



PLATELET-**O**RIENTED
INHIBITION IN **N**EW **T**IA
AND **M**INOR ISCHEMIC **S**TROKE

**ANCILLARY BIOMARKERS STUDY
BLOOD SPECIMEN PROCEDURES
MANUAL**

OUS Sites
DNA and Plasma

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I. DNA AND PLASMA SPECIMEN PROCEDURES

1. SITE REQUIREMENTS for DNA and PLASMA SPECIMEN COLLECTION

Access to the following items is needed for proper specimen processing and storage:

- **Preferred (not required)** - A centrifuge that operates at 1000g and 4°C.
- **Required** – A centrifuge that operates at 1000g.
- **Preferred (not required)** - A –70°C to –90°C freezer. Specimens can be stored for 3 months before shipping. When shipping, specimens will be shipped on dry ice.
- **Required** – A - 20°C, - 30°C or - 40°C freezer. **If using a -20°C, -30°C or -40°C freezer, specimens must be shipped on dry ice within 4 days.**
- Dry ice available for shipping all specimens. (**Refer to your LabCorp Lab Manual for sources of dry ice**).

2. DNA and PLASMA SPECIMEN SAMPLING KITS

LabCorp Clinical Trials will provide sampling kits that will be assembled at LabCorp and sent directly to sites in a reusable container to send the specimens to LabCorp. Each **DNA and Plasma** sampling kit contains the required materials to collect, prepare and return the specimens to LabCorp Clinical Trials. (Additional bulk supplies will be provided to site to supplement sampling kits in the event of breakage or damage.)

Each **DNA and Plasma** sampling kit contains the following:

ITEM	QUANTITY
FORMS	
Laboratory Test Request (Requisition) Form with barcode	1
BLOOD COLLECTION SUPPLIES	
Blood collection:	1
a. 1- 10mL EDTA lavender top tube (vacutainer) with barcode	
b. 1-10ml cryovial tube for buffy coat & left over cells with barcode	
c. 6- 2ml cryovial tubes for plasma with barcodes	
d. 1- Alcohol wipe	
e. 1- Needle	
f. 1- Tourniquet	
g. 1- Gauze pad	
h. 1- Bandage	
i. 1- Non sterile pipettes	

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Biohazard Specimen Bag	1
SHIPPING SUPPLIES	
Kit box (shipping box)	1
Pre-paid & pre-printed air bill	1
Styrofoam shipper	1
Dry Ice shipping label	Multiple
NOTE: Sites must obtain dry ice for shipping specimens – Refer to your LabCorp Manual for sources of dry ice.	

The Laboratory Requisition Form (**Refer to your LabCorp Lab Manual**) and barcode labeled items included inside each sampling kit box are preprinted and contain the **same** barcode number.

➤ **Do not transfer the contents between sampling kits.**

❖ **REORDERING SUPPLIES**

- To reorder supplies, refer to the Kit and Supply Re-Ordering instructions provided in your **LabCorp Lab Manual**.

✓ **When reordering supplies, please allow a minimum of 5 to 7 working days for assembly and delivery of supplies.**

3. DNA and PLASMA SPECIMEN COLLECTION AND PREPARATION

Blood specimens will be collected at study enrollment. Optimally, specimens will be collected at the time of randomization, after the loading dose has been administered to the subject. However, specimens may be collected up to 12 hours after randomization if unavoidable. A hospital phlebotomy laboratory can be used if the Study Coordinator is unable to perform phlebotomy, but the coordinator is responsible for transport of the blood specimen to the lab for centrifugation and arranging for packing and shipping all of the specimens to LabCorp.

❖ **BEFORE COLLECTING SPECIMEN**

- Confirm that Informed Consent for the biomarkers study was obtained.
- Be sure you have selected the correct sampling kit for **DNA and Plasma** specimen collection.
- Check the expiration date for the sampling kit you have selected. (Follow local guidelines for destroying expired sampling kits.)
- Check the LabCorp calendar in your **LabCorp Lab Manual** for any closures due to local and national holidays.

❖ **SPECIMEN COLLECTION AND PREPARATION**

1. Use standard sterile technique for venipuncture.
2. Use opposite arm if there is an IV. If there is an IV in both arms, collect the blood from a vein **distal** to the IV.
3. **Completely fill** the lavender top EDTA tube (**Appendix A**) that is included in the sampling kit.
4. Gently invert the tube at least 6-8 times immediately after collection to avoid clotting. **Avoid exposure to extreme hot or cold temperatures.**

Label the tube with the **POINT Subject ID number and Randomization number (the Randomization # is the study drug bottle #)**.

NOTE: Please use a waterproof or ballpoint pen when filling out all specimen labels and Laboratory Requisition Form.

5. Separate the plasma from the cells by centrifugation immediately or within 30 minutes to 1 hour of collection (**refer to your LabCorp Lab Manual, Centrifugation section**). Please keep tubes upright prior to centrifugation. If you need further assistance with centrifugation, please **refer to your LabCorp Lab Manual** for the contact information for your **LabCorp Project Monitor**.
6. After centrifugation, if you do not see a distinct layer between the blood cells, buffy coat and plasma, please centrifuge the specimen a second time. See **Appendix C** for a diagram of a blood specimen after centrifugation, showing the buffy coat.
7. Using a pipette included in the sampling kit, transfer **0.7mL** of plasma from the lavender top EDTA tube into each of the six, 2ml cryovial tubes. **Fill to the 0.7mL line on the 2ml cryovial tubes. (Appendix D)**

NOTE: Be careful not to disturb the buffy coat layer between the blood cells and the plasma. You should see a distinct layer between the plasma and the cells, called the *buffy coat*.

8. Ensure that the buffy coat it is NOT pipetted in with the plasma. If some of the buffy coat is drawn up with the plasma, please note this at the bottom of the **Lab Requisition Form**.
9. Place the caps on the plasma cryovial tubes.

10. Complete the labels for the six, 2ml cryovial tubes with the **POINT Subject ID number and Randomization number (the Randomization # is the study drug bottle #)**.

NOTE: Please use a waterproof or ballpoint pen when filling out all specimen labels and Laboratory Requisition Form.

11. Using the additional pipette included in the sampling kit, transfer all of the leftover cells and buffy coat from the 10ml EDTA tube to the 10ml cryovial tube.
12. Replace the cap on the 10ml cryovial tube.
13. Place the six, 2ml plasma cryovials and the 10 ml cryovial tube of leftover cells and buffy coat in the Aqu-i-Pak (6-place tube holder with absorbent material).
14. Place the Aqu-i-Pak inside the largest compartment of the biohazard specimen bag and seal. Insert a copy of Laboratory Requisition Form in the outer pocket of the specimen bag (retain a copy for your records).
15. **IMMEDIATELY PLACE THE SPECIMEN BAG IN THE FREEZER IN AN UPRIGHT POSITION.**
16. The specimens must remain in the freezer until **FROZEN** before they can be shipped. Once frozen, the specimens must be shipped frozen on dry ice. Follow the packaging and shipping instructions in your **LabCorp Lab Manual** and ship the 10 ml cryovial tube and six, 2ml cryovial tubes together on dry ice.
17. If the leftover cells and plasma are frozen in a **-20°C, -30°C or -40°C freezer**, specimens must be shipped on dry ice **within 4 days. days**. If specimens are stored in a **-70°C to -90°C freezer**, they may be stored for up to three months before shipping on dry ice.

❖ **PROBLEMS WITH SPECIMEN COLLECTION AND PREPARATION**

If an item in the sampling kit is broken or wasted, replace it with an item from the bulk supplies. There are additional bar-coded labels in each sampling kit. Affix one of the extra bar-coded labels in a way that can be easily scanned (e.g., lengthwise for a tube). Make sure the "Lot Expiration" barcode is not covered. Complete the information required on the label.

4. CENTRIFUGATION

To prepare cell-free plasma, blood specimens must be centrifuged.

Use the nomogram in the **LabCorp Lab Manual** for calculating the speed (RPM or revolutions per minute) for centrifuging the specimen. In order to calculate the speed, you will need to know the **radius** (the distance from the center of the centrifuge you are using to the middle of the tube being centrifuged), and the **RCF**

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(relative centrifugal force, also expressed as “g”; in this study, **g = 1000**). The speed or RPM needs to be calculated once for any specific centrifuge.

Once the speed has been determined, **centrifuge the 10ml EDTA tube at 1000g for 15 minutes**. This will separate the plasma from the cells.

You should see a distinct layer between the plasma and the cells, called the *buffy coat*. **Be careful not to disturb the buffy coat**. It is critical that the buffy coat stays with the leftover cells for use in the DNA extraction that will take place at LabCorp. **If you do not see distinct layers between the cells, buffy coat and plasma please centrifuge the specimen a second time.**

NOTE: If you need further assistance, please contact your **LabCorp Project Monitor (refer to your LabCorp Lab Manual)**.

5. **DNA and PLASMA SPECIMEN STORAGE**

The remaining leftover cells and plasma specimens must be frozen at the site prior to shipping. Samples are to be kept frozen in a -70°C to -90°C, **OR** -20°C, -30°C or -40°C until it is time to ship.

- **If using a -20°C, -30°C or -40°C freezer, specimens must be shipped on dry ice *within 4 days*.**
- **If using a -70°C to -90°C freezer, specimens may be stored for *3 months* before shipping on dry ice.**

During freezer storage, specimens should remain in the biohazard specimen bags in an upright position.

NOTE: Both the remaining cells and extracted plasma will be shipped together on dry ice.

❖ **PRIOR TO SHIPPING**

1. Verify that each of the six, 2ml cryovial tubes and the 10ml cryovial tube is labeled.
2. Verify that each 2ml cryovial label has been completed with **POINT Subject ID number and Randomization number (the study drug bottle number)**.
3. Verify that the label on the 10ml cryovial tube has been completed with the **POINT Subject ID number and Randomization number**.

6. **DNA and PLASMA PACKAGING AND SHIPPING – Please refer to your LabCorp Lab Manual for details.**

1. Assemble the shipping materials.

2. Fill half the shipping box with dry ice.
3. Place the specimen bag on the dry ice in an upright position and pack the remainder of the shipping box with dry ice.

NOTE: Samples from multiple subjects may be shipped in one container and must remain in their individual specimen bags, along with corresponding Lab Requisition Forms.

4. Close and seal the shipping box.
- 5 **Please refer to your LabCorp Lab Manual** for the courier selected for your country and specific information on completing your air bill. Please fill out your air bills completely; the air bill is pre-printed with site name and LabCorp information.
6. Retain a copy for your records.
7. Please refer to your **LabCorp Lab Manual** to determine if a Dry Ice label is pre-affixed to your shipping box or whether you need to affix a provided Dry Ice shipping label to the shipping box.
9. Ship the specimen to LabCorp Clinical Trials. Contact your **LabCorp Laboratory** contact person listed in your **LabCorp Lab Manual** if you need to speak with someone about shipping.

**REMINDER: Specimens can only be shipped
to LabCorp Clinical Trials
MONDAY THROUGH THURSDAY**

II. COMMON PROBLEMS/ERRORS

Drawing up the buffy coat layer when extracting the plasma

If you accidentally draw up some of the buffy coat layer into one of the pipettes, please make a note on the Laboratory Requisition Form. This may affect the accuracy of biomarker levels.

Insufficient separation of cells, buffy coat and plasma layers

Please centrifuge the specimen a second time.

Insufficient dry ice

This may cause specimens to thaw and will affect the accuracy of biomarker levels.

III. LABCORP ADDRESS FOR SPECIMEN SHIPPING

Please refer to your LabCorp Lab Manual

IV. MAYO CLINIC ADDRESS FOR SPECIMEN STORAGE

Mayo Clinic is responsible for long-term DNA and plasma storage.

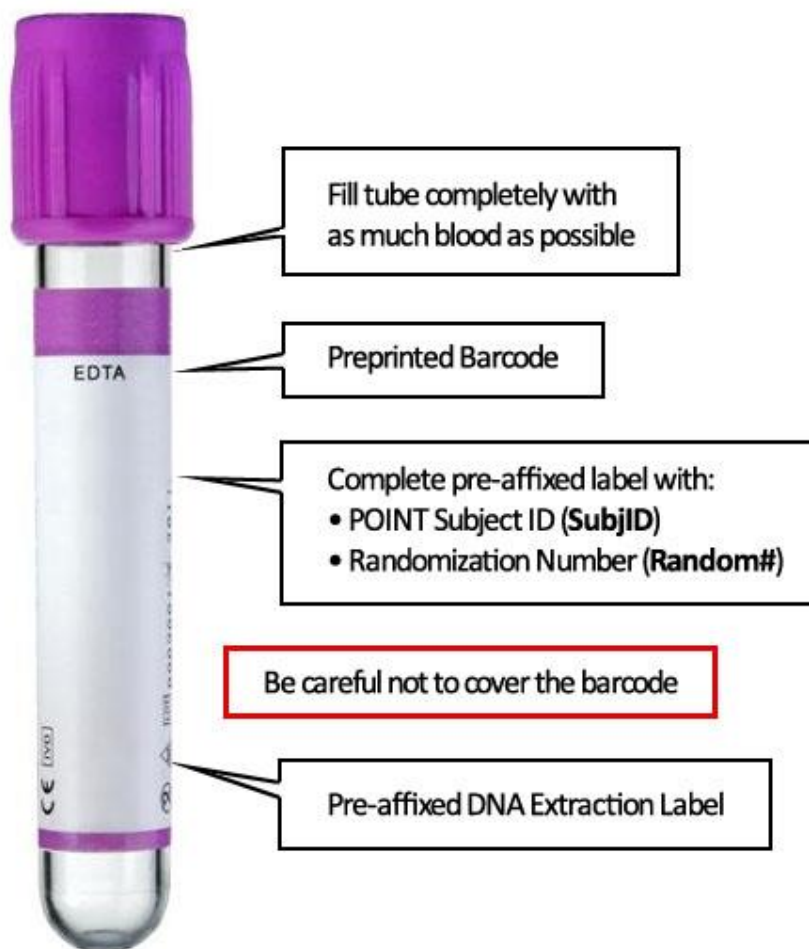
✓ **DO NOT SHIP SPECIMENS DIRECTLY TO MAYO CLINIC.**

Mayo Clinic
ATTN: Owen Ross, PhD
4500 San Pablo Road
Birdsall Room 225
Jacksonville, FL 32224
Phone: (904) 953-6280

V. APPENDICES

Appendix A

10 ML LAVENDER TOP EDTA BLOOD SPECIMEN TUBE



Appendix B

10 ML CRYOVIAL TUBE for LEFTOVER CELLS and BUFFY COAT

Preprinted Barcode

Be careful not to cover the barcode

Complete pre-affixed label with:

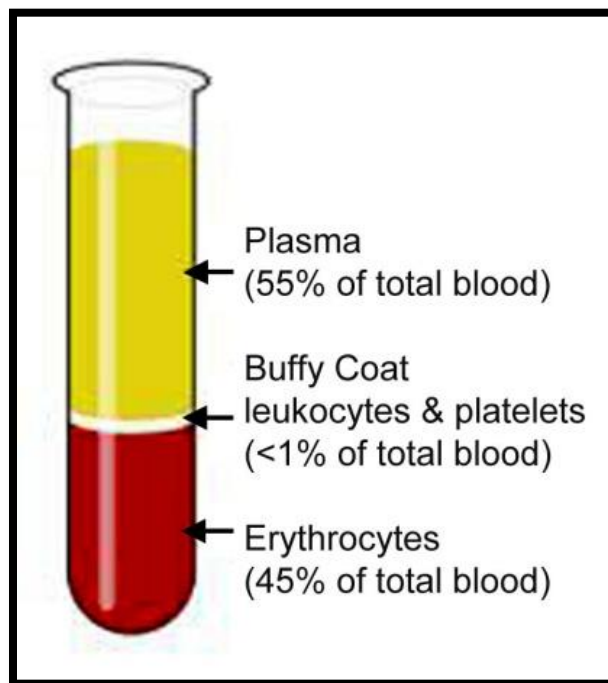
- POINT Subject ID (SubjID)
- Randomization number (Random#)

Pre-affixed DNA Extraction Label



Appendix C

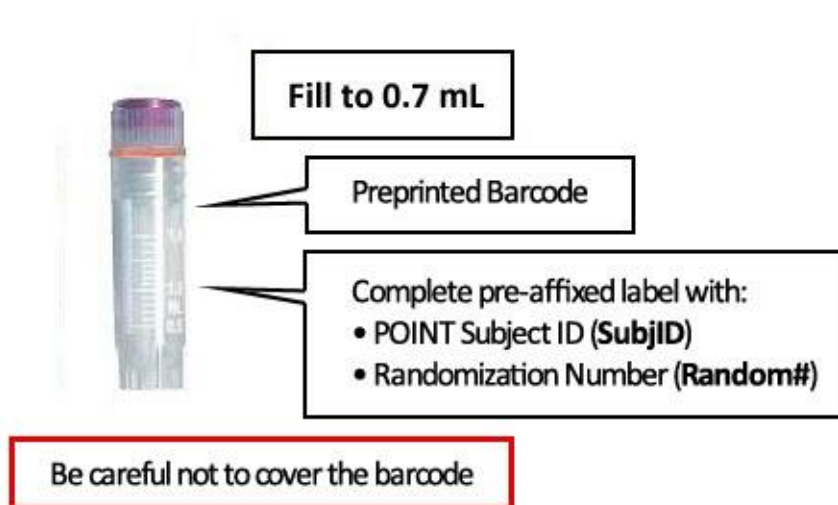
DIAGRAM OF BLOOD SPECIMEN AFTER CENTRIFUGATION



Note: Be careful not to disturb the buffy coat layer between the blood cells and the plasma.

Appendix D

2 ML CRYOVIAL PLASMA TUBE



Using a pipette included in the sampling kit, transfer **0.7 mL** of plasma from the lavender top EDTA tube into each of the six 2ml plasma cryovial tubes.

Fill to the 0.7 mL line on the plasma cryovial tubes.

Note: Be careful not to disturb the buffy coat layer

between the blood cells and the plasma. If some of the buffy coat gets drawn up with the plasma, please note this at the bottom of the Lab Requisition Form.

See **Appendix C** for a diagram of a blood specimen after centrifugation, showing the buffy coat.