CARDIOVASCULAR DISEASE IN AMERICAN INDIANS (PHASE II)

OPERATIONS MANUAL - VOLUME THREE

LABORATORY PROCEDURES

THE NATIONAL HEART, LUNG AND BLOOD INSTITUTE
OF THE NATIONAL INSTITUTES OF HEALTH
THE STRONG HEART STUDY
Cardiovascular Disease in American Indians
(Phase II)

Operational Manual
Volume Three
Laboratory Procedures

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For copies, please contact

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# MANUAL III
## LABORATORY PROCEDURES

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LABORATORY PROCEDURES

1.1 Equipment and Supplies

1. Equipment: A refrigerated clinical centrifuge pre-cooled at 4°C will be required for the separation of plasma from the cells. The centrifuge rotor should have adapters for 16 x 100 mm, 13 x 100 mm and 10⅛ x 64 mm tubes.

Alternatives would include the placement of a non-refrigerated centrifuge in a standard refrigerator via an extension cord.

2. Supplies: A description of the various tubes and supplies that will be needed in the study is presented. Except for the Cryovials (and cap inserts) for frozen samples, all other items can be substituted with equivalent items from the local distributor.

<table>
<thead>
<tr>
<th>Items</th>
<th>Size</th>
<th>Packaging</th>
<th>Material ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic Tubes w/screw on cap (plasma and urine)</td>
<td>14-ml</td>
<td>1000/case</td>
<td>polypropylene</td>
</tr>
<tr>
<td>Cryovials w/screw caps</td>
<td>2-ml</td>
<td>500/case</td>
<td>Corning 25704</td>
</tr>
<tr>
<td>White inserts for Cryovial caps</td>
<td></td>
<td>500/case</td>
<td>Corning 25709-W</td>
</tr>
<tr>
<td>Blue inserts for Cryovial caps</td>
<td></td>
<td>500/case</td>
<td>Corning 25710-B</td>
</tr>
<tr>
<td>Yellow inserts for Cryovial caps</td>
<td></td>
<td>500/case</td>
<td>Corning 25713-Y</td>
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<tr>
<td>Red inserts for Cryovial caps</td>
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<td>500/case</td>
<td>Corning 25711-R</td>
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<td>Transfer Pipets</td>
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<td>500/box</td>
<td>polyethylene</td>
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<td>Transfer Pipets sterile</td>
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<td>polyethylene</td>
</tr>
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<td>Vacutainer, EDTA</td>
<td>10-ml</td>
<td>1000/case</td>
<td>15% solution BD-6457</td>
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<td>Item</td>
<td>Size</td>
<td>Quantity/Case</td>
<td>Code</td>
</tr>
<tr>
<td>----------------------------------------------------</td>
<td>-------</td>
<td>-----------------</td>
<td>--------</td>
</tr>
<tr>
<td>Vacutainer, SST</td>
<td>9.5-ml</td>
<td>1000/case</td>
<td>BD-6510</td>
</tr>
<tr>
<td>Vacutainer, Sodium Citrate</td>
<td>4.5-ml</td>
<td>1000/case</td>
<td>BD-6579</td>
</tr>
<tr>
<td>Vacutainer, Fluoride</td>
<td>5-ml</td>
<td>1000/case</td>
<td>BD-6475</td>
</tr>
<tr>
<td>Vac. Multiple Sample Needles (Butterfly)</td>
<td>21G</td>
<td>200/case</td>
<td>BD-7251</td>
</tr>
<tr>
<td>Vac. Reusable Holder</td>
<td></td>
<td>4 (free with each case of tubes)</td>
<td></td>
</tr>
<tr>
<td>Adhesive tape</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[2x2] Sterile Gauze</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol Wipes</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Latex Gloves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tourniquet</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Urine collection cups</td>
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</tr>
<tr>
<td>Bandaids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Needle Disposal Device</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.2 Procedure for Blood Drawing

Have the following tubes labelled and ready in an ice bucket

**Fasting Sample**
- *Three 10-ml Lavender-top tubes*
- *One 5-ml Gray-top tube*
- *One 4.5-ml Blue-top tube (citrate)*
- *One Special tube*
- *One 9.5-ml Red-Top tube (SST)*

**2-hr Sample**
- *One 5-ml Gray-top tube*

**Other Supplies**
- *Adhesive tape*
- *Tourniquet*
- *Vacutainer sleeve*
- *Vacutainer needle [21G]*
- *alcohol pads*
- *2x2 gauze pads (multiple sample)*
- * bandaids*
- *urine collection cups*

Note: Participants exempted from the GTT will not have a 2-hr sample collected

**NOTE:** Gloves must be worn when drawing blood or handling samples. An appropriate barrier must be used at any time there is a risk of aerosols (such as when opening vacutainer tubes.)

1.2.1 One-Touch Procedure

1) Obtain One-Touch reading from a drop of blood obtained by finger prick. (Using the blood from the venipuncture procedure below will not provide comparable results since there is a difference between capillary blood (fingerstick) and venous blood values.)

2) See One-Touch procedure for calibrating the meter and steps to follow in obtaining a glucose reading. (Consult with the operations manual which can be obtained from Lifescan, Inc. 1-800-227-8862)

1.2.2 Venipuncture Procedure

1) Position the participant in comfortable chair in an environment free from distraction.

2) Query the participant about fasting state. "When was the last time you ate?" Record time since last food or beverage on GTT check list (appendix 6, Volume II). If subject is not fasting, record time and note in comment section what foods or beverage were consumed.
that morning. Be sure to include any additives like cream, sugar, or artificial sweeteners if a beverage was consumed. Regardless of fasting state, proceed with drawing procedure.

3) Inform the participant about the procedure. Use left arm if possible.

4) Assemble all materials; have extra tubes within reach.

5) Apply tourniquet; have subject close fist and palpate for vein. (A vein feels like an elastic tube and bounces when pressure is applied). If the presence of vein is questionable, remove or loosen tourniquet. If the structure remains, it probably was not a vein; if it disappears assume it was a vein. Another technique to assist in locating a vein is to moisten the skin with alcohol as it will decrease the friction and may aid in the palpation of a vein. If the tourniquet has been on for 2 minutes, loosen and reapply before performing venipuncture.

6) Cleanse skin over vein thoroughly using a circular motion from center to periphery. Dry with sterile gauze.

DO NOT TOUCH SKIN AFTER CLEANSING

7) Put gloves on; fit luer adapter needle at end of collection set into Vacutainer sleeve and place lavender top tube into sleeve.

8) Pull skin taut 2 inches below site to keep vein from rolling. With bevel of needle in upright position, enter vein and then push the tube forward as far as it will go. Hold needle in the same direction as vein and at a 15 degree angle to vein.

9) After blood begins to flow, secure butterfly with a piece of tape and loosen the tourniquet.

If blood does not begin to flow, try the following:

a) Move the needle slightly in or out.

b) Rotate needle slightly or lift needle to move bevel away from wall of vein.

c) Try another tube.

d) Loosen tourniquet; blood flow may be impeded if tourniquet is too tight.

* Be sure to watch for signs of hematoma from a vein. If there is any indication of hematoma, immediately remove tourniquet and needle. Place 2x2 gauze over the site, and apply pressure and/or ice pack on site for 5 minutes. If the first attempt to obtain blood is unsuccessful (with the subject's permission) try again on the opposite arm. The same technician should not attempt a venipuncture more than twice.

Strong Heart Study II 11/12/93
Procedure for Blood Drawing
Figure 1. Proper Venipuncture Angle
10) When first tube is filled, remove tube and replace with the next tube. Invert all filled tubes several times and place on ice.

11) Proceed with additional tubes in this order:

   Fasting:  
   3 [10-ml] Lavender top tube  
   1 [5-ml] Gray top tube  
   1 [4.5-ml] Blue top tube  
   1 [9.5-ml] Red top (SST) tube  
   1 Special tube  

   2 Hr:  
   1 [5-ml] Gray top tube

12) After drawing the last tube, remove the tourniquet. Place a gauze on the site of the needle entry and quickly withdraw the needle. Apply pressure to the site. Ask subject to hold the arm straight and hold gauze pad with pressure until told to relax.

13) Record the time the fasting draw is completed on the GTT check list. (See procedure for Glucose Tolerance Test in section 5.6.)

14) Serve glucose beverage; instruct subject to consume it within 3 minutes. Record time on GTT check list.

15) Confirm that bleeding has stopped, and apply pressure bandage at venipuncture site. If bleeding has not stopped, elevate arm and continue to apply pressure until it stops.

16) Affix preprinted labels to tubes, making sure the ID# and tube designation are correct.

17) Give the participant labeled urine specimen cup and instruct him to void into container. Inform him or her where to leave the container.

18) Remove gloves, wash hands, and proceed to next participant.
1.3 Sample Preparation and Storage

The laboratory procedures described in this manual are being implemented in the PENN MEDICAL LABORATORY (PML) of the Medlantic Research Institute.

1.3.1 General Rules for Handling Samples for Lipids and Other Measurements

One important precaution which should always be kept in mind in handling samples for lipids and lipoprotein measurements is that the blood should be cooled (either in the refrigerator or on ice) as soon as the samples are collected, and kept cold until processing is complete and samples are properly stored. Plasma should be separated from the cells within a few hours. Plasma samples should not be allowed to freeze and thaw during any of the handling steps.

1.3.2 Processing of Blood Samples and Urine Sample

The flow diagram in figure 2 illustrates the blood and urine processing procedure of this protocol. A check list is available in the Appendix 1 and should be completed for each participant. Appendix 2 is a check list for quality controls which will require a number of additional tubes of blood.

1. Label vials and tubes for each patient as follows (this should be done before beginning the blood processing). Attach the labels according to the labelling diagram in figure 11.3.1. All tubes are to be labelled before processing is begun:

<table>
<thead>
<tr>
<th>Num/Pat</th>
<th>Container</th>
<th>Label</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14-ml Plasma Tube</td>
<td>LIPIDS</td>
<td>refrigerated (40°C)</td>
</tr>
<tr>
<td>5</td>
<td>2-ml Cryovials without inserts</td>
<td>STORAGE</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>2</td>
<td>2-ml Cryovials without inserts</td>
<td>HbA1c</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>1</td>
<td>14-ml Tube</td>
<td>BUFFY CT</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>3</td>
<td>2-ml Cryovial with blue cap insert</td>
<td>COAG</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>3</td>
<td>2-ml Cryovials with green cap inserts</td>
<td>SPECIAL</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>2</td>
<td>2-ml Cryovials with white cap inserts</td>
<td>INSULIN</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>2</td>
<td>14-ml Urine Tube</td>
<td>URINE</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>2*</td>
<td>2-ml Cryovial with yellow cap inserts</td>
<td>0HR GLUC*</td>
<td>frozen (-70°C)</td>
</tr>
<tr>
<td>2**</td>
<td>2-ml Cryovial with red cap inserts</td>
<td>2HR GLUC**</td>
<td>frozen (-70°C)</td>
</tr>
</tbody>
</table>
* 0Hr Gluc sample will also be used for Plasma Creatinine testing.
** The 2-ml Cryovials with red cap inserts will not be needed for any participant exempt from the GTT

2. Arrange the vials and tubes on ice in the order in which they are listed in step 2.

3. Spin all blood tubes at 40°C for 10 minutes, at 1500 RPM (500 x g).

4. Remove tubes from centrifuge and place them in a rack on ice.

5. **[10-ML] LAVENDER TOP TUBES** (total of 3)
   
   A. Remove stoppers from all three [10-ml] lavender top tubes.

   B. With a fresh, disposable transfer pipet, place approximately 6-7 ml into the 14 ml plasma tube. With the same pipet divide all the remaining plasma between 5 [2-ml] Cryovials labelled "Storage." (approx. 1.0 ml each) Be very careful **not to disturb** the cell layer in the bottom of the Lavender top tubes (leave approximately 0.5 ml of plasma in each tube). Discard pipet after completion of this step.

   C. Do **not** freeze the [14-ml] plasma tube, place in refrigerator for weekly shipment. **Make sure that the cap has been securely closed. Do not freeze this tube.**

   D. Securely cap and freeze the Cryovials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

   E. Using a **sterile** pipet, carefully draw up the buffy coat from the first [10-ml] Lavender top tube and dispense into a prelabelled 14 ml tube labelled "Buffy Ct." The buffy coat is the white layer on top of the red cells. It is rich in white cells. This step is best carried out by positioning the pipet tip slightly above the buffy coat and, while aspirating, carefully moving the tip just over the surface of the buffy coat in a **slow** swirling motion. The resulting aspirate should contain all of the buffy coat material, plus a small amount of red cells and plasma. When aspirating, try not to let the pipet tip actually touch the buffy coat, or an excess amount of red cells will be drawn up. Similarly, suspending the tip too far above the buffy coat results in too much plasma and too few white cells. This should result in a volume of approximately 1.0-1.5 ml.

   F. Using a fresh sterile pipet for each tube, repeat step E with the remaining two [10-ml] Lavender top tubes for the same patient, pipetting into the same 14 ml tube.
G. Immediately after the buffy coats have been dispensed into the 14 ml tube, and the tube securely capped, place the vial on ice, (or immediately into the freezer.) It is important to ensure that the top of the tube is screwed on tightly, otherwise the "O-ring" seal will leak.

H. The tube which contains the buffy coats is to be frozen at -70°C as quickly as possible. (The specimens are stable if kept on ice for up to a maximum of 4 hours, if necessary, however, immediate freezing is preferred.) Once frozen, the samples are to be kept at -70°C until shipment.

I. Transfer at least 1.5 ml of red cells from the last tube into each of the remaining 2 remaining [2-ml] Cryovials without cap inserts (labelled "HbA1c."). Cap and freeze these tubes as quickly as possible at -70°C.

J. Replace the stopper in both of the other vacutainer tubes. Label these tubes with the patient ID labels for "Red Cells" and place the tubes in refrigerator for weekly shipment to Memorial Blood Center of Minneapolis (see shipping instructions in section 1.4.) Make sure that the stopper has been securely replaced. Do not freeze these tubes.

K. Discard the used pipets and the remaining tubes with the red cells.

6. **BLUE TOP TUBE**

A. Remove the stopper from the tube.

B. With a fresh transfer pipet, divide all of the plasma between the three [2-ml] Cryovials labelled "Coag."

C. Using a caps with a blue insert, securely cap the vials.

D. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E. Discard the pipet and tube with the remaining red cells.

7. **SPECIAL TUBE**

A. Remove the stopper from the tube.

B. With a fresh transfer pipet, divide all of the plasma between the three [2-ml] Cryovials labelled "Special."
C. Using a caps with a **green** insert, securely cap the vials.

D. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E. Discard the pipet and tube with the remaining red cells.

8. **RED TOP (SST) TUBE**

A. Remove the stopper from the tube.

B. With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "Insulin."

C. Using a caps with **white** inserts, securely cap the vials.

D. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E. Discard the pipet and tube with the remaining red cells.

9. **URINE SAMPLE**

A. Pour approximately 8 ml of the patient's urine sample into each of 2 tubes labeled "URINE". Discard the remaining sample.

B. Securely cap the tubes and freeze at -70°C.

C. Once frozen, the samples are to be kept at -70°C until shipment.

10. **GRAY TOP TUBES**

A. Remove the stopper from the fasting [5-ml] gray top tube.

B. With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "0hr Gluc."

C. Using caps with **yellow** inserts, securely cap the vials.

D. Discard the pipet and tube with the remaining red cells.

E. Remove the stopper from the 2 hour [5-ml] gray top tube.
F. With a fresh transfer pipet, divide the plasma between the two [2-ml] Cryovials labelled "2hr Gluc."

G. Using caps with red inserts, securely cap the vials.

H. Freeze the vials as quickly as possible at -70°C. Once frozen, the samples are to be kept at -70°C until shipment.

E. Discard the pipets and tubes with the remaining red cells.

1.3.3 Sample storage prior to shipping

Three zip-lock bags will be needed for each participant.

Bag A will be for the refrigerated (40°C) sample to be shipped to Penn Medical Laboratory (Medlantic Research Institute) and should contain the following properly labelled tube:

* 1 [14-ml] plasma tube ("Lipids")

Bag B will be for the refrigerated samples (40°C) to be shipped to Memorial Blood Center of Minneapolis and should contain the following properly labelled tubes:

* 2 [10-ml] LTT Vacutainer tube ("Red Cells")

Bag C will be used for frozen samples (-70°C) to be shipped to Penn Medical Laboratory (Medlantic Research Institute) and should contain the following properly labelled tubes:

* 5 [2-ml] Cryovials for plasma ("Storage")
* 1 [4-ml] Cryovial for buffy coat ("Buffy Ct")
* 2 [2-ml] Cryovials for red cells ("HbA1c")
* 3 [2-ml] Cryovials with blue cap inserts ("Coag")
* 3 [2-ml] Cryovials with green cap inserts ("Special")
* 2 [2-ml] Cryovials with white cap inserts ("Insulin")
* 2 [14-ml] Urine samples ("Urine")
* 2 [2-ml] Cryovials with yellow cap inserts ("0Hr Gluc")
* 2 [2-ml] Cryovials with red cap inserts ("2Hr Gluc")
FIGURE 2  Processing Blood Samples and Urine Sample

**FASTING**

Three [10-ml] Lavender top tubes

Centrifuge (1500 RPM, 10 min 4°C)

Plasma

Red Cells

1 x [14-ml] tube (apx 7 ml)
DO NOT FREEZE
ship cold (blue ice)
("Lipids" label)

5 x [2-ml] Cryovials (apx 1.0 ml each)
(store/ship frozen)
("Storage" label)

One [5-ml] Gray top tube

Centrifuge (1500 RPM, 10 min 4°C)

Plasma (G0)
2 x [2-ml] Cryovials with yellow cap inserts
(store/ship frozen)
("0Hr Gluc" label)

Remove and freeze buffy coats

1 x [4-ml] Cryovial (store/ship frozen)
("Buffy Ct" label)

Aliquot 2 x [2-ml] Cryovials for HbA1c
(store/ship frozen)
("HbA1c" labels)

Red Cells for typing
("Red Cells" label)
DO NOT FREEZE

One [4.5-ml] Blue top tube

Centrifuge (1500 RPM, 10 min 4°C)

Plasma
3 x [2-ml] Cryovials with blue cap insert
(store/ship frozen)
("Coag" labels)

One [9.5-ml] SST Red (tiger) tube

Centrifuge (1500 RPM, 10 min 4°C)

Serum
2 x [2-ml] Cryovials with White cap inserts
(store/ship frozen)
("Insulin" labels)

One Urine sample

DO NOT CENTRIFUGE

apx 8 ml each of two Urine transfer vials
(store and ship frozen)
("Urine" labels)

**2-HOUR**

One [3-ml] Gray top tube

Centrifuge (1500 RPM, 10 min 4°C)

Plasma (G2) 2 x [2-ml] Cryovials with yellow cap inserts (store/ship frozen) ("2Hr Gluc" labels)
ATTACHING BAR CODED LABELS TO SAMPLE VIALS

Small Vials (Cryovials)

Attach label with lines in barcode in horizontal orientation. (see diagram)

Large Vials (SC Tubes)

Attach label with lines in barcode in horizontal orientation.

Align label with top of vial. (see diagram)

FIGURE 3. ATTACHING BAR CODED LABEL TO SAMPLE VIALS
1.4 Shipping

1.4.1 Shipping Schedule

1. Refrigerated plasma samples

Refrigerated plasma samples are to be shipped weekly. Ship the samples in approved insulated containers with adequate refrigerant packs to keep the samples cold. **DO NOT FREEZE THESE SAMPLES.** Samples should not come in direct contact with refrigerated packs.

The samples are to be sent via airfreight, priority overnight delivery, to the following address for receipt by 10:30 AM EST, Monday through Friday. Do not ship on Fridays, weekends, or the day before a holiday:

**Penn Medical Laboratory**
**Medlantic Research Institute**
108 Irving Street N.W.
Washington, DC 20010
(202) 877-5481

2. Refrigerated red cell samples

Refrigerated red cell samples are to be shipped weekly. Ship the samples in approved insulated containers with adequate refrigerant packs to keep the samples cold. **DO NOT FREEZE THESE SAMPLES.** **SAMPLES ARE NOT TO COME INTO DIRECT CONTACT WITH REFRIGERANT PACKS.**

The samples are to be sent via airfreight, priority overnight delivery, to the following address for receipt by 10:30 AM EST, Monday through Friday. Do not ship on Fridays, weekends, or the day before a holiday:

**Paternity Laboratory**
**Memorial Blood Center of Minneapolis**
2304 Park Avenue South
Minneapolis, Minn 55404
(800) 871-3300

3. Frozen samples

Frozen samples are to be shipped on dry ice once every 2 weeks. When packing, place at least 8-9 lbs. of dry ice in the box. Pack tightly and do not add any other packing material.
The samples are to be sent via airfreight, priority overnight delivery, to the following address for receipt by 10:30 AM EST, Monday through Friday. Do not ship on Fridays, weekends, or the day before a holiday:

**Penn Medical Laboratory**
**Medlantic Research Institute**
108 Irving Street N.W.
Washington, DC  20010
(202) 877-5481

1.4.2 Packing

All samples are to be packed according to DOT regulations and in compliance with shipper's requirements. This includes the following:

* All samples are to be securely caped and sealed in a transport bag.
* Shipping containers are to be self contained with sufficient absorbent material surrounding sample bags to a absorb any spillage.
* The exterior of all packages are to be labelled according to the shipper's requirements.

1.4.3 Shipping Slip

A completed shipping slip form should be put into each shipped container. See appendix 4 for samples of forms. Information required for each participant includes the ID code, the number of plasma tubes, the number of special blood tubes, and the number of blood cell tubes. Place a check mark on the *Frozen Shipment Form* next to the ID number of any participant using insulin. Extra labels are to be mailed along with the bag containing the unfrozen plasma and the whole blood (5 ml PTT) drawn on each patient. These labels will be used by the laboratory.

Put the shipping slip in a plastic bag and place the bag on top of the insulated lid before closing the outside cardboard box.

A copy of the shipping slip should be retained by the originating clinic (Appendix 5).

Upon receipt of the samples by PML, a status check list will be sent back to the PI (or pre-designated individual) by FAX. The condition of the samples received will be noted on the list, along with any discrepancies between the shipping form and samples actually received.
1.4.4 Shipment questions or problems

If you have any question regarding the status of a shipment contact either Dr. Michael Paidi, Darlene Allen, MT(ASCP), or George Webb at PML. Special shipments for weeks involving a legal holiday are to be coordinated with the laboratory.

Tel (202) 877-5481
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