# Biospecimen Adult Blood Procedures

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|---|---|
| **Event:** | Pre-Preg, PV1, PV2, Birth, 6M, 12M, 36M, and 60M |
| **Domain:** | Biospecimen |
| **Type of Document:** | Standard Operating Procedures |

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# Biospecimen Adult Blood Procedures

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# Biospecimen Adult Blood Procedures

## 1.0 Overview

Adult blood specimens will be collected from women at the pre-pregnancy visit, pregnant women at two pre-birth visits and the birth visit[1], and from the primary caregiver at the 6-, 12-, 36-, and 60-month visits. All blood collections are non-fasting collections except for the second pregnancy visit blood collection, which is a fasting collection.

Once collected, some blood tubes require centrifugation to be completed within 2 hours. This can be done in the field or after transport and receipt at the Sample Handling Location (SHL). Depending on tube type and location of centrifugation, collected blood tubes will be transported to the SHL at either refrigerated or ambient temperatures. Following receipt and, if necessary, centrifugation at the SHL, blood tubes should be stored at ambient, refrigerated, or frozen temperatures depending on tube type until shipment to the National Children's Study (NCS) Repository. In the future, these biospecimens will be analyzed to obtain information about physiological conditions and environmental exposures.

## 1.1 Requirements/Conditions for Collection

The participant must have provided all appropriate consents and authorizations granting permission for collection of biospecimens as indicated in the NCS Informed Consent Manual of Procedures.

The participant must answer a set of questions to determine their eligibility for blood collection. Eligibility is always assessed during administration of the Adult Blood

[1] Additional details on the adult blood collection at the birth visit can be found in the Biospecimen Birth Procedures.

Instrument at the time of the blood draw. To aid in scheduling of staff or follow-up clinic visits for blood collection, Regional Operations Centers (ROCs) may choose to also establish eligibility of the participant before the blood draw through administration of the Adult Blood Pre-Screening Instrument (Appendix 2). This is done either on the telephone before the blood collection or during the home visit. If the participant meets any exclusion criteria or does not know or refuses to answer the questions that determine eligibility on either instrument, he/she will not be eligible for the blood collection.

Adult blood collections except for the birth visit will occur during the respective study visit in either a home or clinic setting. For non-fasting visits, it is preferable to complete the blood collection near the end of the visit because centrifugation of some blood tubes must be completed within 2 hours of collection. Since the second pregnancy visit is a fasting blood collection, blood collection for this visit should be performed toward the beginning of the visit. Following the collection the participant should be offered a break to eat something.

Centrifugation of blood tubes may be performed at the location where blood is collected (e.g., participant's home, clinic), or at the SHL. Depending on the location where centrifugation will be performed, ROCs may choose to modify the visit flow and change the timing of collection as needed to meet the 2-hour time requirement.

## 1.2 Pre-visit Preparation

Prior to the study visit, ROC staff will:

- Ensure that all materials and supplies are prepared and organized in advance of the study visit. A list of equipment and supplies for each activity is provided in following sections. For details on manufacturer and part numbers of equipment and supplies, refer to the Biospecimen Overview of Activities.
    - Confirm that blood tubes and supplies have not expired.
    - The refrigerated chamber of the transport cooler in which some blood tubes are transported must be preconditioned and contain:

    - Four (4) -1ºC cold packs - ensure these are frozen prior to use and placed in the small chamber of the transport cooler to equilibrate before placing blood specimens in the chamber.
    - Digital thermometer, affixed to the inside wall of the refrigerated chamber.
- Ensure threshold monitors are properly handled prior to use:
    - Never activate threshold monitors before collected specimens are ready for transport.
- Ensure eye-readable, 2-D barcoded Specimen ID labels are affixed to the blood tubes per NCS specifications.
    - Refer to the Biospecimen Overview of Activities for more detailed information on labeling procedures and specifications.
- Plan where centrifugation will be performed to successfully meet the 2-hour time requirement from blood collection to completion of centrifugation.
    - It is preferred that if the blood is collected at a home visit the centrifugation be done at the SHL.
    - Additional considerations include the distance between the blood collection location (e.g., home or clinic) and the SHL, potential travel conditions (e.g., traffic and weather), and the schedule of data collection assignments.

# 2.0 Procedures for Adult Blood Collection

## 2.1 Collection Equipment and Supplies

- Personal Protective Equipment (PPE) per local ROC Exposure Control Plan (e.g., gloves, lab coat, etc.)
- Pre-labeled blood tubes specific for the study visit type packaged in plastic clamshells. Tube requirements for each visit are outlined in Table 1 and further information can be found in Appendix 4.
- Alcohol wipes

- Large bandage
- Sterile gauze, 2” x 2”
- Prescreened 21-gauge butterfly needle assembly unit with 12-inch tubing (prescreened 23- and 25-gauge needles should also be available if needed)
- Tourniquet, single use
- Needle holder, single use
- Amber transport bag with absorbent pad
- Specimen collection tray
- Chux pad
- Hand sanitizer
- Phlebotomy ball
- Biohazard waste bag, approx. 19” x 24”
- Sharps container
- Computer with bar code scanner that includes automated system for electronic data capture
- Adult Blood Instrument – developed by the ROC Information Management System (IMS) Hub according to the Adult Blood Instrument Specifications.
    - Hard copy paper and pencil instrument (PAPI) back up forms are available on the NCS portal for collection of instrument data if an electronic data capture system is unavailable at an NCS visit.

## 2.2 Procedures for Adult Phlebotomy

These procedures assume that the blood collection process and the recording of information about the participant are conducted in such a way that the participant is comfortable, and there is little or no delay in recording information as soon as a response is provided or an activity is completed. Information should be recorded in the Adult Blood Instrument provided by the ROC IMS Hub.

If centrifugation will be performed in the participant's home or in the clinic, it is recommended that the ROC data collector set up the centrifuge prior to the blood draw.

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Explain the blood collection procedures to the participant.
2. If the participant does not refuse the collection, continue with the blood collection process.
    - If the participant refuses the collection, indicate that this is fine, thank the participant for his/her time, and record "participant refusal" as the main reason the specimen was not collected.
3. Read the adult blood collection questions (Appendix 3) to the participant and record the responses.
    - If the participant meets eligibility requirements, continue with the blood collection process.
    - If the participant is unable to provide a blood specimen due to ineligibility, indicate that he/she is ineligible for the blood collection, thank the participant for his/her time, and record the reason for ineligibility as the main reason why the specimen was not collected:
        - If the participant indicated he/she had hemophilia or other blood disorder, record "Hemophilia".
        - If the participant indicated he/she had cancer chemotherapy in the past 4 weeks, record "Cancer chemotherapy"
        - If the participant answered "Don't know" to hemophilia or cancer chemotherapy status, record "Don't know".
        - If the participant refused to answer the hemophilia or cancer chemotherapy status questions, record "Refused".
4. Locate the adult blood collection supplies.
5. Verify that the Specimen ID labels are affixed to the blood tubes and are readable and not damaged.

6. Find a place for the participant to sit while the blood collection is being done. The participant should be able to rest their arm comfortably while holding it straight at the elbow, palm up on a flat surface. There also should be room for the specimen collection tray to be within easy reach.
7. Cover the area where the participant will rest his/her arm with a Chux pad, absorbent side up.
8. Place the specimen collection tray in the selected work area.
9. Lay a Chux pad on top of the specimen collection tray for any spills that might occur and set up supplies.
10. Organize the blood tubes in the appropriate draw order as outlined in Table 1.
    - The 3 ml lavender top, prescreened tube and the 6 ml royal blue top, serum, prescreened tube are collected before the red top tubes, and the red top tubes are collected before additional tubes with additives.
        - The 3 ml lavender top, prescreened tube and the 6 ml royal blue top, serum, prescreened tube are designated for trace metal analysis and must be collected before any other tubes to avoid contamination.
        - The 8.5 ml yellow top ACD and 2.5 ml clear top PAXgene™ tubes are always collected last.

**Table 1. Blood tube draw order by study visit**

| Study Visit | Blood Tube Draw Order | Label Extension |
|---|---|---|
| Pre-pregnancy visit | 3 ml lavender top, prescreened | LP10 |
| | 10 ml red top | RD10 |
| | 10 ml red top | RD11 |
| | 6 ml lavender top | LV15 |
| First pre-birth visit (non-fasting) | 8.5 ml red/gray top SST | SS10 |
| | 10 ml red top | RD10 |
| | 5 ml clear top PPT | PP10 |
| | 6 ml lavender top | LV15 |
| | 8.5 ml yellow top ACD | AD10 |

| Study Visit | Blood Tube Draw Order | Label Extension |
|---|---|---|
| Second pre-birth visit (fasting) | 6 ml royal blue top, serum, prescreened | RS10 |
| | 8.5 ml red/gray top SST | SS10 |
| | 10 ml red top | RD10 |
| | 5 ml clear top PPT | PP10 |
| | 6 ml lavender top | LV15 |
| | 2.5 ml clear top PAXgene™ | PX10 |
| Birth visit | 3 ml lavender top, prescreened | LP10 |
| | 10 ml red top | RD15 |
| | 10 ml red top | RD10 |
| | 6 ml lavender top | LV15 |
| 6-month visit | 6 ml royal blue top, serum, prescreened | RS30 |
| | 8.5 ml red/gray top SST | SS30 |
| | 10 ml red top | RD30 |
| | 5 ml clear top PPT | PP30 |
| | 6 ml lavender top | LV30 |
| | 2.5 ml clear top PAXgene™ | PX30 |
| 12-month visit | 3 ml lavender top, prescreened | LP40 |
| | 10 ml red top | RD30 |
| | 10 ml red top | RD31 |
| | 6 ml lavender top | LV30 |
| 36-month visit | 3 ml lavender top, prescreened | LP40 |
| | 8.5 ml red/gray top SST | SS30 |
| | 10 ml red top | RD30 |
| | 6 ml lavender top | LV30 |
| | 2.5 ml clear top PAXgene™ | PX30 |
| 60-month visit | 3 ml lavender top, prescreened | LP40 |
| | 6 ml royal blue top, serum, prescreened | RS30 |
| | 8.5 ml red/gray top SST | SS30 |
| | 10 ml lavender top | LV50 |

11. Cleanse hands thoroughly with hand sanitizer and put on a lab coat and gloves.
12. Have the participant extend their arm on the Chux pad, palm up and straight at the elbow.
13. Position the arm on the work area so that the veins are readily accessible. Be sure the arm is in a downward position, with the elbow lower than the heart.
    - If necessary, place a prop under the elbow to achieve this position. A rolled-up Chux pad can be used for this purpose.
14. Inspect the arm being used for venipuncture. The veins of choice are those located in the antecubital area.
    - Do not draw blood from an arm that:
        - Has a rash, open sore, is swollen, or shows signs of a recent venipuncture or hematoma.
        - Contains an arterial access such as a fistula or shunt.
    - Avoid areas that have scar tissue or the presence of tendons near the vein.
    - If the arm is not appropriate, inspect the other arm.
15. Apply the tourniquet several inches above the elbow and palpate for a suitable vein.
16. Select a vein that is palpable and well-fixed to surrounding tissue. Palpate even when the vein can be seen.
    - If the veins do not distend rather quickly, the following techniques may be used:
        - Massage the arm from wrist to elbow; this forces blood into the veins.
        - Tap the area sharply with the index and second finger two or three times; this causes the veins to dilate.
        - The arm to be used for venipuncture may be hung at the participant's side without a tourniquet. This allows the veins to fill with blood to their capacity.
        - Examine the other arm. Sometimes the veins in one arm are larger and more suitable for blood draw than in those in the other arm.

17. Open the butterfly needle assembly unit and attach it to the needle holder. If veins appear to require a smaller needle gauge, substitute the 21 gauge needle assembly with a 23 gauge needle assembly.
18. If the tourniquet has been applied for more than 1 minute while searching for a vein, release the tourniquet. Prolonged obstruction of blood flow by the tourniquet is unnecessary and uncomfortable for the participant and may alter certain test results.
    - After 2–3 minutes, reapply the tourniquet but not too tightly.
19. Ask the participant to squeeze the phlebotomy ball or make a tight fist.
20. Cleanse the area with an alcohol wipe.
    - Hold the alcohol wipe with two fingers on one side so that only one side of the alcohol wipe touches the area of the puncture site.
    - Cleanse the area using a circular motion beginning with a narrow radius and moving outward so as not to cross over the already-cleansed area.
    - Allow area to air dry.
    - Do not palpate the vein after cleansing the skin.
21. Remove the cover from the needle.
22. The vein should be fixed or held taut during the puncture. Place the left thumb about 1 inch below the point of entry and pull the skin gently in a downward motion (this stretches the skin and anchors or fixes the vein).
23. Hold the needle in line with the vein, with the bevel up and at a 15° angle with the skin, about 1/2-inch below the proposed point of entry to the vein.
24. Push the needle firmly and deliberately into the vein. When firmly in the vein, blood should appear in the tubing of the needle assembly past the end of the needle. The blood flow into the tubing verifies that the needle has adequately penetrated the selected vein.
25. Quickly push the first blood tube onto the needle in the holder, puncturing the center of the stopper.
    - If no blood enters the tube and no bruise is forming, probe the vein until entry is indicated by blood flowing into the tube.

- If no blood enters the tube and a bruise is forming, remove the tourniquet and then the needle immediately. Do not keep probing as this could cause severe bruising.
    - Place a gauze pad over the puncture site and ask the participant to apply firm pressure to the puncture site for 3 minutes.
    - Ask the participant if a second attempt may be made. If the participant agrees, make a second attempt on the other arm with new sterile collection supplies and new tubes. A second attempt is allowed only after verbal consent from the participant. No more than two attempts should be made.

26. Release the tourniquet after the flow is established in the tube or if the participant becomes uncomfortable. The participant may open his/her fist once blood flow in the first tube is established.
27. When the first tube is filled to capacity, remove it from the holder and place the next tube in the holder.
28. You may drape a clean piece of gauze over the needle to obstruct the participant's view.
29. Gently invert each tube 8–10 times immediately upon removing each tube, while filling the next tube.
30. Cover blood tubes with an amber bag as much as possible. Tubes should have minimal exposure to light.
31. Repeat until all the tubes are filled.
32. When the last tube is filled, carefully withdraw the needle, covering the puncture site with a sterile gauze pad. Remove the needle in a smooth and quick motion. NEVER apply pressure to the gauze until the needle is clear of the puncture site and away from the arm. Applying pressure during the needle withdrawal causes the needle tip to scratch the skin under the gauze.
33. Have the participant hold the gauze pad with mild pressure and raise his/her arm in the air without bending the arm. This procedure helps prevent the formation of a hematoma.

34. Immediately slide the needle safety guard forward to prevent an accidental needle stick. Place the entire used needle assembly in the sharps container. Discard the entire assembly. DO NOT reuse the needle holder for multiple blood draws.
35. Check the venipuncture site.
    - If there is no bleeding from the puncture site, remove the gauze and apply a bandage.
    - Instruct the participant to sit quietly for a few minutes.
    - Observe the participant during this time for adverse effects, e.g., dizziness or fainting.
    - If bleeding continues, keep direct pressure on the site for 5 minutes or more.
36. If the participant had been fasting prior to the blood collection, encourage the participant to eat a snack before continuing with the remaining data collection activities.
37. Record the collection status of each blood tube as either “Full draw”, “Short draw”, or “No draw” for each blood tube.
    - Select “Full draw” to indicate that the blood tube was filled to at least ¾ of the desired capacity.
    - Select “Short draw” to indicate that the blood tube was filled to less than ¾ of the desired capacity.
    - Select “No draw” to indicate that the blood tube was not collected.
    - If the blood tube was not collected or the draw was short, record the reason:
        - If the collection supplies malfunctioned while performing the collection, record “Equipment failure”.
        - If the participant fainted during the procedure, record “Fainting”.
        - If the participant felt light-headed during the procedure, record “Light-headedness”.
        - If the participant developed a hematoma during the procedure, record “Hematoma”.
        - If the participant began to bruise during the procedure, record “Bruising”.

- If the vein collapsed during the procedure, record “Vein collapsed during the procedure”.
- If there was not a suitable vein for the blood collection, record “No suitable vein”.
- If there was another reason the blood tube could not be collected or was a short draw, record “Other” and specify the reason.

38. Record the Specimen ID from each blood tube that has a “full draw” or “short draw” tube status. Recover the blood tubes with the amber transport bag as the Specimen ID is recorded.
39. Record where the blood collection occurred: In the participant’s home, at a clinic, hospital, or other location.
40. Record the date and time the blood was collected.
41. Record the overall status for blood collection: “Collected”, “Partially Collected”, or “Not Collected”. This information may be automatically captured in the Adult Blood Instrument and may not need to be recorded by the data collector.

- If the overall status of the blood collection is “Not Collected”, record the reason:
  - If the participant had a safety exclusion that prevented collection of the blood specimen, record “Safety exclusion”.
  - If the participant has a physical limitation that prevented them from providing a blood specimen, record “Physical limitation.”
  - If participant became ill during the visit or has an emergency that requires termination of the visit, record “Participant ill/Emergency.”
  - If unable to collect blood in any of the tubes and was denied permission for a second attempt, record “Quantity not sufficient”.
  - If there was a language issue due to the participant’s primary language being Spanish record, “Language issue, Spanish.”
  - If there was a language issue due to the participant’s primary language being other than Spanish record, “Language issue, non-Spanish.”

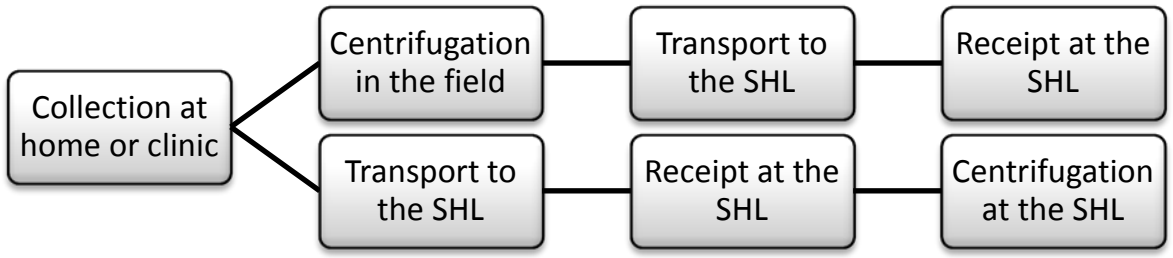
- If the participant has a cognitive disability that prevents them from understanding the instructions, record “Cognitive disability.”
- If there was not a sufficient amount of time for the collection, record “No time.”
- If the specimen was not collected for a reason other than those listed, record “Other” and specify the reason.
- If the participant refused the collection, record “Refused”.
- If it is not known why the specimen was unable to be collected, record “Don’t know”.

42. Record any comments, problems, or concerns pertaining to the adult blood collection.

# 3.0 Adult Blood Transport, Receipt, and Centrifugation Procedures

To reduce variability in blood specimen handling and to preserve the integrity of a wide variety of blood analytes, the NCS has established the following requirement: *Separation of serum or plasma from cells (centrifugation) must be completed within 2 hours of blood collection.* If blood is collected in the home, it is preferred that centrifugation be done following transport and receipt at the SHL, but ROCs may choose to centrifuge blood in the field prior to transport and receipt at the SHL to accomplish centrifugation within 2 hours of collection.

**Figure 1. Possible pathways of adult blood collection, transport, receipt and centrifugation procedures**




The following procedures assume centrifugation will occur at the SHL, following transport and receipt at the SHL. If the tubes will be centrifuged in the field, prior to transport and receipt, see Appendix 1

# 4.0 Adult Blood Transport Procedures

Adult blood specimens will be transported to the SHL at either refrigerated or ambient temperatures depending on tube type and location of centrifugation. Transport temperatures will be monitored using threshold temperature monitors. Table 2 (see Section 4.2) lists the appropriate transport temperature for each blood tube prior to centrifugation.

- Refrigerated tubes will be transported in a plastic clamshell containing upper and lower threshold temperature monitors within an amber transport bag in the biospecimen transport cooler.
- Ambient tubes will be transported in a Styrofoam tube holder containing a lower threshold temperature monitor within an ambient transport bag.

If transport to the SHL will occur following centrifugation, see Appendix 1.

## 4.1 Transport Equipment and Supplies

- PPE per local ROC Exposure Control Plan (e.g., gloves, lab coat, etc.)
- Plastic clamshell
- Upper (20°C) threshold temperature monitor
- Lower (2°C) threshold temperature monitor
- Amber bag with absorbent pad
- Pre-conditioned biospecimen transport cooler with:
  - Four -1ºC cold packs in the refrigerated chamber
  - Digital thermometer, affixed to inside wall of refrigerated chamber
- Ambient transport bag with:
  - Styrofoam 5-tube holder
- Computer with bar code scanner that includes automated system for electronic data capture
- Adult Blood Instrument – developed by the ROC IMS Hub according to the Adult Blood Instrument Specifications.
  - Hard copy PAPI back up forms are available on the NCS portal for collection of instrument data if an electronic data capture system is unavailable at an NCS visit.

## 4.2 Procedures for Transport Prior to Centrifugation

The following procedures should be done immediately after collecting the blood tubes to preserve the integrity of blood specimens and prevent potential loss of analytes. Ensure that the transport cooler has been properly preconditioned. Information should be recorded in the Adult Blood Instrument provided by the ROC IMS Hub.

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Put on a lab coat and gloves if not already on.

2. Place the blood tubes in the refrigerated clamshell or the ambient tube holder according to Table 2.

**Table 2. Transport temperature prior to centrifugation by study visit and tube type**

| Study Visit | Blood Tube Type | Label Extension | Transport Temperature Prior to Centrifugation |
|---|---|---|---|
| Pre-pregnancy visit | 3 ml lavender top, prescreened | LP10 | Refrigerated, NC |
| | 10 ml red top | RD10 | Refrigerated |
| | 10 ml red top | RD11 | Refrigerated |
| | 6 ml lavender top | LV15 | Refrigerated, NC |
| First pre-birth visit (non-fasting) | 8.5 ml red/gray top SST | SS10 | Ambient |
| | 10 ml red top | RD10 | Refrigerated |
| | 5 ml clear top PPT | PP10 | Ambient |
| | 6 ml lavender top | LV15 | Refrigerated, NC |
| | 8.5 ml yellow top ACD | AD10 | Ambient, NC |
| Second pre-birth visit (fasting) | 6 ml royal blue top, serum, prescreened | RS10 | Refrigerated |
| | 8.5 ml red/gray top SST | SS10 | Ambient |
| | 10 ml red top | RD10 | Refrigerated |
| | 5 ml clear top PPT | PP10 | Ambient |
| | 6 ml lavender top | LV15 | Refrigerated, NC |
| | 2.5 ml clear top PAXgene™ | PX10 | Refrigerated, NC |
| Birth visit | 3 ml lavender top, prescreened | LP10 | Refrigerated, NC |
| | 10 ml red top | RD15 | Refrigerated |
| | 10 ml red top | RD10 | Refrigerated |
| | 6 ml lavender top | LV15 | Refrigerated, NC |
| 6-month visit | 6 ml royal blue top, serum, prescreened | RS30 | Refrigerated |
| | 8.5 ml red/gray top SST | SS30 | Ambient |
| | 10 ml red top | RD30 | Refrigerated |
| | 5 ml clear top PPT | PP30 | Ambient |
| | 6 ml lavender top | LV30 | Refrigerated, NC |

| Study Visit | Blood Tube Type | Label Extension | Transport Temperature Prior to Centrifugation |
|---|---|---|---|
| | 2.5 ml clear top PAXgene™ | PX30 | Refrigerated, NC |
| 12-month visit | 3 ml lavender top, prescreened | LP40 | Refrigerated, NC |
| | 10 ml red top | RD30 | Refrigerated |
| | 10 ml red top | RD31 | Refrigerated |
| | 6 ml lavender top | LV30 | Refrigerated, NC |
| 36-month visit | 3 ml lavender top, prescreened | LP40 | Refrigerated, NC |
| | 8.5 ml red/gray top SST | SS30 | Ambient |
| | 10 ml red top | RD30 | Refrigerated |
| | 6 ml lavender top | LV30 | Refrigerated, NC |
| | 2.5 ml clear top PAXgene™ | PX30 | Refrigerated, NC |
| 60-month visit | 3 ml lavender top, prescreened | LP40 | Refrigerated, NC |
| | 6 ml royal blue top, serum, prescreened | RS30 | Refrigerated |
| | 8.5 ml red/gray top SST | SS30 | Ambient |
| | 10 ml lavender top | LV50 | Refrigerated, NC |

NC denotes that the blood tube is transported at this temperature but not centrifuged following receipt.

3. Locate a lower (2ºC) threshold temperature monitor, remove the adhesive, and affix it to the outside of the refrigerated clamshell. Activate the monitor by pushing the button on the front. This monitor will be used to determine whether the temperature of the clamshell falls below 2ºC during transport.
4. Locate the upper (20°C) threshold temperature monitor. Remove the adhesive and affix it to the outside of the refrigerated clamshell (see Exhibit 1 for proper placement). Activate the monitor by pushing the button on the back. “On” will be displayed in blue on the front of the monitor.

**Exhibit 1. Placement of threshold temperature monitors in the refrigerated clamshell**

5. Immediately place the refrigerated clamshell inside an amber transport bag to minimize exposure of the blood tubes to light, and seal the bag. If there are two clamshells, place each clamshell inside a separate amber bag.
6. Position the amber bag securely and upright within the refrigerated chamber of the transport cooler as shown in Exhibit 2.

**Exhibit 2. Proper packing of the refrigerated chamber**


7. If able to measure the temperature of the refrigerated chamber of the biospecimen transport cooler, select “Temperature” and record the temperature in degrees Celsius and whether it is positive or negative.

- If not able to measure the temperature of the refrigerated chamber, record the reason:
    - If unable to measure the temperature because the thermometer is broken, record “Not able to measure – thermometer broken”.
    - If unable to measure the temperature because the thermometer was not available, record “Not able to measure – thermometer not available”.
    - If unable to measure the temperature for another reason, record “Not able to measure – other” and specify reason.
    - If there are not tubes that require refrigerated transport, record “Not applicable”.

8. Document the statuses of the upper and lower threshold monitors in the refrigerated chamber:
    - If the monitor was placed in the refrigerated chamber, record “Yes, in chamber”.
    - If the monitor is not required (e.g., tubes not collected) for the refrigerated chamber, record “No, not required”.
    - If the monitor was not placed in the refrigerated chamber, record “No, not available”.
9. When transporting tubes at ambient temperatures, locate a lower (2°C) threshold temperature monitor, remove the adhesive, and affix it inside the Styrofoam ambient tube holder. Activate the monitor by pushing the button on the front. This monitor will be used to determine whether the temperature of the tube holder falls below 2ºC during transport.
10. Immediately close the lid of the ambient tube holder to minimize exposure of the blood tubes to light.
11. Place the Styrofoam ambient tube holder upright in the ambient transport bag and attach the bag securely to the cooler.
12. Document the status of the lower threshold monitor in ambient tube holder:
    - If the lower threshold monitor was placed in the ambient tube holder, record “Yes, in chamber”.

- If the lower threshold monitor is not required (e.g., tubes not collected) for the ambient tube holder, record “No, not required”.
- If the lower threshold monitor was not placed in the ambient tube holder, record “No, not available”.

13. Confirm that the transport cooler and the ambient transport bag are properly closed for transport.
14. Record any comments, problems, or concerns pertaining to blood tube transport.
15. Record any other additional comments pertaining to the blood collection.

## 4.3 Clean-Up Procedures

1. After completing the blood collection procedures and preparing the blood tubes for transport, make sure the work area is clean.
   - If a spill has occurred, follow the local ROC Exposure Control Plan to clean up the spill.
2. If the blood specimens are the last biospecimens collected at the visit, clean the work area, and pack all collection supplies.
3. Close the sharps container.
   - If this is the last time the sharps container will be used, permanently seal the container and discard it in the biohazard waste bag.
4. After performing clean-up procedures discard gloves and cleanse hands with hand sanitizer.
5. Be sure any trash is packed properly in the biohazard waste bag for transport. All supplies used for the blood collection should be placed into a biohazard waste bag for disposal as medical waste at the SHL.

# 5.0 Adult Blood Receipt Procedures

Following transport to the SHL, blood specimens are to be receipted, centrifuged as appropriate, and stored at refrigerated (2 - 10°C), frozen (-30°C or below), or ambient (15 - 28°C) temperatures until they are shipped to the NCS Repository.

## 5.1 Receipt Equipment and Supplies

- PPE per local ROC Exposure Control Plan (e.g., gloves, lab coat, etc.)
- Bleach wipes
- Biohazard waste bag, approx. 19” x 24”
- Computer with bar code scanner that includes automated system for electronic data capture
- Biospecimen Tracking System - developed by the ROC IMS Hub to capture information for the Operational Data Element (ODE) tables
    - Hard-copy PAPI or other backup mechanism for recording ODEs in the event that the automated Biospecimen Tracking System is inaccessible.

## 5.2 Procedures for Adult Blood Receipt

These procedures assume that the blood receipt process and the recording of information about the process are conducted in such a way that the biospecimen integrity is maintained and there is little or no delay in recording information in the Biospecimen Tracking System as soon as an activity is completed.

Note: Data such as location of receipt, staff receipting the specimen, as well as the date and time the specimen is receipted and stored at the SHL may be automatically captured in the Biospecimen Tracking System and in that case may not need to be entered by the ROC data collector.

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Put on a lab coat and gloves.
2. Open the transport cooler and record the temperature of the refrigerated chamber using the digital thermometer located inside the chamber (in °Celsius). If the thermometer is not present, record this information.
3. Remove the clamshell from the refrigerated chamber.

4. Check the upper (20°C) threshold temperature monitor on the refrigerated clamshell to determine if the temperature went above 20°C during transport (see Exhibits 3 and 4). If the upper threshold temperature monitor is triggered, a blue color will be displayed beside the number of hours as in Exhibit 4.

**Exhibit 3. Untriggered upper threshold temperature monitor**


**Exhibit 4. Triggered upper threshold temperature monitor**


5. Check the lower (2°C) threshold temperature monitor in the refrigerated clamshell and the ambient tube holder, if used, to determine if the temperature went below 2°C during transport. As shown in Exhibit 5, a lower threshold temperature monitor that has been triggered will display a red color in the display window (right monitor), whereas an untriggered monitor will remain clear (left monitor).

**Exhibit 5. Lower threshold temperature monitor**

6. Record the status of the threshold temperature monitors as “Triggered” or “Not Triggered.”
    - Record whether the upper threshold monitor is triggered.
        - If it is triggered, record the number of hours triggered.
    - Record whether the refrigerated chamber lower threshold monitor is triggered.
    - Record whether the ambient tube holder lower threshold monitor is triggered.
7. Confirm that the specimens are at the appropriate temperature, labeled with a Specimen ID, and that they contain no personal identifying information.
8. Record the Specimen ID from each blood tube and the condition of the specimens upon receipt (e.g., damaged, thawed, or other additional comments).
9. Record any other additional comments about the condition of the specimen upon receipt at the SHL

# 6.0 Adult Blood Centrifugation Procedures

The type of blood tube used for collection and the potential analytes that will be measured in each tube determine whether a particular blood tube should be shipped to the NCS Repository as whole blood (i.e., non-centrifuged), or whether serum or plasma must be separated from blood cells (i.e., centrifuged) prior to shipping. Not all blood tubes are centrifuged prior to shipment; Table 3 (see Section 6.2) displays centrifugation specifications by visit type and blood tube type. For those tubes not centrifuged, ensure they are placed at the proper storage temperature while the other tubes are centrifuged.

ROCs should centrifuge tubes with either the Unico® Power Spin LX or the Unico® Porta Spin centrifuge (required by NCS for all centrifuged blood specimens), regardless of whether centrifugation is performed in the field or at the SHL. ROC Staff should adhere to the following recommendations when performing centrifugation:

- Recommended temperature range for centrifugation is 15º C to 25º C.
- Each portable centrifuge should have a small thermometer affixed to the outside lid.

- Plan for use of balance tubes, as appropriate.
- The Unico® Power Spin LX is variable speed and the setting for “BLOOD” should be selected for the 15 minute spin cycle. This “BLOOD” setting speed is approximately 3300-3500 RPM or 1400 maximum RCF.
- The Unico® Porta Spin is fixed speed, therefore no speed setting needs to be selected for the 15 minute spin cycle. The pre-set spin speed is approximately 3300-3500 RPM or 1350 maximum RCF.

## 6.1 Centrifugation Equipment and Supplies

- PPE per local ROC Exposure Control Plan (e.g., gloves, lab coat, etc.)
- Hand sanitizer
- Bleach wipes
- Centrifuge with thermometer affixed to outside lid
- Balance Tubes
- Computer with bar code scanner that includes automated system for electronic data capture
- Biospecimen Tracking System - developed by the ROC IMS Hub to capture information for the ODE tables
    - Hard-copy PAPI or other backup mechanism for recording ODEs in the event that the automated Biospecimen Tracking System is inaccessible.

## 6.2 Procedures for Centrifugation

These procedures assume that the blood centrifugation process and the recording of information about the process are conducted in such a way that the biospecimen integrity is maintained and there is little or no delay in recording information in the Biospecimen Tracking System as soon as an activity is completed.

Note: Data such as location of centrifugation, staff centrifuging the specimen, as well as the date and time the specimen is centrifuged at the SHL may be automatically

captured in the Biospecimen Tracking System and will not need to be entered by the ROC data collector.

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Record ID of the centrifuge being used.
2. Refer to Table 3 to determine which blood tubes require centrifugation. For those tubes not centrifuged, ensure they are placed at the proper storage temperature (see Table 4 and Section 7).

**Table 3. Centrifugation specifications by study visit and blood tube type**

| Study Visit | Blood Tube Type | Label Extension | Centrifugation Within 2 Hours of Collection |
|---|---|---|---|
| Pre-pregnancy visit | 3 ml lavender top, prescreened | LP10 | |
| | 10 ml red top | RD10 | X |
| | 10 ml red top | RD11 | X |
| | 6 ml lavender top | LV15 | |
| First pre-birth visit (non-fasting) | 8.5 ml red/gray top SST | SS10 | X |
| | 10 ml red top | RD10 | X |
| | 5 ml clear top PPT | PP10 | X |
| | 6 ml lavender top | LV15 | |
| | 8.5 ml yellow top ACD | AD10 | |
| Second pre-birth visit (fasting) | 6 ml royal blue top, serum, prescreened | RS10 | X |
| | 8.5 ml red/gray top SST | SS10 | X |
| | 10 ml red top | RD10 | X |
| | 5 ml clear top PPT | PP10 | X |
| | 6 ml lavender top | LV15 | |
| | 2.5 ml clear top PAXgene™ | PX10 | |

| Study Visit | Blood Tube Type | Label Extension | Centrifugation Within 2 Hours of Collection |
|---|---|---|---|
| Birth visit | 3 ml lavender top, prescreened | LP10 | |
| | 10 ml red top | RD15 | X |
| | 10 ml red top | RD10 | X |
| | 6 ml lavender top | LV15 | |
| 6-month visit | 6 ml royal blue top, serum, prescreened | RS30 | X |
| | 8.5 ml red/gray top SST | SS30 | X |
| | 10 ml red top | RD30 | X |
| | 5 ml clear top PPT | PP30 | X |
| | 6 ml lavender top | LV30 | |
| | 2.5 ml clear top PAXgene™ | PX30 | |
| 12-month visit | 3 ml lavender top, prescreened | LP40 | |
| | 10 ml red top | RD30 | X |
| | 10 ml red top | RD31 | X |
| | 6 ml lavender top | LV30 | |
| 36-month visit | 3 ml lavender top, prescreened | LP40 | |
| | 8.5 ml red/gray top SST | SS30 | X |
| | 10 ml red top | RD30 | X |
| | 6 ml lavender top | LV30 | |
| | 2.5 ml clear top PAXgene™ | PX30 | |
| 60-month visit | 3 ml lavender top, prescreened | LP40 | |
| | 6 ml royal blue top, serum, prescreened | RS30 | X |
| | 8.5 ml red/gray top SST | SS30 | X |
| | 10 ml lavender top | LV50 | |

3. Prepare for centrifugation:
    - Before initiating centrifugation, verify that all tubes requiring centrifugation at the visit (PPT clear top tube, royal blue top serum tube, red top tube, and/or the SST red/gray top tube) are sufficiently clotted. Wait at least 30 minutes from time of collection for sufficient clotting to occur. If a blood tube has clotted, a large clump of cells will be visible and will adhere to the wall of the tube upon gentle inversion of the tube. It may take up to 45–60 minutes for a tube to clot completely.
        - If a tube has not clotted within 60 minutes of the blood draw, record this information as a centrifuge comment. Tubes should be handled as normal and centrifuged and shipped to the NCS Repository for special handling.
4. Place the tubes requiring centrifugation directly into the centrifuge.
5. Balance the blood tubes appropriately with balance tubes.
6. Close the lid securely and centrifuge for 15 minutes at 3300-3500 RPM.
7. Record centrifugation start time and date.
8. Upon completion of centrifugation, open the lid and remove each tube carefully.
    - Blood tubes should be stored at the appropriate temperatures, as soon as possible following centrifugation (see Section 7.0).
9. Record the centrifugation end time and date for all tubes.
10. If able to measure centrifuge temperature using the thermometer on the outside lid, record the centrifuge temperature in degrees Celsius.
    - If not able to measure the centrifuge temperature, record the reason.
11. Inspect each tube for hemolysis and record whether hemolysis is observed.
12. Record any additional comments, problems, or concerns about the centrifugation procedure; this would include if a tube was broken during centrifugation or if a tube did not clot.

# 7.0 Adult Blood Storage Procedures

Blood specimens will be stored until shipment to the NCS Repository in pressure bags at either refrigerated (2 - 10°C), frozen (-30°C or below), or ambient (15 - 28°C) temperatures depending on the blood tube type (see Table 4).

These procedures assume that the blood storage process and the recording of information about the process are conducted in such a way that the biospecimen integrity is maintained and there is little or no delay in recording information in the Biospecimen Tracking System as soon as an activity is completed.

Note: Data such as location of storage, staff storing the specimen, as well as the date and time the specimen is stored at the SHL may be automatically captured in the Biospecimen Tracking System and will not need to be entered by the ROC data collector.

**Table 4. Blood tube storage specifications by study visit and tube type**

| Study Visit | Blood Tube Type | Label Extension | Storage Temperature |
|---|---|---|---|
| Pre-pregnancy visit | 3 ml lavender top, prescreened | LP10 | Frozen |
| | 10 ml red top | RD10 | Refrigerated |
| | 10 ml red top | RD11 | Refrigerated |
| | 6 ml lavender top | LV15 | Refrigerated |
| First pre-birth visit (non-fasting) | 8.5 ml red/gray top SST | SS10 | Refrigerated |
| | 10 ml red top | RD10 | Refrigerated |
| | 5 ml clear top PPT | PP10 | Refrigerated |
| | 6 ml lavender top | LV15 | Refrigerated |
| | 8.5 ml yellow top ACD | AD10 | Ambient |
| Second pre-birth visit (fasting) | 6 ml royal blue top, serum, prescreened | RS10 | Refrigerated |
| | 8.5 ml red/gray top SST | SS10 | Refrigerated |
| | 10 ml red top | RD10 | Refrigerated |
| | 5 ml clear top PPT | PP10 | Refrigerated |
| | 6 ml lavender top | LV15 | Refrigerated |

| Study Visit | Blood Tube Type | Label Extension | Storage Temperature |
|---|---|---|---|
| | 2.5 ml clear top PAXgene™ | PX10 | Frozen |
| Birth visit | 3 ml lavender top, prescreened | LP10 | Frozen |
| | 10 ml red top | RD15 | Refrigerated |
| | 10 ml red top | RD10 | Refrigerated |
| | 6 ml lavender top | LV15 | Refrigerated |
| 6-month visit | 6 ml royal blue top, serum, prescreened | RS30 | Refrigerated |
| | 8.5 ml red/gray top SST | SS30 | Refrigerated |
| | 10 ml red top | RD30 | Refrigerated |
| | 5 ml clear top PPT | PP30 | Refrigerated |
| | 6 ml lavender top | LV30 | Refrigerated |
| | 2.5 ml clear top PAXgene™ | PX30 | Frozen |
| 12-month visit | 3 ml lavender top, prescreened | LP40 | Frozen |
| | 10 ml red top | RD30 | Refrigerated |
| | 10 ml red top | RD31 | Refrigerated |
| | 6 ml lavender top | LV30 | Refrigerated |
| 36-month visit | 3 ml lavender top, prescreened | LP40 | Frozen |
| | 8.5 ml red/gray top SST | SS30 | Refrigerated |
| | 10 ml red top | RD30 | Refrigerated |
| | 6 ml lavender top | LV30 | Refrigerated |
| | 2.5 ml clear top PAXgene™ | PX30 | Frozen |
| 60-month visit | 3 ml lavender top, prescreened | LP40 | Frozen |
| | 6 ml royal blue top, serum, prescreened | RS30 | Refrigerated |
| | 8.5 ml red/gray top SST | SS30 | Refrigerated |

| Study Visit | Blood Tube Type | Label Extension | Storage Temperature |
|---|---|---|---|
| | 10 ml lavender top | LV50 | Refrigerated |

## 7.1 Storage Equipment and Supplies

- PPE per local ROC Exposure Control Plan (e.g., gloves, lab coat, etc.)
- 6-slot tube sleeve
- Amber bag with absorbent pad
- Pressure bag, approx. 7” x 12”
- Styrofoam 2-tube shipper with absorbent pad – For yellow top ACD tubes only
- Refrigerator/freezer, 4°C/-20°C
- Refrigerator storage bin
- Freezer, -30°C
- Temperature monitoring and alarm system for freezer, refrigerator and room temperature
- Computer with bar code scanner that includes automated system for electronic data capture
- Biospecimen Tracking System - developed by the ROC IMS Hub to capture information for the ODE tables
    - Hard-copy PAPI or other backup mechanism for recording ODEs in the event that the automated Biospecimen Tracking System is inaccessible.

## 7.2 Storage Procedures for Refrigerated, Ambient, and Frozen Blood Tubes

### 7.2.1 Procedures for Storage of Refrigerated Blood Tubes

See Table 4 (Section 7.0) for list of blood tubes and their storage temperature by visit type.

*Refrigerated blood tubes should not be stored in a pressure bag with any other refrigerated biospecimens. For specific details on the number of biospecimens that can be stored in each pressure bag, refer to the Biospecimen Overview of Activities.*

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Put on a lab coat and gloves.
2. Place the blood tubes in the 6-slot tube sleeve.
3. Immediately place the tube sleeve inside the amber transport bag, seal the bag, and place it into a pressure bag.
    - Do not seal the pressure bag until the shipment to the NCS Repository is prepared. This is important because temperature threshold monitors will need to be placed inside the pressure bag before shipping.
    - Up to 12 blood tubes in 2 tube sleeves can be placed in one amber bag.
4. Store the pressure bag so that the blood tubes are in an upright position in the refrigerator storage bin until the shipment to the NCS Repository is prepared.
5. Record the storage information for every specimen in the pressure bag including the storage location where the specimen is stored and the time placed in storage.
6. If any problems, other than temperature events (Section 7.3), occur during the storage of the blood specimens prior to shipment, record a comment to describe the problem and resolution.
7. When the pressure bag is removed to be placed in a shipping container, record the date and time it is removed from storage.

### 7.2.2 Procedures for Storage of Ambient Blood Tubes

The yellow top ACD tubes are not centrifuged and are stored ambient in a Styrofoam 2-tube shipper in a pressure bag until shipment to the NCS Repository.

*Ambient blood tubes should not be stored in a pressure bag with any other ambient biospecimens. For specific details on the number of biospecimens that can be stored in each pressure bag, refer to the Biospecimen Overview of Activities.*

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Put on a lab coat and gloves.
2. Place the tube in a Styrofoam 2-tube shipper and close the lid.
    - Only include one blood tube per Styrofoam 2-tube shipper.
3. Place the 2-tube shipper into a pressure bag. Do not seal the pressure bag until the shipment is prepared.
4. Keep the pressure bag at ambient temperature until the shipment to the NCS Repository is prepared.
5. Record the storage information for every specimen in the pressure bag, including the storage location where specimen is stored and the time placed in storage.
6. If any problems, other than temperature events (Section 7.3), occur during the storage of the blood specimens prior to shipment, record a comment to describe the problem and resolution.
7. When the pressure bag is placed in a shipping container, record the date and time it is removed from storage.

### 7.2.3 Procedures for Storage of Frozen Blood Tubes

The 3 ml lavender top, prescreened tubes and the 2.5 ml clear top PAXgene™ tubes are not centrifuged and must be stored frozen in a pressure bag.

*Multiple types of frozen biospecimens may be stored together in the same pressure bag, as long as they are not overcrowded. Urine, saliva, and breast milk specimens should be stored upright; blood tubes should be stored on their side until they are frozen to reduce the possibility of tube breakage due to the expansion of tube contents during freezing. For specific details on the number of biospecimens that can be stored in each pressure bag, refer to the Biospecimen Overview of Activities.*

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Put on a lab coat and gloves.
2. Place the tube(s) to be frozen in a tube sleeve.
3. Place the tube sleeve into an amber transport bag.
4. Place the amber transport bag containing the blood tubes in a pressure bag. Do not seal the pressure bag until the shipment is prepared.
5. Store the blood tubes on their side in the pressure bag and place the pressure bag in the freezer storage bin.
    - Once the blood tubes are frozen, they may be placed in an upright position and should be kept frozen until the shipment to the NCS Repository is prepared.
6. Record the storage information for every specimen in the pressure bag including the storage location where the specimen is stored and the time placed in storage.
7. If any problems, other than temperature events (Section 7.3), occur during the storage of the specimens prior to shipment, record a comment to describe the problem and resolution.
8. When the pressure bag is removed from the freezer to be placed in a shipping container, record the date and time it is removed from storage.

## 7.3 Temperature Events

If during storage the location where the specimens are stored experiences temperatures outside the low or high limit described in Table 5, refer to the Biospecimen Overview of Activities for information on documenting temperature events in the Biospecimen Tracking System.

**Table 5. Upper and lower temperature limits for storage locations**

| Storage Location | Low limit | High limit |
|---|---|---|
| Biospecimen Refrigerator | 2°C | 10°C |
| Biospecimen Freezer | n/a | -25°C |
| Ambient temperature of SHL | 15°C | 28°C |

Refer to the Biospecimen Shipping Procedures for information on the shipment of biospecimens.

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# Appendix 1:

# Adult Blood Procedures for Centrifugation in the Field and Transport

## 1.0 Adult Blood Centrifugation Procedures

If the tubes must be centrifuged in the field in order to meet the 2-hour time requirement from blood collection to completion of centrifugation, follow the procedures below. After centrifugation and transport are complete, continue with receipt of blood tubes (Section 5) and storage of blood tubes (Section 7) of the Adult Blood Collection Procedures.

The type of blood tube used for collection and the potential analytes that will be measured in each tube determine whether a particular blood tube should be shipped to the NCS Repository as whole blood (i.e., non-centrifuged), or whether serum or plasma must be separated from blood cells (i.e., centrifuged) prior to shipping. Not all blood tubes are centrifuged prior to shipment; Table 1-1 displays centrifugation specifications by visit type and blood tube type. For those tubes not centrifuged, ensure they are placed at the proper transport temperature while the other tubes are centrifuged.

ROCs should centrifuge tubes with either the Unico® Power Spin LX or Unico® Porta Spin centrifuge (required by NCS for all centrifuged blood specimens) regardless of whether centrifugation is performed at the SHL, at the participant's home, or at another location. ROC staff should adhere to the following recommendations when performing centrifugation:

- Recommended temperature range for centrifugation is 15ºC to 25ºC.
- Each portable centrifuge must have a small thermometer affixed to the outside lid.
- Plan for use of balance tubes, as appropriate.
- It is recommend that centrifuges are not left in extreme hot or cold temperature conditions (e.g., in a car) before centrifugation.
- The Unico® Power Spin LX is variable speed and the setting for "BLOOD" should be selected for the 15 minute spin cycle. This "BLOOD" setting speed is approximately 3300-3500 RPM or 1400 maximum RCF.

- The Unico® Porta Spin is fixed speed therefore no speed setting needs to be selected for the 15 minute spin cycle. The preset spin speed is approximately 3300-3500 RPM or 1350 maximum RCF.

### 1.1 Centrifugation Equipment and Supplies

- PPE per local ROC Exposure Control Plan (e.g., gloves, lab coat, etc.)
- Hand sanitizer
- Bleach wipes
- Biohazard waste bag, approx. 19” x 24”
- Centrifuge with thermometer affixed to outside lid
- Balance Tubes
- Adult Blood Instrument – developed by the ROC IMS Hub according to the Adult Blood Instrument Specifications
- Hard copy PAPI back up forms are available on the NCS portal for collection of instrument data if an electronic data capture system is unavailable at an NCS visit.

### 1.2 Procedures for Centrifugation

These procedures assume that the blood centrifugation process and the recording of information about the process are conducted in such a way that the biospecimen integrity is maintained and there is little or no delay in recording information as soon as an activity is completed.

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. If blood will be centrifuged at the collection location, record the following:
    - That it will be centrifuged at the collection location.
    - Equipment ID of the centrifuge
2. Refer to Table 1-1 to determine which blood tubes require centrifugation.

**Table 1-1. Blood centrifugation specifications by study visit and blood tube type**

| Study Visit | Blood Tube Type | Label Extension | Centrifugation Within 2 Hours of Collection |
|---|---|---|---|
| Pre-pregnancy visit | 3 ml lavender top, prescreened | LP10 | |
| | 10 ml red top | RD10 | X |
| | 10 ml red top | RD11 | X |
| | 6 ml lavender top | LV15 | |
| First pre-birth visit (non-fasting) | 8.5 ml red/gray top SST | SS10 | X |
| | 10 ml red top | RD10 | X |
| | 5 ml clear top PPT | PP10 | X |
| | 6 ml lavender top | LV15 | |
| | 8.5 ml yellow top ACD | AD10 | |
| Second pre-birth visit (fasting) | 6 ml royal blue top, serum, prescreened | RS10 | X |
| | 8.5 ml red/ gray top SST | SS10 | X |
| | 10 ml red top | RD10 | X |
| | 5 ml clear top PPT | PP10 | X |
| | 6 ml lavender top | LV15 | |
| | 2.5 ml clear top PAXgene™ | PX10 | |
| Birth visit | 3 ml lavender top, prescreened | LP10 | |
| | 10 ml red top | RD15 | X |
| | 10 ml red top | RD10 | X |
| | 6 ml lavender top | LV15 | |
| 6-month visit | 6 ml royal blue top, serum, prescreened | RS30 | X |
| | 8.5 ml red/gray top SST | SS30 | X |
| | 10 ml red top | RD30 | X |
| | 5 ml clear top PPT | PP30 | X |
| | 6 ml lavender top | LV30 | |

| Study Visit | Blood Tube Type | Label Extension | Centrifugation Within 2 Hours of Collection |
|---|---|---|---|
| | 2.5 ml clear top PAXgene™ | PX30 | |
| 12-month visit | 3 ml lavender top, prescreened | LP40 | |
| | 10 ml red top | RD30 | X |
| | 10 ml red top | RD31 | X |
| | 6 ml lavender top | LV30 | |
| 36-month visit | 3 ml lavender top, prescreened | LP40 | |
| | 8.5 ml red/gray top SST | SS30 | X |
| | 10 ml red top | RD30 | X |
| | 6 ml lavender top | LV30 | |
| | 2.5 ml clear top PAXgene™ | PX30 | |
| 60-month visit | 3 ml lavender top, prescreened | LP40 | |
| | 6 ml royal blue top, serum, prescreened | RS30 | X |
| | 8.5 ml red/gray top SST | SS30 | X |
| | 10 ml lavender top | LV50 | |

3. Before preparing for centrifugation, place the blood tubes (except the yellow top ACD tube) that do not require centrifugation in a plastic clamshell. Put the plastic clamshell in an amber transport bag and place it at 2-10 °C. The 8.5 ml yellow top ACD tube should be placed in the Styrofoam tube holder and put in the ambient transport bag.
4. Prepare for centrifugation:
    - Before initiating centrifugation, verify that all tubes requiring centrifugation at the visit (clear top PPT tube, royal blue top serum tube, red top tube, and/or the red/gray top SST tube) are sufficiently clotted. Wait at least 30 minutes from time

of collection for sufficient clotting to occur. If a blood tube has clotted, a large clump of cells will be visible and will adhere to the wall of the tube upon gentle inversion of the tube. It may take up to 45–60 minutes for a tube to clot completely.

- If a tube has not clotted within 60 minutes of the blood draw, record this information as a centrifuge comment. Tubes should be handled as normal and centrifuged and shipped to NCS Repository for special handling.

5. Place tubes requiring centrifugation directly into the centrifuge.
6. Balance the blood tubes appropriately with balance tubes.
7. Close the lid securely and centrifuge for 15 minutes at 3300-3500 RPM.
8. Record centrifuge start time and date.
9. Upon completion of centrifugation, open the lid and remove each tube carefully.
   - Blood tubes should be stored at the appropriate temperatures, as soon as possible following centrifugation (see Section 7.0).
   - If a tube breaks during centrifugation follow the clean-up procedures (Section 3 of Appendix 1)
10. Record the centrifugation end time and date for all tubes.
11. If able to measure centrifuge temperature using the thermometer on the outside lid, select “Temperature” and record the centrifuge temperature in degrees Celsius.
    - If not able to measure the centrifuge temperature, record the reason:
      - If unable to measure the temperature because the thermometer is broken, record “Not able to measure – thermometer broken”.
      - If unable to measure the temperature because the thermometer was not available, record “Not able to measure – thermometer not available”.
      - If unable to measure the temperature for another reason, record “Not able to measure – other” and specify reason.
12. Inspect each tube for hemolysis and record information as applicable:
    - If all tubes helmolyzed, record “Yes, all tubes hemolyzed”.

- If at least one of the tubes hemolyzed and at least one of the tubes did not hemolyze, record “Yes, at least one tube hemolyzed and at least one tube did not hemolyze”.
- If none of the tubes hemolyzed, record “No, none of the tubes hemolyzed”.

13. Select all tubes in which hemolysis was noted.
14. Record any comments, problems, or concerns about the centrifugation procedure; this would include if a tube was broken during centrifugation or if a tube did not clot.

## 2.0 Adult Blood Transport Procedures Following Centrifugation

After centrifugation has taken place in the field, adult blood specimens will be transported in the biospecimen transport cooler to the SHL, where they will be receipted and stored until shipment to the NCS Repository, see Table 1-2. Considerations for transport of blood tubes:

- All adult blood specimens will be transported to the SHL at refrigerated temperature (2 - 10°C) following centrifugation, except for the yellow top ACD tube.
    - Refrigerated tubes will be transported in a plastic clamshell containing upper and lower threshold temperature monitors within an amber colored bag in the biospecimen transport cooler.
- If an 8.5 ml yellow-top ACD tube was collected at the visit, this tube will be transported at ambient temperatures (15 – 28 °C) in a Styrofoam tube holder with a low temperature monitor within the ambient transport bag.

### 2.1 Transport Equipment and Supplies

- PPE per local ROC Exposure Control Plan (e.g., gloves, lab coat, etc.)
- Hand sanitizer
- Plastic clamshell
- Upper (20°C) threshold temperature monitor
- Lower (2°C) threshold temperature monitor

- Amber bag with absorbent pad
- Pre-conditioned biospecimen transport cooler with:
  - Four -1ºC cold packs in refrigerated chamber
  - Digital thermometer, affixed to inside wall of refrigerated chamber
- Ambient transport bag with:
  - Styrofoam 5-tube holder
- Adult Blood Instrument – developed by the ROC IMS Hub according to the Adult Blood Instrument Specifications
  - Hard copy PAPI back up forms are available on the NCS portal for collection of instrument data if an electronic data capture system is unavailable at an NCS visit.

## 2.2 Procedures for Transport Post-Centrifugation

These procedures assume that the transport process following centrifugation and the recording of information about the process are conducted in such a way that the biospecimen integrity is maintained and there is little or no delay in recording information as soon as an activity is completed.

*Always use standard precautions when handling biospecimens. Refer to local ROC Exposure Control Plan.*

1. Put on a lab coat and gloves, if not already on.
2. Remove the amber bag from the refrigerated chamber (containing the non-centrifuged tubes) and immediately place the centrifuged tubes in the refrigerated clamshell. If necessary for the visit type, use a second refrigerated clamshell.
3. Locate a lower (2ºC) threshold temperature monitor, remove the adhesive, and place it on the outside of the refrigerated clamshell. Activate the monitor by pushing the button on the front. This monitor will be used to determine whether the temperature of the clamshell falls below 2ºC during transport.
4. Locate the upper (20°C) threshold temperature monitor. Remove the adhesive and affix it to the outside of the refrigerated clamshell (see Exhibit 1-1 for proper

placement). Activate the monitor by pushing the button on the back. "On" will be displayed in blue on the front of the monitor.

**Exhibit 1-1. Placement of threshold temperature monitors in the refrigerated clamshell**

5. Immediately place the refrigerated clamshell inside an amber transport bag to minimize exposure of the blood tubes to light, and seal the bag. If there are two clamshells, place each clamshell inside a separate amber bag.
6. Position the amber bag securely and upright within the refrigerated chamber of the transport cooler as shown in Exhibit 1-2.

**Exhibit 1-2. Proper packing of the refrigerated chamber**

7. If able to measure the temperature of the refrigerated chamber of the biospecimen transport cooler, select “Temperature” and record the temperature in degrees Celsius and whether it is positive or negative.
    - If not able to measure the temperature of the refrigerated chamber, record the reason:
        - If unable to measure the temperature because the thermometer is broken, record “Not able to measure – thermometer broken”.
        - If unable to measure the temperature because the thermometer was not available, record “Not able to measure – thermometer not available”.
        - If unable to measure the temperature for another reason, record “Not able to measure – other” and specify reason.
        - If there are not tubes that require refrigerated transport, record “Not applicable”.
8. Document the statuses of the upper and lower threshold monitors in the refrigerated chamber:
    - If the monitor was placed in the refrigerated chamber, record “Yes, in chamber”.
    - If the monitor is not required (e.g., tubes not collected) for the refrigerated chamber, record “No, not required”.
    - If the monitor was not placed in the refrigerated chamber, record “No, not available”.
9. When transporting the 8.5 ml yellow top ACD tubes at ambient temperatures, locate a lower (2°C) threshold temperature monitor, remove the adhesive, and affix it inside the Styrofoam ambient tube holder. Activate the monitor by pushing the button on the front. This monitor will be used to determine whether the temperature of the tube holder falls below 2ºC during transport.
10. Immediately close the lid of the ambient tube holder to minimize exposure of the blood tubes to light.
11. Place the Styrofoam ambient tube holder upright in the ambient transport bag and attach the bag securely to the cooler.
12. Record the status of the lower threshold monitor in the ambient tube holder:

- If the lower threshold monitor was placed in the ambient tube holder, record “Yes, in chamber”.
- If the lower threshold monitor is not required (e.g., tubes not collected) for the ambient tube holder, record “No, not required”.
- If the lower threshold monitor was not placed in the ambient tube holder, record “No, not available”.

13. Confirm the cooler is properly closed for transport.
14. Record any comments, problems, or concerns pertaining to the blood tube transport.
15. Record any other comments, problems, or concerns pertaining to the blood specimen collection.

## 3.0 Clean-Up Procedures for Home, Clinic, or Other Location Centrifugation

1. After completing the centrifugation procedures, unplug the centrifuge.
2. Clean the inside and outside of the centrifuge with bleach wipes. Any spills on the inside should be cleaned appropriately.
    - If a blood tube breaks during centrifugation, close the centrifuge lid securely and place the entire unit in an empty biohazard waste bag. Tie the biohazard waste bag closed and do not open the bag or attempt to remove the broken blood tube from the centrifuge until it has been transported safely to the SHL.
    - If a balance tube breaks during centrifugation, remove the blood tubes and store according to procedures. Do not attempt to remove the broken balance tube from the centrifuge until it has been transported safely to the SHL.
3. After performing clean-up procedures discard gloves and cleanse hands with hand sanitizer.
4. Be sure any trash is packed properly in the biohazard waste bag for transport. All supplies used for the blood collection should be placed into a biohazard waste bag for disposal as medical waste at the SHL.

After centrifugation and transport to the SHL, continue with receipt (Section 5) and storage of blood tubes (Section 7) in the Adult Blood Procedures.

# APPENDIX 2:

# Administration of the Adult Blood Pre-Screening Instrument

## 1.0 Overview

Participants must answer a set of questions to determine their eligibility for blood collection. Eligibility is always assessed during the administration of the Adult Blood Instrument at the time of the blood draw. To aid in scheduling of staff or follow-up clinic visits for blood collection, ROCs may choose to also establish eligibility of participants before the blood draw through administration of the Adult Blood Pre-Screening Instrument. This is done either on the telephone before the blood collection or during the home visit.

## 2.0 Procedures for Administration of the Adult Blood Pre-Screening Instrument

These procedures assume that the process and the recording of information about the participant are conducted in such a way that the participant is comfortable, and there is little or no delay in recording information as soon as a response is provided. Information should be recorded in the Adult Blood Pre-Screening Instrument provided by the ROC IMS Hub.

1. Explain the blood prescreening procedures to the participant.
2. Read the following adult blood prescreening questions to the participant and record the responses.
    - *Do you have hemophilia or any bleeding disorder?*
        - Record the participant's response of "Yes," "No," "Don't know," or "Refused" to answer.
        - If the participant answers "Yes" or "Don't know," or refuses to answer, the participant will not be eligible for the blood collection.
    - *Have you had cancer chemotherapy within the past 4 weeks?*

- Record the participant's response of "Yes," "No," "Don't know," or "Refused" to answer.
- If the participant answers "Yes" or "Don't know," or refuses to answer, the participant will not be eligible for the blood collection.

3. If the participant answers "Yes" or "Don't know," or refuses to answer either question, indicate that he/she is not eligible for the blood collection and thank him/her for the time.
4. If the participant answers "No" to both questions, indicate that he/she is eligible for the blood collection. Thank the participant for answering the questions.
5. Record any comments pertaining to the administration of the Adult Blood Pre-Screening Instrument.
6. Proceed to scheduling the blood collection.

## APPENDIX 3:

## Field-by-Field Instructions for Adult Blood Collection Questions

Participants will be asked the following questions at all visits when blood is drawn. Information should be recorded in the Adult Blood Instrument provided by the ROC IMS Hub. Instructions for completing each item are provided below.

1. Do you have hemophilia or any bleeding disorder?
    - Record the participant's response of "Yes", "No", "Don't know", or "Refused".
    - If the participant answers "Yes" or "Don't know," or refuses to answer, blood will not be collected.
2. Have you had cancer chemotherapy within the past 4 weeks?
    - Record the participant's response of "Yes", "No", "Don't know", or "Refused".
    - If the participant answers "Yes" or "Don't know," or refuses to answer, blood will not be collected.
3. Have you had any problems with a blood draw in the past?
    - Record the participant's response of "Yes", "No", "Don't know", or "Refused".
    - If the participant answers "Yes," go to question 4.
    - If the participant answers "No," "Don't know," or refuses to answer, go to question 5.
4. What problems have you had with a blood draw in the past? (Mark all that apply)
    - Record the types of problems that the participant experienced during previous blood draws.
    - If the participant reports a problem that is not included in the list record Other-specify and briefly describe the problem.
    - If the participant refuses to answer or does not remember specifically what type of problem was experienced in the past, record and go to question 5.
5. When was the last time you had anything to eat or drink other than water?

- Record the date and time the participant reports last eating or drinking anything other than water.

6. Have you had sweetener or milk added to a drink, such as coffee or tea, in the last 8 hours?
    - Record the participant's response.
    - "Sweetener" includes sugar, honey, and flavored creamers. If the participant consumed an artificial sweetener in coffee, tea, or a diet soda, record "No."
7. Have you had alcohol such as beer, wine, or liquor in the last 8 hours?
    - Record the participant's response.
8. Have you chewed gum, or used breath mints, lozenges, cough drops, or other cough or cold remedies in the last 8 hours?
    - Record the participant's response.
9. Have you used antacid, laxatives, or anti-diarrheal medications in the last 8 hours?
    - Record the participant's response.
10. Have you taken a dietary supplement such as vitamins or minerals in the last 8 hours?
    - Record the participant's response.
11. Has a doctor ever told you that you had diabetes?
    - Record the participant's response.
    - If the participant answers "Yes", go to question 12.
    - If the participant answers "No" and is pregnant probe "*This includes gestational diabetes*". If the participant still answers "No" after probe, prepare to draw participant's blood.
    - If the participant is not pregnant and answers that she had gestational diabetes while pregnant, indicate that this does not include gestational diabetes and prepare to draw the participant's blood.
    - If the participant answers "No" and is not pregnant, prepare to draw the participant's blood.

12. Have you taken any insulin in the last 8 hours?

- Record the participant’s response and prepare to draw the participant’s blood.

## APPENDIX 4:

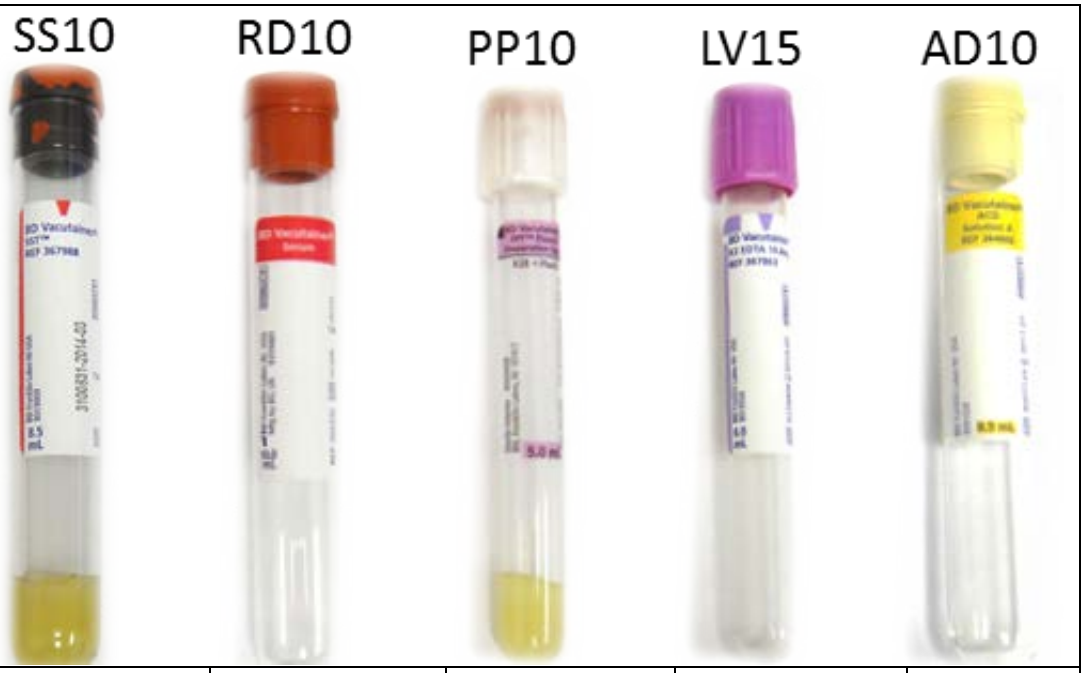
## Specifications for Blood Tubes used for Adult Blood Collection

**Table 1. Specifications for handling of adult blood tubes collected at the pre-pregnancy visit**

| | | | | |
|---|---|---|---|---|
| **Blood tube picture** |  |  |  |  |
| **Tube type** | 3 ml Lavender top, prescreened | 10 ml Red top | 10 ml Red top | 6 ml Lavender top |
| **Transport temp. prior to centrifugation** | Refrigerated | Refrigerated | Refrigerated | Refrigerated |
| **Centrifugation within 2 hours** | | X | X | |
| **Transport temp. after centrifugation** | Refrigerated, NC | Refrigerated | Refrigerated | Refrigerated, NC |
| **Storage temperature** | Frozen | Refrigerated | Refrigerated | Refrigerated |

NC denotes tube is transported at this temperature but not centrifuged

**Table 2. Specifications for handling of adult blood tubes collected at the first pregnancy visit (PV1)**

| | | | | | |
|---|---|---|---|---|---|
| **Blood tube picture** |  | | | | |
| **Tube type** | 8.5 ml Red/gray top SST | 10 ml Red top | 5 ml Clear top PPT | 6 ml Lavender top | 8.5 ml Yellow top ACD |
| **Transport temp. prior to centrifugation** | Ambient | Refrigerated | Ambient | Refrigerated | Ambient |
| **Centrifugation within 2 hours** | X | X | X | | |
| **Transport temp. after centrifugation** | Refrigerated | Refrigerated | Refrigerated | Refrigerated, NC | Ambient, NC |
| **Storage temperature** | Refrigerated | Refrigerated | Refrigerated | Refrigerated | Ambient |

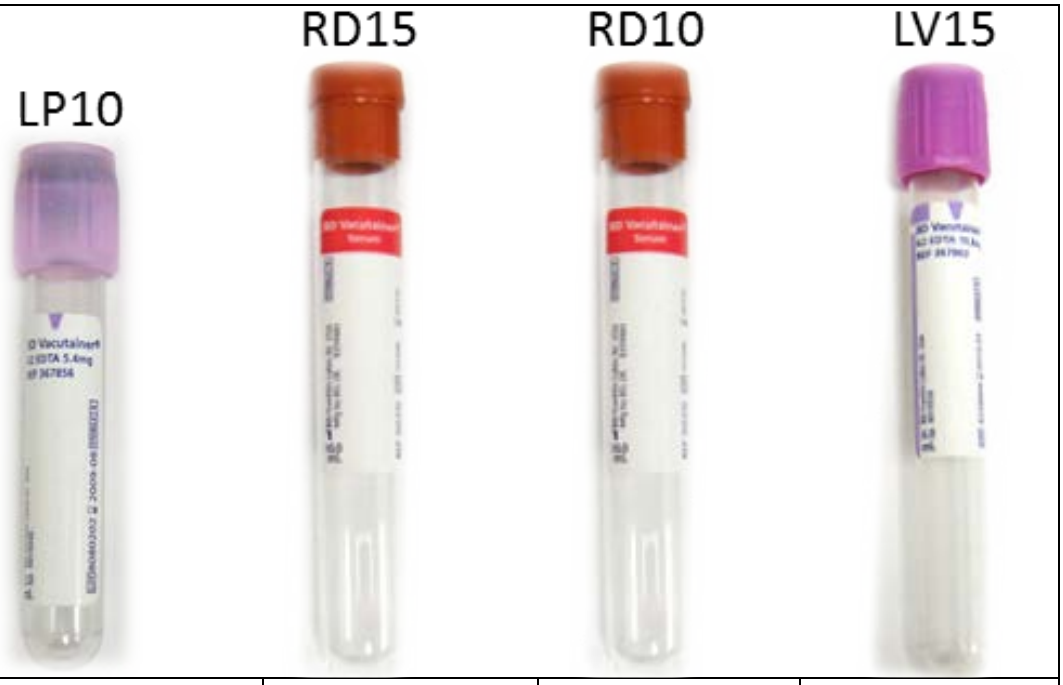
NC denotes tube is transported at this temperature but not centrifuged

**Table 3. Specifications for handling of adult blood tubes collected at the second pregnancy visit (PV2)**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Blood tube picture** |  |  |  |  |  |  |
| **Tube type** | 6 ml Royal blue top, serum, prescreened | 8.5 ml Red/gray top SST | 10 ml Red top | 5 ml Clear top PPT | 6 ml Lavender top | 2.5 ml Clear top PAXgene™ |
| **Transport temp. prior to centrifugation** | Refrigerated | Ambient | Refrigerated | Ambient | Refrigerated | Refrigerated |
| **Centrifugation within 2 hours** | X | X | X | X | | |
| **Transport temp. after centrifugation** | Refrigerated | Refrigerated | Refrigerated | Refrigerated | Refrigerated, NC | Refrigerated, NC |
| **Storage temperature** | Refrigerated | Refrigerated | Refrigerated | Refrigerated | Refrigerated | Frozen |

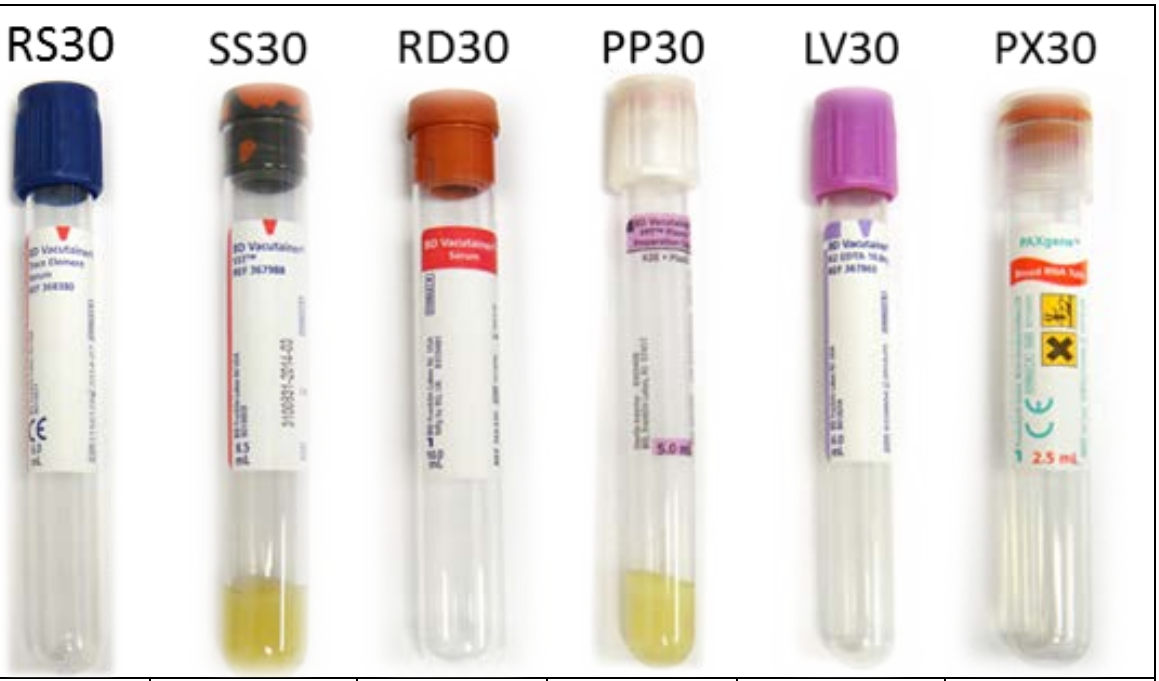
NC denotes tube is transported at this temperature but not centrifuged

**Table 4. Specifications for handling of adult blood tubes collected at the birth visit**

| | | | | |
|---|---|---|---|---|
| **Blood tube picture** |  | | | |
| **Tube type** | 3 ml Lavender top, prescreened | 10 ml Red top | 10 ml Red top | 6 ml Lavender top |
| **Transport temp. prior to centrifugation** | Refrigerated | Refrigerated | Refrigerated | Refrigerated |
| **Centrifugation within 2 hours** | | X | X | |
| **Transport temp. after centrifugation** | Refrigerated, NC | Refrigerated | Refrigerated | Refrigerated, NC |
| **Storage temperature** | Frozen | Refrigerated | Refrigerated | Refrigerated |

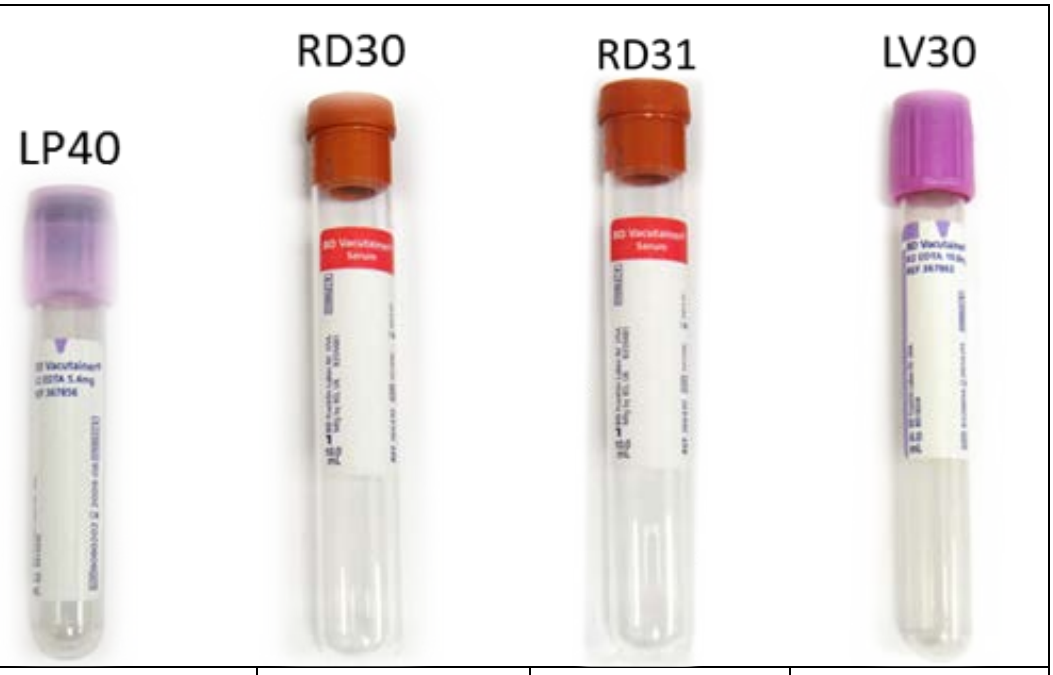
NC denotes tube is transported at this temperature but not centrifuged

**Table 5. Specifications for handling of adult blood tubes collected at the 6-month visit**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Blood tube picture** |  | | | | | |
| **Tube type** | 6 ml Royal blue top, serum, prescreened | 8.5 ml Red/gray top SST | 10 ml Red top | 5 ml Clear top PPT | 6 ml Lavender top | 2.5 ml Clear top PAXgene™ |
| **Transport temp. prior to centrifugation** | Refrigerated | Ambient | Refrigerated | Ambient | Refrigerated | Refrigerated |
| **Centrifugation within 2 hours** | X | X | X | X | | |
| **Transport temp. after centrifugation** | Refrigerated | Refrigerated | Refrigerated | Refrigerated | Refrigerated, NC | Refrigerated, NC |
| **Storage temperature** | Refrigerated | Refrigerated | Refrigerated | Refrigerated | Refrigerated | Frozen |

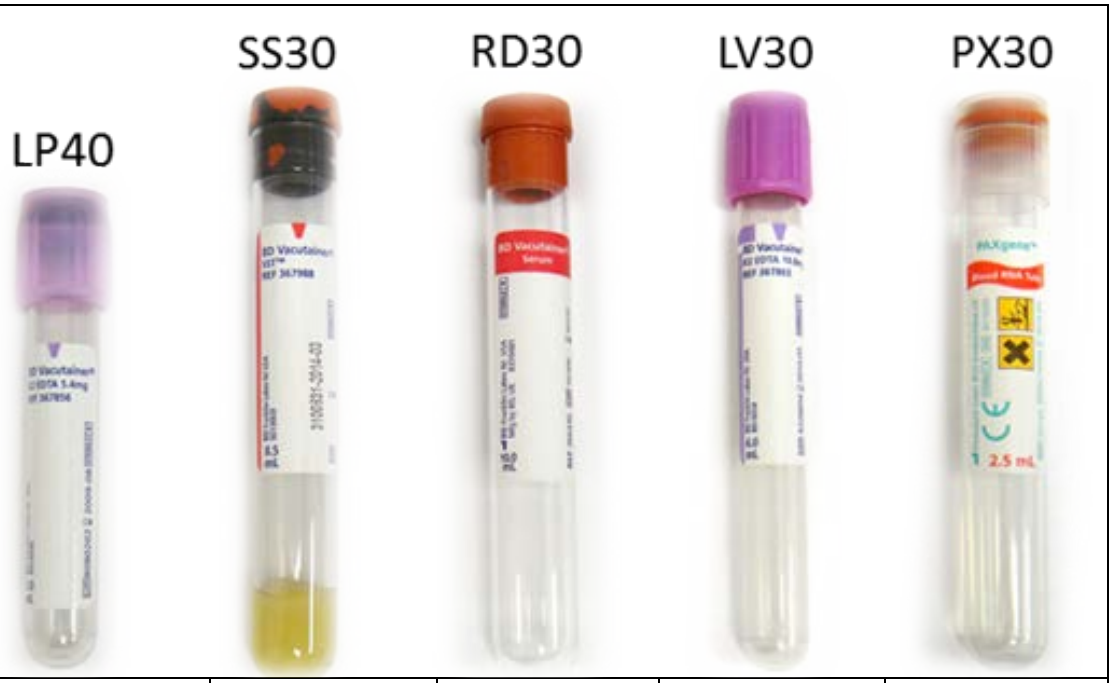
NC denotes tube is transported at this temperature but not centrifuged

**Table 6. Specifications for handling of adult blood tubes collected at the 12-month visit**

| | | | | |
|---|---|---|---|---|
| **Blood tube picture** |  | | | |
| **Tube type** | 3 ml Lavender top, prescreened | 10 ml Red top | 10 ml Red top | 6 ml Lavender top |
| **Transport temp. prior to centrifugation** | Refrigerated | Refrigerated | Refrigerated | Refrigerated |
| **Centrifugation within 2 hours** | | X | X | |
| **Transport temp. after centrifugation** | Refrigerated, NC | Refrigerated | Refrigerated | Refrigerated, NC |
| **Storage temperature** | Frozen | Refrigerated | Refrigerated | Refrigerated |

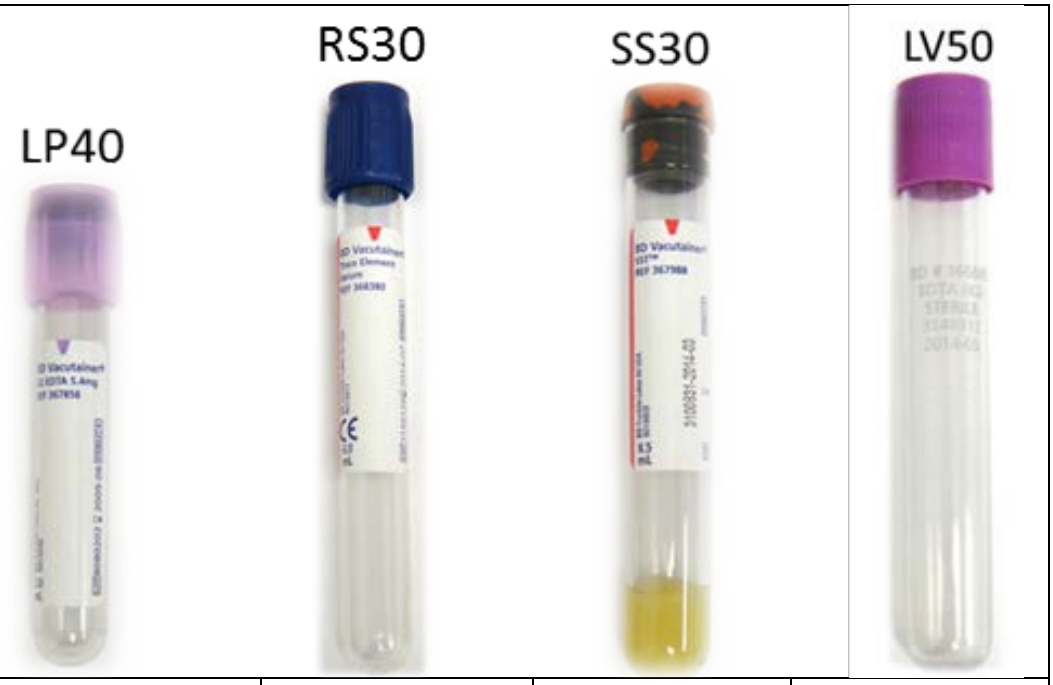
NC denotes tube is transported at this temperature but not centrifuged

**Table 7. Specifications for handling of adult blood tubes collected at the 36-month visit**

| | | | | | |
|---|---|---|---|---|---|
| **Blood tube picture** |  | | | | |
| **Tube type** | 3 ml Lavender top, prescreened | 8.5 ml Red/gray top SST | 10 ml Red top | 6 ml Lavender top | 2.5 ml Clear top PAXgene™ |
| **Transport temp. prior to centrifugation** | Refrigerated | Ambient | Refrigerated | Refrigerated | Refrigerated |
| **Centrifugation within 2 hours** | | X | X | | |
| **Transport temp. after centrifugation** | Refrigerated, NC | Refrigerated | Refrigerated | Refrigerated, NC | Refrigerated, NC |
| **Storage temperature** | Frozen | Refrigerated | Refrigerated | Refrigerated | Frozen |

NC denotes tube is transported at this temperature but not centrifuged

**Table 8. Specifications for handling of adult blood tubes collected at the 60-month visit**

| | | | | |
|---|---|---|---|---|
| **Blood tube picture** |  | | | |
| **Tube type** | 3 ml Lavender top, prescreened | 6 ml Royal blue top, serum, prescreened | 8.5 ml Red/gray top SST | 10 ml Lavender top |
| **Transport temp. prior to centrifugation** | Refrigerated | Refrigerated | Ambient | Refrigerated |
| **Centrifugation within 2 hours** | | X | X | |
| **Transport temp. after centrifugation** | Refrigerated, NC | Refrigerated | Refrigerated | Refrigerated, NC |
| **Storage temperature** | Frozen | Refrigerated | Refrigerated | Refrigerated |

NC denotes tube is transported at this temperature but not centrifuged