air waybill and Class 9 Dry Ice label, or FedEx may reject or return your package.

10. Complete the FedEx air waybill with the following info-
    a. Section 1, "From": fill in your name, address and phone number
    b. Section 6, "Special Handling and Delivery Signature Options": under "Does this shipment contain dangerous goods?" check the boxes for "Yes, Shipper's Declaration not required" and "Dry Ice". Enter the number of packages (1) x the net weight of dry ice in kg.

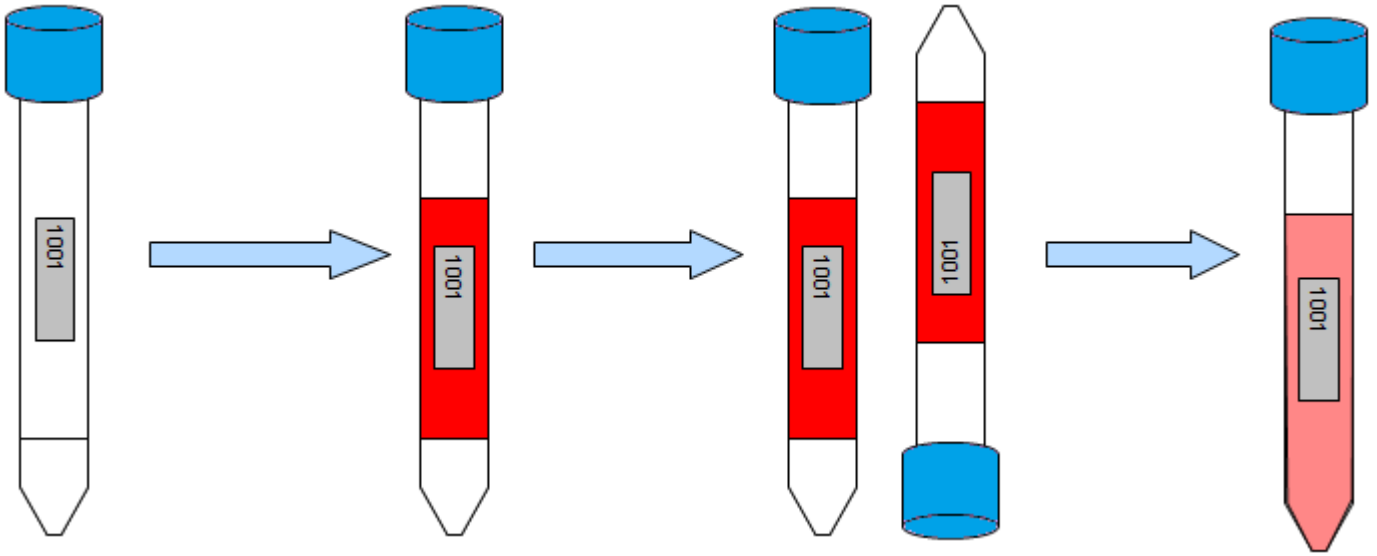
11. Complete the Class 9 UN 1845 Dry Ice label (black and white diamond) with the following info-
    a. Your name and return address
    b. Net weight of dry ice in kg
    c. Consignee name and address: Coriell Institute, 403 Haddon Ave, Camden, NJ 08103
    d. Do not cover any part of this label with other stickers, including pre-printed address labels.

12. Apply all provided warning labels and the completed FedEx air waybill to outside of package, taking care not to overlap labels.

13. Hold packaged samples in -80°C freezer until time of FedEx pick-up/drop-off.

APPENDIX E

2.7ml Light-blue Top Sodium citrate Tube for PT/PTT Analysis



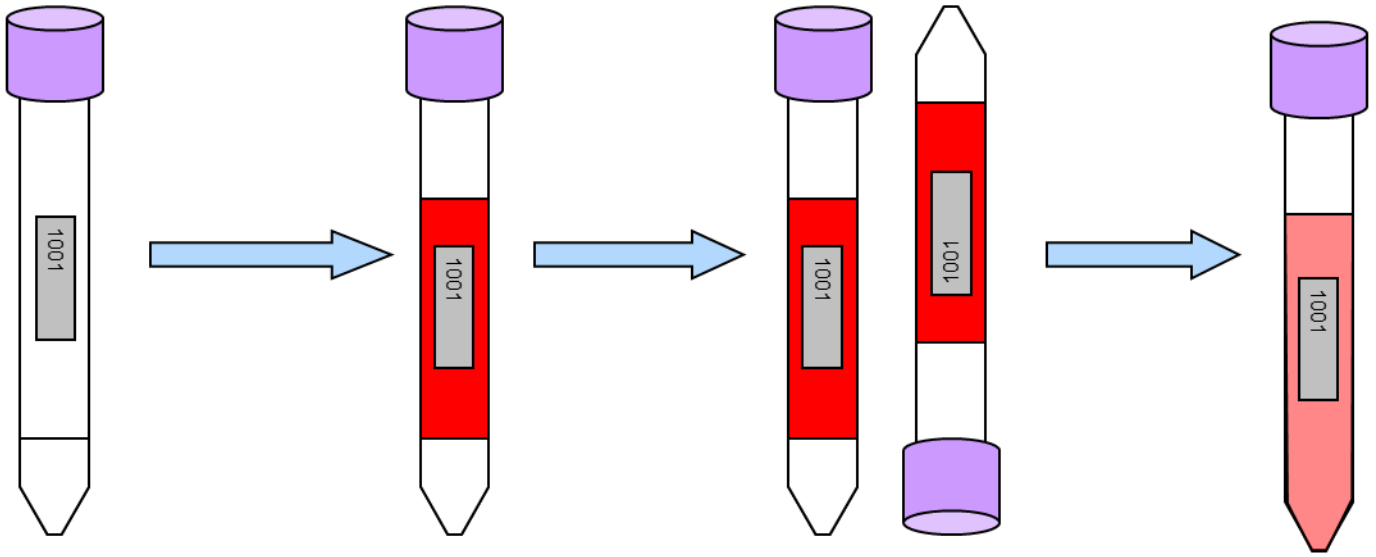
1: Store tube at room temperature. Label according to site format (no BioFIND label).

2: Collect blood, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

3: Immediately after blood draw, **invert tube 3-4 times** to mix sample.

4: Immediately after inversion, prepare sample for shipment to local laboratory.

10ml Lavender Top EDTA Tube for CBC, Platelet Analysis



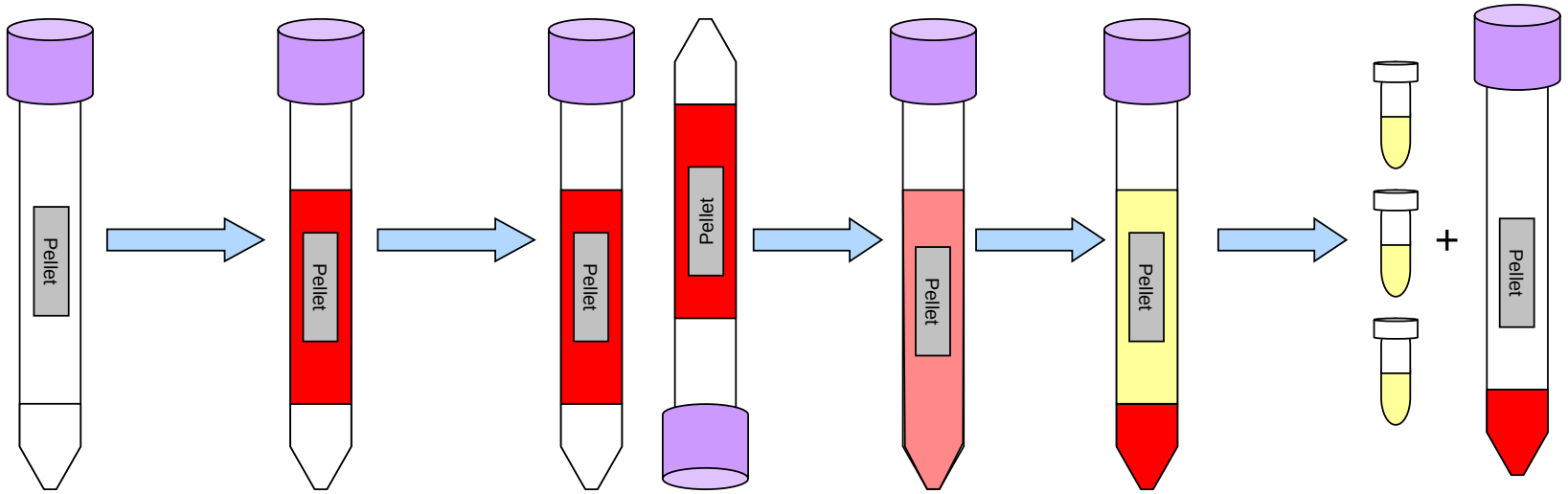
1: Store tube at room temperature. Label according to site format (no BioFIND label).

2: Collect blood, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

3: Immediately after blood draw, **invert tube 8-10 times** to mix sample.

4: Immediately after inversion, prepare sample for shipment to local laboratory.

10ml Lavender Top EDTA Tube for Plasma and Pellet



1: Store tubes at room temperature, label with pre-printed "Pellet" labels prior to blood draw.

2: Collect blood in "Pellet" Tube, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

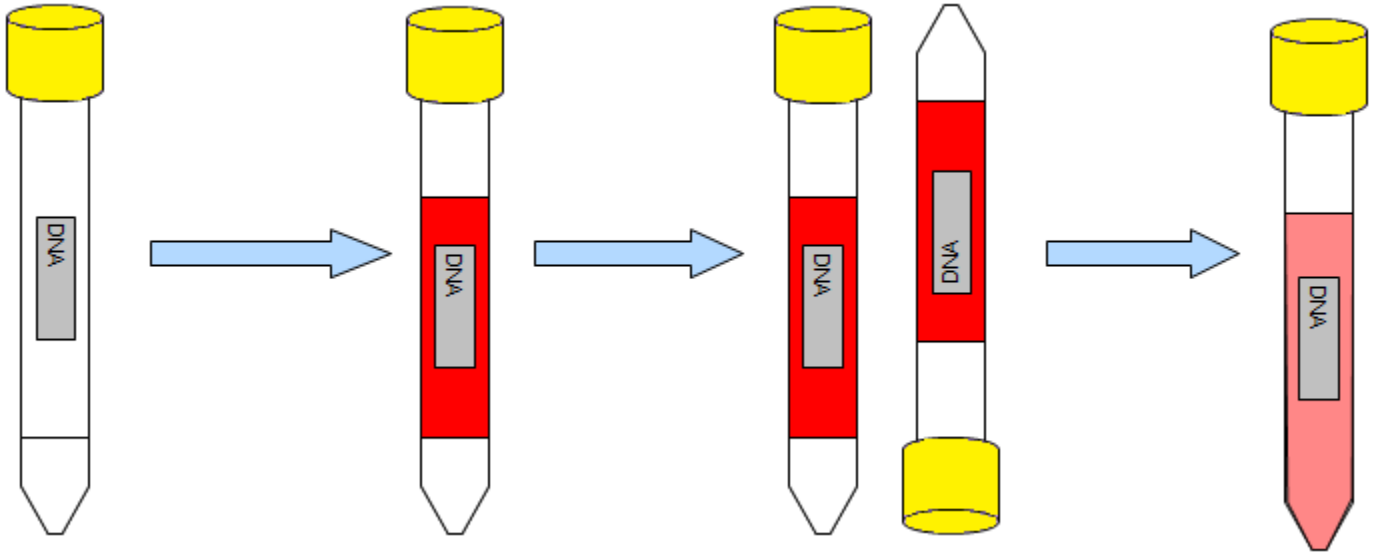
3: Immediately after blood draw**, invert tubes 8-10 times** to mix samples.

4: Within 30 minutes of blood draw, centrifuge samples at 4^0C, 1500 x g for 15 minutes.

5: Label micro centrifuge tubes with preprinted "Plasma" labels. Use pipette to aliquot 1 ml samples of plasma. Store plasma aliquots frozen at -80^0C until shipment.

APPENDIX H

8.5ml Yellow Top ACD Tube for DNA Extraction



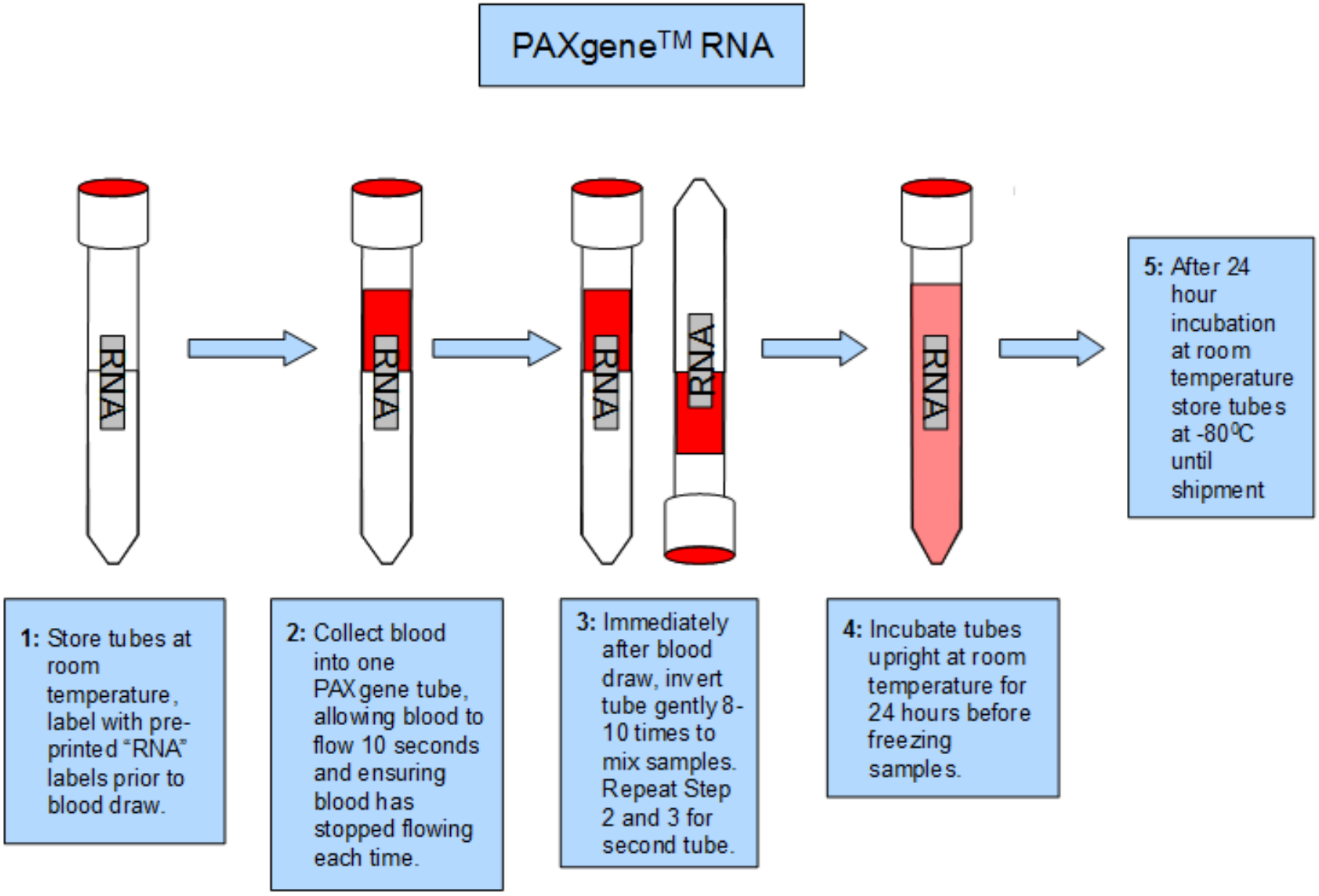
1: Store tube at room temperature, label with pre-printed "DNA" label prior to blood draw.

2: Collect blood, allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

3: Immediately after blood draw, **invert tube 8-10 times** to mix sample.

4: Immediately after inversion, insert into shipping container. Ship ambient to Coriell.

APPENDIX I

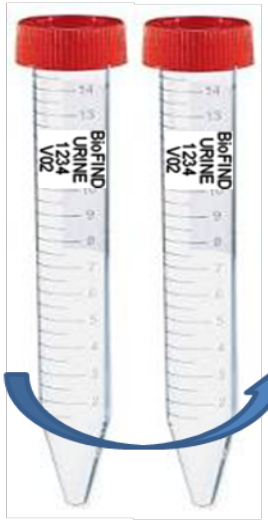

PAXgene™ RNA
RNA
RNA
RNA
RNA
RNA
5: After 24 hour incubation at room temperature store tubes at -80°C until shipment
1: Store tubes at room temperature, label with pre-printed "RNA" labels prior to blood draw.
2: Collect blood into one PAXgene tube, allowing blood to flow 10 seconds and ensuring blood has stopped flowing each time.
3: Immediately after blood draw, invert tube gently 8-10 times to mix samples. Repeat Step 2 and 3 for second tube.
4: Incubate tubes upright at room temperature for 24 hours before freezing samples.

APPENDIX J

URINE COLLECTION

1: Collect urine into the 50 ml collecting cup

2: Pour 10 ml urine to the 15 ml conical tube



3: Within 30 minutes of collection, centrifuge samples at 4^{0}C, 2500 x g for 15 minutes.

4. Transfer 10 ml to pre-labeled 15-ml tubes

5. Freeze on dry ice

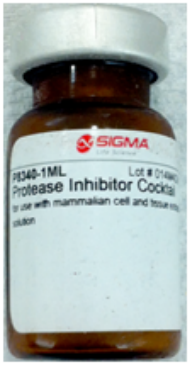
6. Store urine aliquots frozen at -80^{0}C Keep at site

7. Store urine aliquots frozen at -80^{0}C until shipment

APPENDIX K

Saliva Collection - PIC Preparation (prior to scheduled visit)

Upon receipt
100X PIC



1: Store 1-ml 100X PIC according to manufacturer recommendation

Thaw at room temp on bench top when aliquoting

10 Aliquots
100X PIC
(100 µl ea)

2: Aliquot 100 µl into each 1.5 ml microcentrifuge tube

Label "100x PIC" with permanent marker on the tube and date

3: Store PIC aliquots at -20°C

At day of visit
100X PIC
(thaw at RT)

4: At day of visit, remove one 100X PIC aliquot from -20°C
Thaw at room temperature

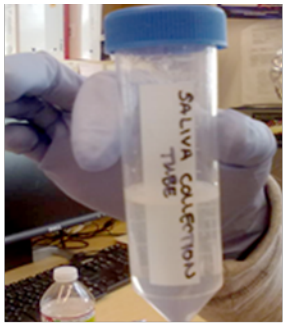
Dilute to
10X PIC

At day of visit
Dilute to 10X PIC
(1 ml after dilution)

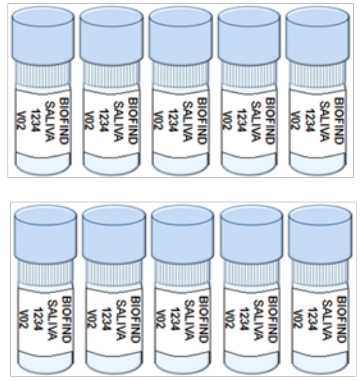
5: Dilute to 10X by adding 900 µl sterile ddH_2O. Invert few times to mix. Keep at room temp until ready for use

APPENDIX L

SALIVA COLLECTION



+



Volume of saliva transferred to a 15 ml tube	Volume of 10X PIC to add to sample
1.0 ml	100 µl (0.1 ml)
1.5 ml	150 µl (0.15 ml)
2.0 ml	200 µl (0.2 ml)
5.0 ml	500 µl (0.5 ml)
10.0 ml	Entire 1 ml of 10X PIC

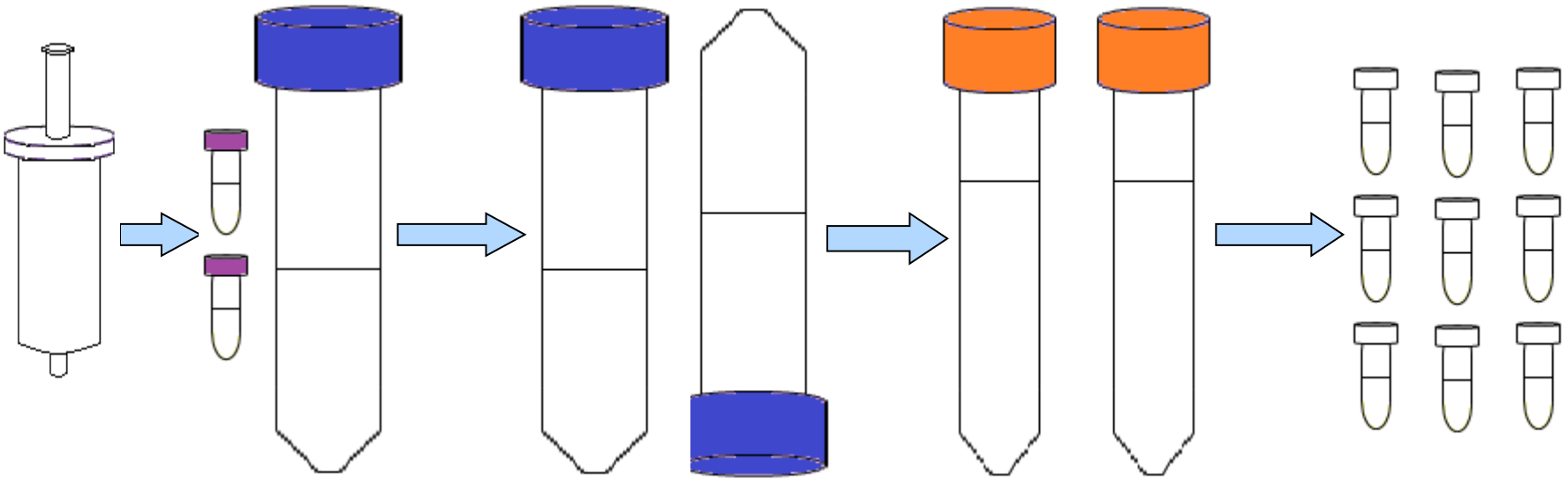
3: Within 30 minutes of collection, centrifuge samples at 4^0C, 2000 x g for 15 minutes

4: Label the 2 ml microcentrifuge tubes with pre-printed label "Saliva". Place the labeled tubes on ice

5. Aliquot 0.5 ml of centrifuged saliva to each 2 ml microcentrifuge tube. Store saliva aliquots frozen at -80^0C until shipment.

APPENDIX M

CSF Preparation



1: Collect CSF in syringes supplied in LP Tray Kit. Transfer first 2 mls into purple-top microcentrifuge tubes for lab analysis. Transfer remaining CSF into 50 ml conical tube

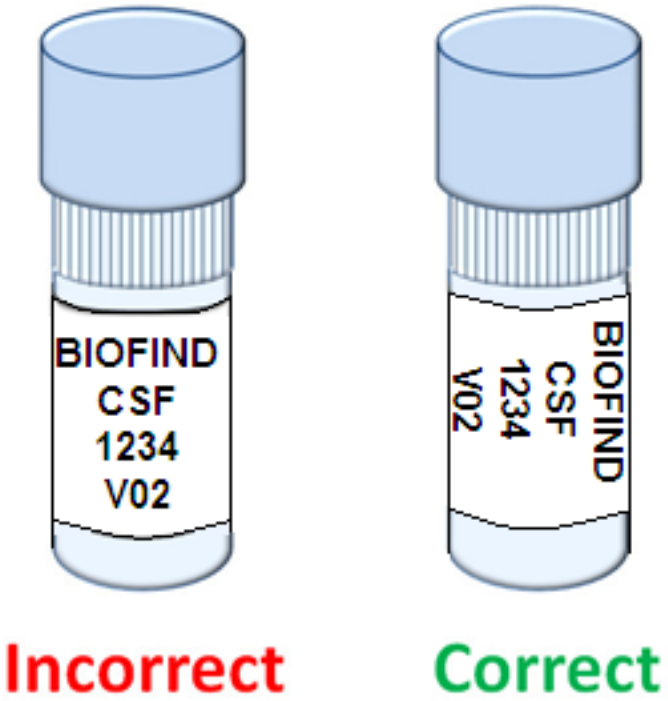
2: Gently invert 50 ml conical tube 3-4 times.

3: **Within 15 minutes of CSF collection**, transfer total CSF from 50 ml conical tube into two 15 ml conical tubes, ensuring that there is an equal volume in each tube. Centrifuge tubes at room temperature, 2000 x g for 10 minutes.

4: Label microcentrifuge tubes with preprinted "CSF" labels. Use micropipette to aliquot 1 ml samples of CSF. Freeze immediately; store CSF aliquots at -80 ^{0}C until shipment.

APPENDIX N

ALIQUOT TUBE LABELING DIAGRAM



BioFIND Lab Worksheet

Subject ID: _____ Visit: _____ Date: _____

Blood Draw Order

Visit 01

1) **PT/PTT**: Light blue top sodium citrate tube
2) **CBC, platelets**: Lavender top EDTA tube
3) **Plasma/Pellet:** Lavender top EDTA tube
4) **DNA**: Yellow top ACD tube

Sample Collection Flow Chart – Visit 01

<table>
<tr><td colspan="5">Visit 01</td></tr>
<tr><td>Sample</td><td colspan="4">0 min 15min 30min 24 hour</td></tr>
<tr><td>PT/PTT
Whole-blood: Light blue top sodium citrate tube</td><td colspan="4">Ship ambient to local lab</td></tr>
<tr><td>CBC, platelets
Whole-blood: Lavender top EDTA tube</td><td colspan="4">Ship ambient to local lab</td></tr>
<tr><td rowspan="3">Plasma/Pellet
Whole-blood: Lavender top EDTA tube</td><td colspan="2">Centrifuge within 30 min. of collection</td><td colspan="2"></td></tr>
<tr><td>. . . .</td><td>Aliquot</td><td>Freeze at -80°C</td><td></td></tr>
<tr><td colspan="4">1500g ×15 min. 4 °C, transfer plasma to aliquot tubes, retain pellet; freeze immediately at -80°C</td></tr>
<tr><td>DNA
Whole-blood: Yellow top ACD tube</td><td colspan="4">Ship ambient to Coriell; must be received within 5 days of collection</td></tr>
</table>

BioFIND Lab Worksheet

Subject ID: _____ Visit: _____ Date: _____

Sample Collection and Blood Draw Order

Visit 02

2) Saliva
3) Urine
4) **RNA**: PAXgene™ tubes (x3)
5) **Plasma/Pellet**: Lavender top EDTA tubes (x3)
6) **CSF**: Lumbar Puncture

Sample Collection Flow Chart – Visit 02

<table>
<tr><td colspan="5">Visit 02</td></tr>
<tr><td>Sample</td><td colspan="4">0 min 15min 30min 24 hour</td></tr>
<tr><td rowspan="2">Saliva</td><td colspan="2">Add PIC immediately, centrifuge within 30 min. of collection</td><td>Aliquot</td><td>Freeze at -80°C</td></tr>
<tr><td colspan="4">2000g x 15 min. 4°C, transfer saliva to aliquot tubes; freeze immediately at -80°C</td></tr>
<tr><td>Urine</td><td colspan="4">2500g x 15 min. 4°C, transfer urine to new 15-ml tubes; freeze immediately at -80°C</td></tr>
<tr><td>RNA
Whole-blood: PAXgene tubes</td><td colspan="3">…….
Sit ambient 24 hrs (at minimum >2 hrs)</td><td>Freeze at -80°C</td></tr>
<tr><td rowspan="3">Plasma/Pellet
Whole-blood: Lavender top EDTA tubes</td><td colspan="2">Centrifuge within 30 min. of collection</td><td colspan="2"></td></tr>
<tr><td>. . . .</td><td>Aliquot</td><td>Freeze at -80°C</td><td></td></tr>
<tr><td colspan="4">1500g ×15min 4 °C, transfer plasma to aliquot tubes, retain pellets; freeze immediately at -80°C</td></tr>
<tr><td rowspan="4">CSF
Lumbar Puncture</td><td colspan="4">Transfer initial 2 mls to purple-top aliquot tubes; ship ambient to local lab</td></tr>
<tr><td colspan="2">Centrifuge within 15 min. of collection</td><td colspan="2"></td></tr>
<tr><td>. . . .</td><td>Aliquot</td><td>Freeze at -80°C</td><td></td></tr>
<tr><td colspan="4">2000g x 10 min. 18-25 °C, transfer CSF to aliquot tubes; freeze immediately at -80°C</td></tr>
</table>

2. *Data Recording*

Food ***Intake, Date*** _____ ***Pre-Lab Time*** _____ ***Pre-LP Time***_____ ***Low-Fat Diet Menu*** Yes / No / Fasted
PD Med **Last** ***Intake, Date*** _____ ***Time*** _____

Sample	Time at Collection	Volume Obtained (mL)	Time at Centrifugation	Time at Aliquoting	No. of Aliquots	Time at Freezing		Freezing Method	
								Dry Ice	-80°C Freezer
Visit 01									
PT/PTT						*Ambient*			
CBC/platelets						*Ambient*			
Plasma/Pellet									
DNA						*Ambient*			
Visit 02									
RNA						Date	Time		
Plasma/Pellet									
SALIVA									
URINE									
CSF									

APPENDIX P

Low-Fat Diet Menu Suggestions

Due to the interference of lipid content in blood specimens collected for biomarker evaluation in the BIOFIND study, it is **strongly advised that samples be collected after an 8 hour fast (no food or drink except fluids such as water, tea, black coffee)**. If fasting is not achievable, a subject should be on a low-fat diet for at least 8 hours prior to blood collection.

Below is a list of suggested sample menus that could be consumed prior to blood collection. These lists are not all inclusive and sites should use their best judgment in this process.

Foods that are allowed prior to blood collection:

Sample Breakfast Items:

Dry whole-wheat toast
Fruit salad – no dressing
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Dry cereal – (without nuts/ no granola; no milk)
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Plain oatmeal or other cooked whole grain cereal
Topped with fresh or dried fruit (no butter, milk or cream)
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Dry whole-wheat toast
Poached egg-whites or egg substitute
Clear tea or coffee (no milk or cream)
Fruit or vegetable juice

Sample Lunch Items:

Turkey breast sandwich on whole wheat bread
Lettuce and Tomato and Mustard
Clear beverage
Flavored gelatin

Plain pasta with plain marinara sauce– no butter or cheese
Side of steamed vegetables or green salad
Clear beverage
Flavored gelatin

Steamed chicken breast (lean, without skin)
Side of steamed vegetables or green salad
Clear beverage
Flavored gelatin

Large tossed green salad with assorted vegetables (no dressing or cheese)
Clear beverage
Flavored gelatin

Cucumber sandwich on whole-wheat bread
Lettuce, tomatoes, shredded carrots, onions or other vegetables
Clear beverage
Flavored gelatin

Clear broth with vegetables and pasta
Fruit salad – no dressing
Clear beverage
Flavored gelatin

Low-Fat Diet Menu Suggestions (continued)

Foods to avoid prior to blood collection:

Avoid: All fats and nuts such as:

- Butter
- Cream
- Bacon fat
- Lard
- All oils
- All margarine
- All nuts
- Peanut butter
- Coconut
- Whole seeds such as pumpkin and sunflower

Avoid: All milk and dairy products such as:

- All whole milk products
- All cheeses
- All products containing cheese
- Cheeses spreads such as cream cheese
- Sour cream
- All ice cream
- Milk chocolate

Avoid: High fat prepared foods and foods naturally high in fat:

- All red meats or meats containing fat such as pork
- Fatty meats such as:
    - Luncheon meats
    - Organ meats
    - Bacon
- Fatty fish such as:
    - Salmon
    - Mackerel
- Salad dressing and mayonnaise
- Buttered, au gratin, creamed or fried vegetables.
- Fried foods
- Fried snacks such as:
    - Chips
    - Crackers
    - French fries
- Gravies and sauces
- Baked goods & frosting

APPENDIX Q

CORIELL INSTITUTE FOR MEDICAL RESEARCH

NINDS Biomarker Sample Submission Non-Conformance Report

This form is to be completed by the NINDS Repository personnel when a sample has been received and issues are noted. Completed form is to be emailed or faxed to submission site coordinators and Coordination Centers.

Site #/Name: _____

Subject ID: _____ Visit Type: _____

Received by: _____ Date: _____

Your shipment was received with the observed problem(s) checked below. Please take note so that your future shipments are received without incident.

	Ambient temperature samples (e.g. Yellow Top ACD blood tube for DNA extraction) shipped on Friday or Saturday (domestic sites only)
	Ambient temperature samples (e.g. Yellow Top ACD blood tube for DNA extraction) received after 5-days of collection (domestic and international sites)
	Low volume (< 4ml) in Yellow Top ACD blood tube
	Frozen samples shipped on Thursday, Friday or Saturday (domestic sites only)
	Frozen samples arrived on Saturday or Sunday (International sites only)
	Advanced notice of shipment not provided
	Shipment notification does not match Shipment Notification form received with samples
	No Shipment Notification form included in package
	Shipment Notification form incomplete
	Package contents do not match Shipment Notification form
	Package received has little/no dry ice
	Signs of sample thawing present
	Samples submitted in non-standard tubes
	Sample tubes damaged/cracked
	Samples not labeled appropriately/labels peeling off
	CSF/SER/PL samples pink in color
	Unexpected sample(s) received (specify in Comments section below)
	Other (specify in Comments section below)

Comments:

_____

_____

This form is ( ) emailed ( ) faxed to _____ **on** _____
Name **Date**

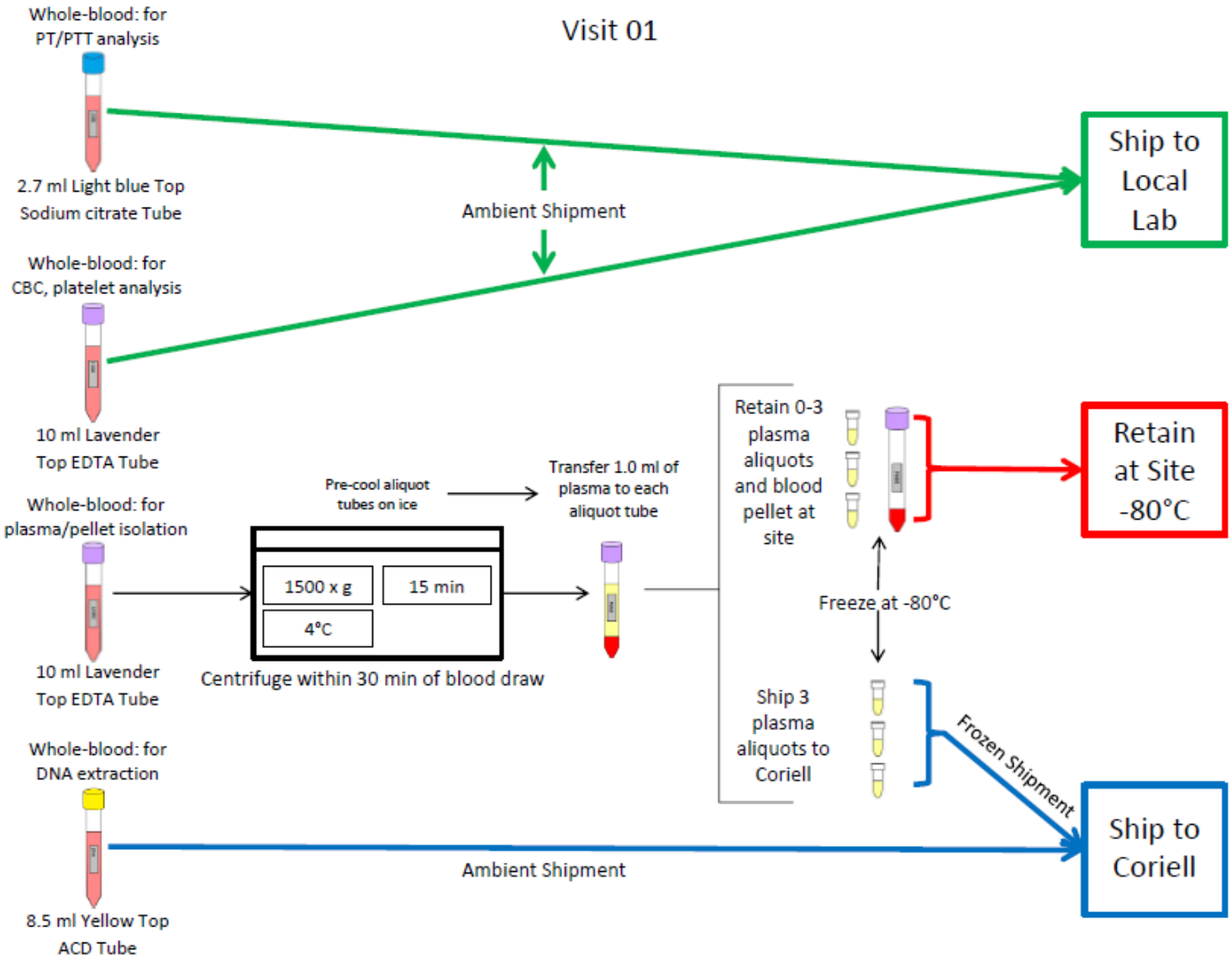
BioFIND Lab Sample Collection and Processing Summary – Baseline Visit/Visit 01

Sample Type	Tube Type	Number of Tubes Supplied in Kit	BioFIND Label	Processing/ Aliquoting	Tubes Shipped to Coriell	Tubes Retained at Site	Tubes Shipped to Local Lab
Whole-blood: for PT/PTT analysis	2.7 ml Light blue top Sodium Citrate Tube	1	N/A	N/A	0	0	1
Whole-blood: for CBC, platelets analysis	10ml Lavender Top EDTA Tube	1	N/A	N/A	0	0	1
Whole-blood: for isolation of plasma	2 ml microcentrifuge tubes	6		1 ml plasma aliquots in each 2 ml microcentrifuge tube	3	0-3	0
	10ml Lavender Top EDTA Tube	1		Retain blood pellet in EDTA tube	0	1	0
Whole-blood: for DNA extraction	8.5 ml Yellow Top ACD Tube	1		N/A	1	0	0
TOTAL V01		10			4	1-4	2

BioFIND Lab Sample Collection and Processing Summary – Baseline Visit/Visit 01 (continues)

Visit 01			
Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/Aliquoting
Whole-blood: for PT/PTT analysis	2.7 ml Light blue top Sodium Citrate Tube	1	• Collect blood into tube using your institution's recommended procedure for standard venipuncture technique • Immediately after blood collection, gently invert/mix (180 degree turns) the tube 3-4 times • Ship to local lab for PT/PTT analysis
Whole-blood: for CBC, platelets analysis	10ml Lavender Top EDTA Tube	1	• Collect blood into tube using your institution's recommended procedure for standard venipuncture technique • Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8-10 times • Ship to local lab for CBC, platelets analysis
Whole-blood: for isolation of plasma	2 ml micro-centrifuge tubes	6	• Place 2 ml microcentrifuge aliquot tubes on ice prior to procedure, but after labeling, so they are pre-cooled • Collect blood into tube using your institution's recommended procedure for standard venipuncture technique • Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8-10 times • Within 30 minutes of blood collection, centrifuge at 4°C for 15 minutes at 1500 x g • Using a micropipette, transfer 1.0 ml of blood plasma (top layer) into each labeled, pre-cooled, aliquot tube • Retain the used EDTA tube containing the pelleted blood sample; freeze and store EDTA tube at -80°C • Freeze all samples immediately following processing by transferring to -80°C Freezer. If samples cannot be immediately transferred to -80°C Freezer, prepare a sufficient amount of dry ice for immediate freezing • Store all samples at -80°C; ship 3 plasma aliquots to Coriell
	10ml Lavender Top EDTA Tube	1	
Whole-blood: for DNA extraction	8.5 ml Yellow Top ACD Tube	1	• Collect blood into tube using your institution's recommended procedure for standard venipuncture technique • Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8-10 times • Ship to Coriell for DNA extraction

BioFIND Lab Sample Collection and Processing Summary – Baseline Visit/Visit 01 (continues)



BioFIND Lab Sample Collection and Processing Summary – Final Visit/Visit 02

Sample Type	Tube Type	Number of Tubes Supplied in Kit	BioFIND Label	Processing/ Aliquoting	Tubes Shipped to Coriell	Tubes Retained at Site	Tubes Shipped to Local Lab
Saliva	50 ml & 15 ml conical tube	1+2		0.5 ml saliva aliquots in each 2 ml tube	6	0-4	0
	2 ml tube	10					
Urine	50 ml cup	1		N/A	1	1	0
	15 ml conical tube	4					
Whole-blood: for RNA extraction	2.5 ml PAXgene Tube	3		N/A	2	1	0
Whole-blood: for isolation of plasma	2 ml tubes	18		1 ml plasma aliquots in each 2 ml tube	9	0-9	0
	10ml Lavender Top EDTA Tube	3		Retain blood pellet in EDTA tube	2	1	0
CSF	50 ml conical tube	1	N/A	Combine and mix total CSF	N/A		
	15 ml conical tubes	2		Divide and spin total CSF			
	2 ml tubes	18		1 ml CSF aliquots in each 2 ml tube	10	0-8	0
	2 ml purple-top tubes	2		1 ml CSF aliquots in each 2 ml purple-top tube	0	0	2
TOTAL V02		65			30	2-24	2

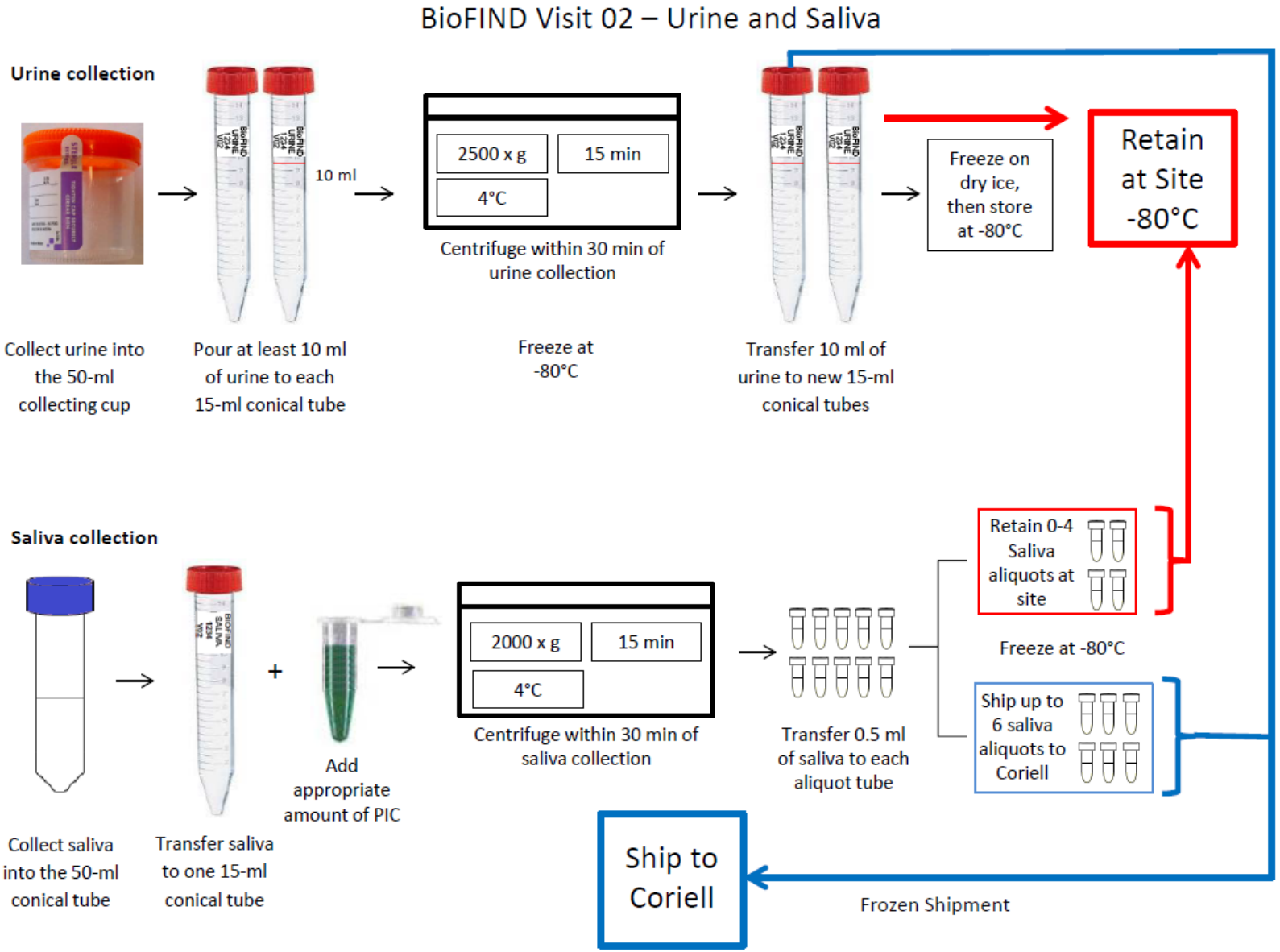
BioFIND Lab Sample Collection and Processing Summary – Final Visit/Visit 02 (continues)

BioFIND Visit 02 (Saliva, Urine)			
Sample Type	Tube Type	Number of Tubes Supplied in Kit	Processing/Aliquoting
Saliva	50 ml conical tube	1	• Label and place 50 ml conical tube on ice. Collect saliva into the 50 ml tube • Transfer saliva using a serological pipette or a micropipetter to a 15 ml conical tube • Add appropriate PIC according to chart in Appendix L • Immediately after adding PIC , mixing samples by either vortexing or vigorously inverting/mixing (180 degree turns) the tube 20 – 30 seconds • Within 30 minutes of saliva collection, centrifuge at 4°C for 15 minutes at 2000 x g • Using a micropipette, transfer 0.5 ml of saliva into each labeled, pre-cooled, 2 ml aliquot tube • Freeze all samples immediately following processing by transferring to -80°C Freezer. • Store all samples at -80°C; ship up to 6 saliva aliquots to Coriell
	15ml conical tube	2	
	2 ml micro-centrifuge tubes	10	
Urine	50 ml orange top cup	1	• Label collecting cup and all 15-ml conical tubes • Collect urine specimen in the collection cup • Pour 10-ml of the urine into two 15-ml conical tubes • Within 30 min of collection, centrifuge at 4°C, 2500x g for 15 min • Using transfer pipette, transfer urine to two fresh 15-ml tubes • Immediately after transferring urine to new tubes, freeze tubes by insert tubes in dry ice • Store all samples at -80°C; ship 1 15-ml tube of urine to Coriell
	15ml conical tube	4	

BioFIND Lab Sample Collection and Processing Summary – Final Visit/Visit 02 (continues)

BioFIND Visit 02 (RNA, Plasma, CSF)			
Sample Type	**Tube Type**	**Number of Tubes Supplied in Kit**	**Processing/Aliquoting**
Whole-blood: for RNA extraction	2.5 ml PAXgene Tube	3	• Collect blood into tubes using your institution's recommended procedure for standard venipuncture technique • Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8-10 times • Incubate tubes UPRIGHT at room temperature (18°C to 25°C) for 24 hours, or a minimum of 2 hours if it is not possible to incubate for 24 hours • After incubation at room temperature, transfer tubes to -80°C freezer • Store all samples at -80°C; ship 2 tubes to Coriell
Whole-blood: for isolation of plasma	2 ml micro-centrifuge tubes	18	• Place 2 ml microcentrifuge aliquot tubes on ice prior to procedure, but after labeling, so they are pre-cooled • Collect blood into tube using your institution's recommended procedure for standard venipuncture technique • Immediately after blood collection, gently invert/mix (180 degree turns) the tube 8-10 times • Within 30 minutes of blood collection, centrifuge at 4°C for 15 minutes at 1500 x g • Using a micropipette, transfer 1.0 ml of blood plasma (top layer) into each labeled, pre-cooled, aliquot tube • Retain the used EDTA tubes containing the pelleted blood samples; freeze and store EDTA tubes at -80°C • Freeze all samples immediately following processing by transferring to -80°C Freezer. If samples cannot be immediately transferred to -80°C Freezer, prepare a sufficient amount of dry ice for immediate freezing • Store all samples at -80°C; ship 9 plasma aliquots and 2 blood pellets to Coriell
	10ml Lavender Top EDTA Tube	3	
CSF	50 ml conical tube	1	• Label aliquot tubes prior to CSF collection • Perform lumbar puncture using the atraumatic technique • Collect CSF into syringes (if a noticeably bloody tap, discard first 1-2 mls), take the first 2 mls of CSF from the first syringe, place in the CSF labs tubes (1 ml in each purple-top microcentrifuge tube), and send it to the local lab for routine diagnostic tests • Collect an additional 15-20 mls of CSF and transfer to 50 ml conical polypropylene tube, mix gently by inverting 3-4 times • Within 15 minutes of collection, transfer the CSF from the 50 ml conical tube to the two 15 ml conical tubes • Spin the CSF sample at 2000 x g for 10 minutes at Room Temperature [64^{0}F - 77^{0}F (18°C to 25°C)] • Using a micropipette, transfer 1.0 ml of supernatant directly into aliquot tubes • Freeze samples immediately following processing by transferring to -80°C Freezer. If samples cannot be immediately transferred to -80°C Freezer, prepare a sufficient amount of dry ice for immediate freezing • Store all samples at -80°C; ship 10 CSF aliquots to Coriell
	15 ml conical tubes	2	
	2 ml micro-centrifuge tubes	18	
	2 ml purple-top micro-centrifuge tubes	2	

BioFIND Lab Sample Collection and Processing Summary – Final Visit/Visit 02 (continues)



BioFIND Lab Sample Collection and Processing Summary – Final Visit/Visit 02 (continues)