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**INTRODUCTION**

This Manual of Operations addresses the SHOW standards applied to the collection of bio-specimens from Survey Participants (SPs) and to the implementation of the SHOW Laboratory Operations. It is designed to standardize specimen collection and processing across teams and sites to ensure the quality and biological integrity of the collected samples in addition to assuring quality control measures are applied to this critical study component.

Using procedures in this manual, specimens from consenting participants are collected and processed by SHOW staff and transported for testing and storage, subject and specimen information are entered into the biospecimen database, and specimens are stored in the SHOW biorepository. Procedures specific to the storage of specimens in the biorepository are detailed in a separate Biorepository Manual.

**A. SURVEY SITE PREPARATION**

**1. Laboratory/Site Preparation**

This section details steps needed to prepare the space that is designated as the laboratory area. This may be in a permanent facility or in a designated sample collection site. Visits completed in a participant’s home require slight adjustments to these procedures.

Certified phlebotomists are handling blood and urine samples of SPs. Per Department of Transportation and International Air Transport Association (IATA) rules and regulations, these samples are considered non-regulated substances, unless SPs report infectious substances, e.g. TB, HIV, Hepatitis B and C, etc. See Bloodborne Pathogens (BBP) Exposure Control Plan, S:\MOO-MOPs\2018 MOO-MOP\3. Laboratory\Bloodborne Pathogens Exposure Control Plan 2018.doc:

All SHOW biosafety procedures must be followed, as outlined by the SHOW Biosafety Protocol B00000823, that can be accessed via ARROW, [https://arrow.wisc.edu/](https://arrow.wisc.edu/)

At the start of each week, the phlebotomist receives the participant folders for the week. The phlebotomist first confirms they have folders for all scheduled SPs. The phlebotomist then reviews the paperwork for each participant. The phlebotomist checks the folders for a copy of the signed consent form, looking over which procedure(s) the SP agreed to do, and confirms correct SPIDs on the labels, laboratory form and payment form.

**a. Supply Readiness/Stock Inventory**

At the end of each week, each phlebotomist conducts an inventory of their laboratory stock to be sure they have enough of the following supplies for handling the scheduled number or approximately 25 subject specimens in the following week.

Laboratory Stock contents:
- Non-Latex Gloves in various sizes
- Laboratory coats
- Safety glasses
- Safety shield
• Centrifuge
• Balancing tubes
• Tube racks for draw tubes
• Tube racks for cryovials
• Barcode labels
• Extra needles of the two sizes (21G and 23G BD Vacutainer Push Button Blood Collection Set)
• Marshfield Lab requisition forms
• Freezer and refrigerator thermometers, as appropriate
• Sharps containers
• Extra gauze (order when down to two packages)
• Extra alcohol wipes (order when down to two boxes)
• Hand Sanitizer
• 1mL Cryovials for serum and plasma (order when down to 2000)
• 2mL Cryovials for urine
• Cryovial freezer boxes
• PaxGene freezer boxes
• Plastic pipettes
• Band aids
• Tourniquets
• Sterile urine cups
• 15 ml transfer tubes
• 50 ml transfer tubes
• Cleansing wipes (for urine collection)
• Styrofoam container for dry ice
• Small cooler/Styrofoam containers for transport of specimens to Marshfield Labs drop off locations.
• BioHazard bags
• Unexpired collection tubes (extra tubes – need approximately -5-10 of each)
  ▪ Gold SST 5mL (gel barrier; Marshfield Laboratories provides)
  ▪ 10mL Redtops
  ▪ 3mL lavender tubes (Marshfield Laboratories provides)
  ▪ 10mL lavender tubes
  ▪ PAXgene Blood mRNA tube – 2.5mL blood volume
  ▪ Protein Paper Collection Cards for Blood Spots
• Enough SP kits for next week's appts, each contains:
  ▪ 1 Gold SST 5mL (gel barrier; Marshfield Laboratories provides)
  ▪ 2, 10mL Redtops
  ▪ 2, 3mL lavender tubes (Marshfield Laboratories provides)
  ▪ 2, 10mL lavender tubes
  ▪ 1, PAXgene Blood mRNA tube
  ▪ Vacutainers
  ▪ Alcohol wipes
  ▪ Gauze
  ▪ 2, 15 ml transfer tubes for pooling plasma and serum
  ▪ 1, 50 ml transfer tube for pooling urine sample
  ▪ 1mL Cryovials for serum and plasma
  ▪ 2mL Cryovials for urine
  ▪ Cryovial freezer box
• Plastic pipettes
• Band aids
• Tourniquet
• Sterile urine cup
• Cleansing wipes
• Dried blood spot kits
• Oragene saliva collection kits
• Large conical tubes

b. Area Preparation

Laboratory and phlebotomy counter preparation. At the start of each day, wearing non-latex gloves, the phlebotomist wipes surfaces with clinical-grade wipes.

Tube and vial preparation. All tubes and vials must be labeled before any samples are collected from the participant. This includes the urine specimen cup and bag, all vacutainer tubes, and all cryovials.

Site set-up. Exact site set-up will depend on the space and resources available in each sample collection site. Generally, SHOW staff should set-up the site to ensure processing is done as precisely and efficiently. In the ideal situation, three spaces are set-up, one for reviewing paperwork and asking SP pre-venipuncture questions, one for the venipuncture procedure, and one for processing samples. Three stations are recommended to increase participant comfort and ensure sample integrity but may not be possible in all spaces.

• Phlebotomy station
  o Clean area with clinical-grade (e.g. Kimtech) wipe
  o Put down lined absorbent pad (e.g. chux)
  o Set out all venipuncture supplies, including labeled tubes in proper draw order
  o Set out SP file
  o Ensure sharps container is within easy reach
  o Ensure gloves are within easy reach
  o Cover carpeting with plastic (or in homes, with lined absorbent pad)

• Processing station
  o Clean area with clinical-grade wipe
  o Put down lined absorbent pad
  o Place labeled cryovials in cryovial stand, with graduations facing out
    ▪ The most convenient set-up for cryovials in the stand is plasma in front with graduations facing front, urine in the middle with graduations facing front, and serum in the back with graduations facing back
    ▪ Leave lids on cryovials until as close to transfer as possible
  o Place labeled conical tubes upright in tube stand
  o Place labeled Marshfield transfer tube upright in tube stand
  o Place plastic pipettes on the absorbent pad, not in the tube stand
  o Ensure Marshfield form is within easy reach
  o Place timer where visible during processing
  o Ensure biohazard disposal bag (if using) and sharps container is within easy reach
  Cover carpeting with plastic

Restroom Preparation. At the start of each day, the phlebotomist checks the restroom for sufficient stock of paper towels and toilet paper. At the sample collection site, check that any pass through is in working order and that the restroom toilet, sink, countertop, and floor are clean and ready for SP use.
Site set-up in homes. Visits conducted in the home require slightly amended procedures to ensure safety and comfort. In general, travel time must be incorporated, staff must understand guidelines for making decisions about safety, and supply packing and preparation is critical. Specifically, the phlebotomist must set up on a flat surface, ideally a dining table but a counter or side table would work. Staff should confirm with the participant that the surface can be cleaned with a standard solution like Clorox Wipes. Absorbent pads should be placed in the draw area and on the floor beneath the draw area. After completion of venipuncture, staff should carefully and quickly repack supplies and leave as soon as possible in order to begin processing. More detailed instructions can be found at "S:\MOO-MOPs\2018 MOO-MOP\3. Laboratory\Home Visit Best Practices.docx".

2. Survey Participant (SP) Preparation

This section details preparations made and instructions given to subjects who are completing biospecimen collection processes.

The phlebotomist reviews each day’s schedule of SPs in advance as well as the consent signature pages to determine any possible specimen collection exclusions.

The phlebotomist is responsible for welcoming the SPs to the Sample Collection Site. The participants must be given enough time to feel comfortable, both before and after the blood collection. In many cases the most memorable part of the experience for the participant will be the contact with, and the attitude and competence of, the phlebotomist. Prior to the collection of biospecimen(s), staff assure consent for the donation of the specimen(s) by not only reviewing the written documentation but also affirming consent.

SP support, caring, listening, and observational skills ensure meeting the needs of the subject.

a. General Instructions

1. The phlebotomist greets and introduces themselves by name and title or role.
2. This phlebotomist verifies the SP’s name and checks it off on the schedule.
3. The surveyor confirms that the SP is or is not willing to give biospecimens and which ones, based on the indications on the consent signature page that was signed in the home.
4. If the SP’s wishes regarding biospecimens have changed since the consent, then the phlebotomist prepares to re-consent the SP prior to any further procedures.
5. The phlebotomist does any re-consenting necessary. There is no coercion of SPs to have blood drawn and the SP may at any time change his/her mind.
6. The phlebotomist confirms the SP’s SPID#, name with the name printed on the schedule, and that correct SPID# is on the labels.
7. The phlebotomist confirms SP fasting by asking the last time ate or drank anything.
8. The SP is seated while the venipuncture is performed.

b. Participant Preparation for Urine Specimen

- If SP is unable to urinate at the time, collect urine whenever possible. Encourage participants to stay hydrated even if they fasted for the visit. Participants who have difficulty producing a urine specimen may be offered a glass of water. However, do not collect samples after acute fluid load (>24 ounces) or after participant exertion.
• Female participants urinate directly into a specimen collection container, or they may use the Sage Commode Specimen and Measuring System #2500 for urine collection (follow instructions provided) if they prefer. Male participants should urinate directly into a specimen collection container.

Instructions to Participant. Participants are given a brown paper bag which contains the supplies needed to collect the specimen and on the outside of the bag are typed instructions for collecting the urine. The appropriate (male vs. female) instructions are verbally reviewed with the subject and the subject is directed to the appropriate restroom for completion of the procedure. The following are the typed instructions:

1. Wash hands thoroughly.
2. Remove the lid of the container being careful not to touch the inside: open the towelette packs.
3. WOMEN: Stand in a squatting position, using the towelette, wiping from front to back, cleanse the area around the urethra (where the urine comes out). Repeat with a clean wipe, throw used wipe in trash.
4. MEN: Using the towelette, wipe the end of the penis using a circular motion (the foreskin of an uncircumcised male must first be retracted. Repeat with a clean wipe, throw used wipes in trash.
5. Begin urinating in toilet.
6. Touching the outside of the container, bring the container into the stream until the container is half full.
7. Finish urinating in toilet.
8. Cover the container, wipe the outside of the container, and place back in the paper bag.
9. Bring specimen to SHOW staff member.

Instructions are detailed in appendices 4 and 5.

c. Subject Preparation for Blood Specimen Collection

• Nine tubes of blood of various sizes are collected, each containing about 1–2 teaspoons (5–10 ml) of blood. If participants are concerned about the volume of blood drawn, reassure them that the total amount of blood drawn is less than 3.5 tablespoons. The phlebotomist may also assure participants that more than six times as much blood (450 ml) is collected when they donate a unit of blood.

• The phlebotomist confirms participant fasting by asking the last time the participant ate or drank anything other than water.

Participants are not allowed to have consumed anything other than water to be considered fasting. They cannot consume diet soda, coffee or tea with or without cream or sugar or artificial sweeteners, flavored water, alcohol, gum, mints, lozenges, cough drops, cold remedies, antacids, anti-diarrheals, laxatives, or dietary supplements such as vitamins and minerals to be considered fasting.

• SPs that are extremely apprehensive about giving blood are reassured that the blood draw is designed to be as painless as possible. While checking SPs veins for best puncture site, the phlebotomist tries to draw the SP into conversation about themselves to help them relax and to build rapport. The phlebotomist provides positive statements upon finding good clear veins.
Phlebotomy in truly anxious SPs may need to be delayed to a later point in the visit. Under no circumstances is an SP forced or coerced into providing blood.

Assisting participants who are extremely apprehensive about giving blood. The phlebotomist explains to the SP that the blood draw is designed to be as painless as possible. The SP is asked to relax in their chair so the phlebotomist can check the veins in the participant’s arms, without actually drawing blood. If the SP has "good veins," the phlebotomist reassuringly says, "Oh, you have good veins; there should be no problem." If possible in the space, the phlebotomist also offers the option of lying down if the participant is still apprehensive or feels they may become dizzy or light-headed. It may help to let the participant return later for the blood draw or offer a visit to their home if that would help and is logistically feasible.

Assisting participants who look or feel faint
- This reaction is not unusual and does not require calling 911 unless there is a seizure or more serious reaction
- The phlebotomist has the SP remain in the chair and sit, if necessary, with head between knees until his/her color returns and he/she feels better.
- A basin is provided if the SP feels nauseated.
- A cold wet cloth may be placed on the back of the neck.
- If the SP faints, the phlebotomist will help them to the floor, removing anything in the way that may cause harm to the participant. The phlebotomist sits with the SP until he or she regains consciousness.
- If the person continues to feel ill, the Field Team Manager is consulted.
- If SP does not regain consciousness, 911 is called and an incident report form is completed.

B. BIOLOGICAL SPECIMENS COLLECTION AND PROCESSING

1. Urine Specimens

This section details the procedure for obtaining a urine sample that is a “clean-catch midstream” specimen (to minimize normal skin flora contamination) and the steps for processing and storage of this sample.

a. Definitions

- Sterile container: A container for specimens that is certified as being manufactured and packaged to prevent contamination of its interior surfaces with bacteria or viruses.
- Clean Catch: Method of obtaining a urine sample so that it is free of contaminating matter from the external genital area.

b. Equipment and Supplies

- 2 mL cryovials
- 50 ml transfer tube
- Cleansing wipes
- Sterile urine collection container
- Fine point pen to label container
• SPID Labels
• Restroom with hand washing supplies
• Clean-catch directions
• Sage Commode Specimen and Measuring System #2500 (optional)
• Pipettes
• Centrifuge

Forms:
• Laboratory Form (see Appendix 3)

c. Forms and Labeling

• The 20XX Laboratory Form must be labeled with the correct pre-printed barcode SPID label even if no specimens are collected. Complete the first column of the form noting in ink the status of the urine specimen and any comments on why refused, failed, not attempted, barrier, etc., are written in the last column on the right.

• A refusal to provide a urine specimen is documented on the laboratory tests form as is the reason for refusal.

• Urine samples are labeled throughout the collection and processing stages to ensure they are correctly coded. Always place each SP’s SPID labels on the collection container prior to collection and on the 2 mL cryovials just prior to filling.

d. Urine Processing

(This paragraph also repeated under B.2. Blood Specimens, d. Survey Participant Labels.)

SHOW HQ supplies each sheets of identification number (ID) 2D-coded labels with each SP folder, for labeling draw tubes, working tubes, and cryovials. The procedure for creating labels for the phlebotomists can be found at S:\MOO-MOPs\Office procedures\Current office procedures.

• 12 labels for the various, tubes, vials and urine containers
• 12 labels for the draw tubes, paper bags, and other labeling
• 12 labels for plasma 0.5ml
• 10 labels for serum 0.5ml
• 4 date/time labels for Marshfield Labs and 10ml lavender tubes
• 5 extra labels

• Verify the label is on the urine container and the SP is identified. Label 12 2mL cryovials with urine label.

• Pipette equal amounts of urine into two 15 ml conical centrifuge tubes and centrifuge it for 5 minutes at 3200 rpm. The ideal amount of urine in each conical tube is between 8-14 ml of urine. Do not use the breaking mechanism to slow the centrifuge as it can cause disruption of the sediment prior to decantation. All specimens must be centrifuged in capped tubes to prevent any biohazard aerosols.

• Transfer urine using a pipette into 50 ml tube being careful not to disturb the sediment at the
bottom of the conical centrifuge tube. If the sediment is disturbed, the sample must be re-spun and pipetted accordingly. Combine urine from both 15 ml tubes into one 50 ml tube. Cap the tube and mix urine by gentle tube inversion 10 times. This process is necessary to ensure that every aliquot is representative of the whole sample.

- Pipette 1.5 mL of the centrifuged urine into each previously prepared 2mL cryovial, 12 total. Discard the remaining urine. Do not overfill the tubes. There must be room for the urine to expand when frozen.
- Freeze and store 2mL vials upright in cryovial box on dry ice or in provided -20°C freezer for later transfer to the biorepository’s -80°C freezer. If using dry ice, be sure samples are covered completely and there is a layer of dry ice on the bottom of the styrofoam container.

**e. Forms Completion**

- It is important that the form is completed concurrently with individual steps of procedure being completed, especially documentation of times.
- See the urine sample field on the Laboratory Form. Complete first column (status) as to whether samples were:
  - (D) donated
  - (R) refused; SP refused to give urine sample
  - (F) failed to provide; SP was unable to provide sample when attempted
  - (NA) not attempted; SP was willing, but it was not attempted for some reason
- Enter the amount of urine in the centrifuge tube in the middle column of the form.
- Explain any refusals (R) or not attempted (NA) or other difficulty with urine collection in handwritten comments.

Refer to Section, **C. BIOSPECIMEN DISTRIBUTION**, for protocols on packaging and shipping urine (and blood) samples to SHOW headquarters and eventually to the biorepository.

**2. Blood Specimens**

Blood samples collected and processed by the SHOW lab phlebotomists are the foundation for all biological testing to be completed immediately, and for storage and future unspecified research. The most important steps—and potentially the most variable—are the collection and processing of the blood samples. If samples are not correctly drawn and processed, the laboratory results may be compromised.

Phlebotomists are assigned to the procedure of biospecimen collection and processing according to protocol. Various members of the SHOW team, including the Associate Director, the Administrative Program Manager, the Biobank Director, the Phlebotomy Supervisor and the Field Team Supervisor, are responsible for monitoring quality control at the SHOW sites and through review of the data forms. Marshfield Laboratories (MLab) is responsible for checking sample conditions upon arrival at the lab, performing the immediate assays, and reporting results.

**Related documents**
a. Definitions

- **Bloodborne pathogens**: pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV), hepatitis C virus and human immunodeficiency virus (HIV).

- **Contaminated sharps** mean any contaminated object that can penetrate the skin including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed ends of dental wires.

- **Contaminated** means the presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

- **Personal protective equipment** is specialized clothing or equipment worn by an employee for protection against a hazard. General work clothing (e.g., uniforms, pants, shirts, or blouses) not intended to function as protection against a hazard are not considered to be personal protective equipment.

- **Universal precautions** are an approach to infection prevention and control. According to the concept of universal precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, and other bloodborne pathogens.

- **Hemolysis** is the rupturing of red blood cells and the release of their contents into surrounding fluid (blood plasma). This can be caused by improper technique during collection of blood specimens, by the effects of mechanical processing of blood. This includes improper tube mixing and incorrectly filled tubes.

b. Equipment and Supplies

Equipment is mobile to designated sample collection sites throughout the state. Most of the laboratory supplies will be stored in plastic totes, with the centrifuge safely stored in a padded box or tote. The necessary supplies and equipment include the following:

- Lab coats and gloves
- Chair/folding stool – provided or on-site
- Plastic cart with wheels (or plastic tray with compartments) for supplies
- Blood tube racks
- Lab mats and wipes
- Participant ID labels
- Pens
- Unexpired collection tubes
- Tourniquets
- 21-gauge Butterfly needles with adapter (BD #7251)
- Vacutainer holders
- Timer/stopwatch
- Scissors
- Forceps for grasping caps if needed
- Surgical tape/paper tape
- Band aids
- Gauze (2x2-inch)
- Blood spill kit
- Eye wash kit
- Biohazards waste container
- Needle/sharps container
- Brawny paper towels
- Clinical grade wipes or a spray bottle of approved biohazard disinfectant solution

c. Safety Issues and Precautions for Handling Blood Specimens

In accordance with the Occupational Safety and Health Administration (OSHA) regulations on bloodborne pathogens (BBP), the SHOW phlebotomist is trained to follow the standard laboratory safety protocol as outlined below. Also see the BBP Exposure Control Plan.

- Always use non-permeable lab coats, latex gloves, and face shields when handling any blood in any situation in which splashes, spray, spatter, or droplets of blood may be generated and eye, nose, or mouth contamination can be reasonably anticipated.
- Follow 'Universal Precautions' when handling any blood products.
- Immediately place contaminated needles and sharps in a puncture-resistant, leak-proof container. Never recap or break needles.
- Decontaminate work surfaces at least once a day and after any contact with blood or other potentially infectious material with clinical grade wipes or other EPA-approved disinfectant.
- Hepatitis B vaccine is offered to all unvaccinated phlebotomists who handle blood. Documentation of vaccination, or phlebotomist’s refusal to be vaccinated, is kept on file at the SHOW headquarters.
- Maintain training records on each employee documenting phlebotomy and laboratory training. Records are kept at the SHOW headquarters in personnel files. Maintain a sharps injury log for each phlebotomist which includes where and how an injury occurred, type and brand of device involved. A sharps injury or other blood exposure must be reported on the day of the event to the Phlebotomy supervisor and Principal Investigator. PI will submit a report of biological exposure via: https://ehs.wisc.edu/first-report-of-biological-exposure-or-release-event/

d. Survey Participant Labels

1. SHOW HQ supplies (ID) 2D-coded labels with each SP folder.
   - 12 labels for the various, tubes, vials and urine containers
   - 12 labels for the draw tubes, paper bags, and other labeling
   - 12 labels for plasma 0.5ml
   - 10 labels for serum 0.5ml
   - 4 date/time labels for Marshfield Labs and 10ml lavender tubes
2. Each set of participant barcode labels has the same sample ID number. Labels are applied on the long side (on the length) of the tube, not wrapped around the tube.

3. Blood samples must be precisely labeled prior to the collection and processing stages to ensure they are correctly coded.

4. Once the SP confirms that they will have a blood draw, select a sheet of MLab accession labels and place one accession label on the laboratory form to link it to the SP.

5. Cross-check the labels with each participant’s ID number prior to venipuncture.

6. Labeled blood collection tubes are placed in a tube holder system. This tray may be temporarily labeled with the SP’s name and SPID# in order to keep specimens of multiple SPs separate and clearly identified.

e. Laboratory Forms

1. The Laboratory Form provides a vital link between the various sample ID numbers and the SPID numbers and facilitates the efficient collection of urine, central laboratory, plasma, serum, whole blood (for mRNA) and DNA samples. In addition, the Laboratory Form facilitates the monitoring of phlebotomy and other quality assurance parameters and provides information critical to the interpretation of the assay results.

2. Formerly, the Laboratory Inventory and Activity Form documented the sample aliquots for entry into the biorepository data system, documented samples selected for quality control (QC) and linked real SPID of the donor to the QC SPID. Transfer of samples to Prevention Genetics was also noted on this form. As of December 2018, use of the Laboratory Inventory and Activity Form, also called the Sample Activity Log, was discontinued due to implementation of FreezerWorks.

f. Venipuncture Procedure

Preparation

1. Venipuncture is performed with fully gloved hands.

2. Approximately 51 mL of blood will be drawn from each participant and collected into eight tubes. The order in which the tubes are collected is extremely important and must be done as follows:

   - Tube 1: 5 mL Gold top (SST with gel barrier – 1 tube for MLab
   - Tube 2: 10mL Red top – for serum
   - Tube 3: 10mL Red top – for serum
   - Tube 4: 10 mL EDTA lavender top – for plasma and DNA
   - Tube 5: 10 mL EDTA lavender tops – for plasma and DNA
   - Tube 6: 3mL EDTA lavender top – for MLab for CBC
   - Tube 7: 3mL EDTA lavender top – for MLab for HgA1C
   - Tube 8: 2.5mL PAXgene red top – for mRNA
Also reference the Biospecimen Processing Outline, Appendix 1.

3. Venipuncture is standardized for the **sitting position**. Phlebotomists may have participants clench their fists (moderately) during phlebotomy for up to two minutes. Venipuncture is typically performed with 21-gauge butterfly needles but 23g butterfly needles are available for individuals with very small veins.

4. Phlebotomist **arranges draw tubes in order of draw** in the tube rack within easy reach. Phlebotomist assembles butterfly apparatus and vacutainer holders, gauze, and alcohol prep prior to tourniquet application.

5. Phlebotomist **applies tourniquet**.

6. Phlebotomist examines participant’s arms for the **best site for venipuncture**. **Release tourniquet**.

7. Phlebotomist cleanses venipuncture site by **wiping with alcohol prep pad in a circular motion from center to periphery**. Allow area to dry. Do not attempt to dry the area by fanning or blowing on the site. **Do not touch the site after cleaning**.

**Venipuncture**

8. **Tourniquet is re-applied**. Phlebotomist documents start time. It is best to **release the tourniquet as soon as possible** after flow has been established. The tightened tourniquet should be on **no longer than two minutes**; if it is necessary to have it on longer than two minutes, phlebotomist loosens the tourniquet and then re-applies. However, this may result in cessation of blood flow, especially in sick and/or elderly participants, and may result in the need for a second venipuncture.

9. The participant’s arm is firmly grasped, **using thumb to draw the skin taut to anchor the vein**. The thumb should be one or two inches below the venipuncture site.

10. **With the needle bevel upward**, the phlebotomist inserts the needle into the vein in a smooth continuous motion. Venipuncture is performed with a 21-gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly has a small, thin-walled needle that minimizes trauma to the skin and vein. Using 12 inches of tubing allows **tubes to be changed without any movement of the needle in the vein**.

11. The participant's **arm is maintained in a flat or downward position** while keeping the **tube below the puncture site** when the needle is in the vein. It may be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.

12. The phlebotomist should avoid retouching the site after cleaning it. If it is absolutely necessary to re-palpate, the phlebotomist **MUST** change gloves and re-clean the puncture site. Cleaning the tip of the glove to palpate the collection site deviates from the Best Practice standard.
Blood Collection

13. The phlebotomist grasps the flange of the vacutainer holder and gently pushes the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle. (Minimize turbulence whenever possible. Small steps, such as slanting the needle in the vacutainer to have the blood run down the side of the tube instead of shooting all the way to the bottom, may result in significant improvement.)

14. The phlebotomist notes the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the draw. If the flow rate is very slow, the needle may not be positioned correctly. The phlebotomist will then move the needle slightly without causing discomfort to the participant. The needle may not be adjusted more than 2 times and any “digging” for placement is strictly prohibited.

15. If the collection tube does not fill, the phlebotomist will try another tube of the same type. (Partially-filled plasma tubes are not acceptable if less than two-thirds full. Partially-filled serum tubes are okay but will result in a reduced number of aliquots. If a tube is not completely filled, this will be clearly noted on the Laboratory Form.)

16. The phlebotomist keeps a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shut-off valve and cessation of blood flow. Do not vary pressure or reintroduce pressure after completion of the draw.

17. Each vacutainer is filled until the vacuum is exhausted and blood flow ceases. If a vacutainer tube fills only partially, the tube is removed, and another is attached without removing the needle from the vein. Tubes less than half full are not acceptable, but those for MLab should be sent if no full tube can be obtained.

18. The Phlebotomist assesses the subject's response to the blood draw by asking the subject how they are doing and checking for any sign of emotional or physical distress.

Completion of Blood Draw

19. When the blood flow ceases, the phlebotomist removes the tube from the vacutainer holder. The shut-off valve re-covers the point and stops blood flow until the next tube is inserted (if necessary).

20. Lavenders are to be gently inverted several times after being drawn (see 26. Mixing, Clotting Time, etc., below).

21. Tourniquet is released, if still applied.

22. To remove the needle, the phlebotomist lightly places clean gauze over venipuncture site. The needle is removed quickly, and pressure immediately applied to the site with a gauze pad. If using a butterfly needle and while the needle is still in the patient's arm, depress the black button to retract the needle. The needle will slide out of the venipuncture site and lock into place. Do not impede the device retraction. This safety device on a butterfly needle will prevent accidental needle sticks to the Phlebotomist. The participant is asked to hold the gauze
23. The phlebotomist applies a bandage either directly to the skin if bleeding has stopped, or over the gauze pad if bleeding continues.


25. Phlebotomist records on Laboratory Form the start and stop time of the venipuncture.

26. If the participant continues to bleed, the phlebotomist applies pressure to the site with a gauze pad. If necessary, tightly wrap a gauze bandage around the pad and leave in place for at least 15 minutes.

Post-Draw Handling

27. Mixing, Clotting Time, Centrifuge and Storage Time:
   - Tube 1 (Gold - SST) is inverted once and left to clot for 40 minutes at room temperature and then centrifuged 10 minutes.
   - Tubes 2 and 3 (Red - Serum) are not mixed but placed in rack at room temperature for at least 40 minutes, and then centrifuged within 1 hour for 10 minutes.  
     Note: Tubes 1, 2 and 3 are typically spun at the same time.
   - Tubes 4 and 5 (Lavender – 10 ml Plasma) are gently inverted 5-6 times immediately after draw, then 4-5 times more (inverted 10 times total) and centrifuged immediately for 10 minutes.
   - Tubes 6 and 7 (Lavender – 3 ml Plasma) are gently inverted 5-6 times before being placed in biohazard specimen bag and refrigerated immediately.
   - Tube 8 (PAX gene – 2.5mL RNA) is gently inverted 8-10 times before stored upright for a minimum of 2 hours, no more than 24 at ambient room temperature before transfer to a dry ice or a -20 freezer.

28. The phlebotomist cleans up the venipuncture area (if necessary) and disposes of needle and tubing in the appropriate biohazard sharps containers.

29. The phlebotomist completes remainder of the Laboratory Tests Form.

g. Special Considerations for Venipuncture

1. Modified draw. Some participants may choose to only have certain tests or specimens drawn (for example, no DNA or no blood, or no urine). If the participant chooses to have only certain tests completed, the SHOW phlebotomist notes this on the Laboratory Form and only draws and processes those specimens that are indicated. Please note that for those refusing DNA donations but consenting to all other procedures, SHOW still draws all the tubes including the large lavenders, but after removing the plasma for the biobank, the phlebotomist discards the remainder of the large lavender tubes that were meant for DNA extraction.

2. Difficulty venipuncture. If unable to get into the vein or get any blood, the phlebotomist will ask the SP if he/she can try again. If the SP refuses, a note is made on the Laboratory Form that the labs were not drawn and the reason. If the SP agrees to another venipuncture, a second attempt
may be made. If a third attempt is required, it must be completed by another staff member. **No more than three attempts are made** to obtain a blood sample and only if the participant agrees.

3. **Procedures for a difficult draw.** If a blood sample is not forthcoming, the following manipulations may be tried:

   - If there is a sucking sound, the phlebotomist may **turn the needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.**
   - If no blood appears, the phlebotomist may **move the needle slightly in hope of entering the vein. Do not probe.** If not successful, the tourniquet is released, and the needle removed. A second attempt can be made on the other arm.
   - The **phlebotomist may loosen the tourniquet and reapply it more loosely.** It may have been applied too tightly, thereby stopping the blood flow. The tourniquet should remain on for no longer than two minutes at a time.
   - Venipuncture will **not be attempted more than three times.**
   - The phlebotomist reassures the participants that **inability to obtain a clean venipuncture is not any sign of a medical problem** on their part.
   - If venipuncture is unsuccessful, the phlebotomist notes this on the Laboratory Tests Form.

4. **If a collection tube does not fill,** another tube of the same type is tried. Partially-filled plasma tubes (lavender) may be harder to process so every effort is made to fill them at least half full, but anything obtained is retained and the laboratory determines if they can utilize it. Serum tubes less than half full are acceptable but will yield a reduced number of aliquots. If the tube is not completely filled, this is noted on the Laboratory Form.

5. **If NO tubes are collected** (blood flow ceases, difficult venipuncture, etc.), this is noted on the Laboratory Tests Form. **Collection tubes are always to be filled in the order specified, except as noted, below.** If the SP is willing, another attempt is made to complete the draw, collecting only those tubes that were not filled in the first attempt.

**h. Processing Blood Specimens**

1. Phlebotomists begin initial processing according to the schedule outlined below. They must wear personal protective equipment (non-permeable lab coats, gloves, splatter shields) during processing.

2. A table of the processing for all the tubes is outlined in Appendix 1, Biospecimen Processing Outline. The instructions are by the priority of draw. **Note: serum/plasma should be removed from cell pellet within five minutes of centrifugation.**

3. **One (1) Gold top 5ml plastic SST tube** is used for chemistry tests (Serum Creatinine, Glucose, Total Cholesterol, and Total HDL-Cholesterol) run by Marshfield Laboratories within 48 hours.

   - This tube is **inverted once** and kept at **room temperature for 40 minutes of clot time (maximum of 60 minutes), then centrifuged for 10 minutes at 3200 rpm.**
   - Serum is transferred to the provided transfer tube. It is labeled with SPID and MLab barcode labels and a date\time SPID label. The tube is placed in a specimen biohazard bag and refrigerated until pickup by Marshfield Labs (MLab) or for drop off at MLab site.
4. **Two (2) 10 ml plastic Red top tubes** are used to collect serum for the SHOW Biorepository and the serum will be stored in small cryovials for use by future researchers. These tubes are the core of the Biorepository.

   - These **tubes are not inverted and are set to clot for 40 minutes and then centrifuged for 10 minutes at 3200 rpm.** Note: remove tubes from centrifuge as soon as it has completed spinning and place in rack so that tubes are upright, not leaning, to maintain buffy coat separation.

   - Serum is transferred using a pipette into a 15 ml tube being careful not to disturb the cell pellet at the bottom of the conical centrifuge tube. If the pellet is disturbed, the sample must be re-spun. Combine serum from both collection tubes into a 15 ml tube. Cap the tube and mix sample by gentle tube inversion 10 times. This process is necessary to ensure that every aliquot is representative of the whole sample.

   - Serum is pipetted off and deposited in 0.5mL quantities into 1mL cryovials labeled with SHOW SPID 2D-coded labels. Note that cryovials are only half full.

   - Re-centrifuge samples for 10 minutes if needed (“gelled-clot” has formed).

5. **Two (2) 10 mL plastic Lavender top tubes** are used to collect plasma for the SHOW Biorepository and the remaining buffy coat and red cells are frozen. One tube is sent to Prevention Genetics for DNA extraction and storage. The second tube is stored in the SHOW biorepository. Please note: **These tubes are drawn on all blood donors even if the Survey Participant has refused to donate DNA.** The processing phlebotomist checks the consent signature page again to identify DNA refusers and thus lavender tubes with the red cells are discarded after the plasma is removed. Only the most recently signed signature page should be used to determine this. These tubes are labeled with a SPID barcode and a date\time label.

   - These **tubes are inverted gently 10 times and centrifuged immediately for 10 minutes at 3200 rpm.**

   - Plasma is transferred using a pipette into a 15 ml tube being careful not to disturb the cell pellet at the bottom of the conical centrifuge tube. If the pellet is disturbed, the sample must be re-spun. Combine plasma from both collection tubes into a 15 ml tube. Cap the tube and mix sample by gentle tube inversion 10 times. This process is necessary to ensure that every aliquot is representative of the whole sample.

   - Plasma is pipetted off and deposited into 0.5mL quantities into 1ml cryovials labeled with SHOW SPID 2D-coded labels.

   - Remaining buffy coat and red cells are frozen in the upright position on dry ice or in local -20 °C freezer until transfer to -80°C freezer. Once frozen, tubes are placed in a small biohazard bag with the SPID and date written on the bag by a black permanent marker. Transfer to the -80 freezer in the SHOW Administrative office will occur weekly at the end of the collection site week.

6. **Two (2) 3mL plastic Lavender top tubes** are for the determination of the complete blood count (CBC) and Hemoglobin A1C (HbA1c). These tubes are provided by MLab. These are the **lowest priority tubes** and may not be drawn if the blood draw line collapses and venipuncture is not redone.

   - These **tubes are inverted gently 5-6 times, then immediately labeled. Both tubes are placed in specimen biohazard bags and refrigerated for pickup** by the MLab courier.
7. **The single PAXgene red top tube** is for collection of mRNA. The tube does not require processing beyond gentle inversion 8-10 times immediately upon the end of the draw and resting upright for a minimum of two hours, but not longer than 24 hours, at ambient temperature. The tube can then be placed in a -20 freezer or freezing on dry ice until transfer to -80°C freezer.

i. **Preparing aliquots**

1. Aliquots are prepared from serum, plasma and urine after transferring sample into 15- or 50-ml tube and adequate mixing. If two tubes of the same type are collected, sample from both tubes is transferred to one tube. This step is necessary to ensure that every aliquot is representative of the whole sample. Preparing aliquots involves removing the serum or plasma in small amounts (e.g., 0.5 mL) by pipette and placing it into the appropriate color-coded cryovials (provided). Color-coding is predetermined and used to identify sample type.

2. A new pipette is used for each tube type, i.e. one pipette for 10mL EDTA, one for 10mL red top, etc.

3. Plasma or serum of like tubes is pooled from the same participant.

4. Prior to 2018, SHOW did not specify minimum or maximum numbers of cryovials. In 2018 the procedure was changed to specify aliquot numbers to maximize long-term storage space. See item 8 for specific aliquot counts.

5. Collection and draw tubes are discarded in a red biohazard bag or sharps container after pooling and transfer is completed.

6. **Description of aliquots:**

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Type</th>
<th>Cryovial size</th>
<th>Number of aliquots</th>
<th>Color Code</th>
<th>Volume per Cryovial</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 and 3</td>
<td>10mL Red</td>
<td>1mL</td>
<td>10</td>
<td>Yellow Cap</td>
<td>0.5mL Serum</td>
</tr>
<tr>
<td>4 and 5</td>
<td>10mL Lavender</td>
<td>1mL</td>
<td>12</td>
<td>Red Cap</td>
<td>0.5mL Plasma</td>
</tr>
<tr>
<td>urine</td>
<td>2 ml</td>
<td></td>
<td>12</td>
<td>Blue cap</td>
<td>1.5 ml</td>
</tr>
</tbody>
</table>

7. **EDTA plasma cryovials.** Pool and transfer, by volumes specified in the table above, into **RED** topped cryovials. Cryovials are frozen in an upright position at -20°C or on dry ice until transfer to a -80°C freezer.

8. **Serum cryovials.** Pool and transfer serum at room temperature. Carefully pipette 0.5 mL of pooled serum into each of **YELLOW**-capped cryovials. Cryovials are frozen in an upright position at -20°C or on dry ice until transfer to a -80°C freezer.
j. Special Circumstances for Processing and Preparing Aliquots

1. If centrifugation cannot be performed within 40 minutes of collection, specimens are processed as soon as possible after that time. The time of collection and centrifugation is recorded by the phlebotomist on the Laboratory Form.

2. If serum and plasma cryovials cannot be frozen at -20°C or on dry ice within ten minutes of transfer to cryovials, the phlebotomist does it as soon as possible after that, with notation on the Laboratory Form.

3. If blood collection is incomplete, the tubes as drawn are used and as many cryovials of serum and plasma as possible are filled—note quantities on Laboratory Form.

k. Processing Completion

1. The Laboratory Form is part of each SP’s data file and must be kept with that file and be forwarded with all other participant paperwork to SHOW HQ. The Marshfield Laboratory Requisition/Order Forms incorporates all specimens collected on all SPs on that date prior to the pickup time. A separate requisition is used for those drawn after courier pick-up to assure next day pick-up. Copies of all Marshfield Laboratory Requisition/Order Forms are retained and sent to HQ at the end of each week.

Completed, frozen cryovials from each participant are packed into one freezer box. Frozen 2 mL urine tubes are placed in remaining space in freezer box. Samples from two SPIDs can be put into one box. No labeling of boxes is needed. All work areas are wiped down with clinical grade wipes or approved biohazard disinfectant.

2. Cryovials are arranged in their proper racks for the next day’s blood processing.

3. Data from the laboratory forms is entered into CASES AFTER processing and storing of all samples including PAX gene is completed. If PAXgene tube is placed into freezer/dry ice the next day after collection, please finish documenting this step on the form first and then enter into cases. This process records/sends information both to data management as well as the biorepository storage record system.

3. Blood Spots

Blood spots are collected when either < 5 mL of blood was collected in the 10 mL EDTA lavender top tubes or if a study participant refuses blood draw but is willing to provide blood spots. If a participant refuses DNA storage, they are still eligible to provide a blood spot.

The blood spot specimen should only be collected if:

- a) The SP refuses to have their blood drawn OR b) the SP had their blood drawn, but the volume is insufficient.

Related documents

- Laboratory Form
- Laboratory Inventory and Activity Form
a. **Equipment and Supplies**

- Latex gloves
- Survey Participant (SP) ID labels

b. **Preparation, Collection, and Processing**

- See [Dried Blood Spot Manual of Procedures](#) (Appendix 6)

4. **Saliva Specimens**

Saliva is collected from those subjects who are willing to provide a specimen for DNA storage for future research when either < 5 mL of blood was collected in the 10 mL EDTA lavender top tubes or if a study participant refuses blood draw but is willing to provide saliva. The saliva specimen contains buccal epithelial cells and white blood cells. These cells contain DNA, which will be extracted by Prevention Genetics and stored for future research.

The saliva specimen should **only** be collected if:

- The SP has indicated on the informed consent a willingness to give a DNA sample to the SHOW Biobank AND
- The SP refuses to have their blood drawn OR the SP had their blood drawn, but the volume is not enough. [Related documents](#)

- [Laboratory Tests Form](#)
- [Laboratory Inventory and Activity Form](#)

a. **Equipment and Supplies**

- Oragene® DNA Self-Collection Kit, TUBE Format OG-100
- Latex gloves
- Survey Participant (SP) ID labels

b. **Preparation**

- Saliva samples must be precisely labeled with SPID number and date to ensure they are correctly coded. Collection containers are to be labeled prior to collection.

c. **Preparation of Participants for Saliva Collection**

- The phlebotomist verifies that the participant has had nothing to eat or drink, and has not smoked or chewed tobacco in the 30 minutes prior to saliva collection. If they have, the collection is delayed until the time interval is met.

d. **Saliva Collection**

The following are the Oragene® instructions for saliva collection.
1. Remove the big cap from the top of the collection tube and ensure that the solution in the cap is clear and colorless. Be careful not to puncture or contaminate this cap (e.g., do not touch it with an ungloved hand, touch it to any surface, or cough or sneeze on it). Also, minimize the amount of time that the cap is off the tube to minimize the chance for contamination. Do not touch the inside of the tube.

2. Give the tube to the participant and ask that they spit into the tube until the amount of liquid saliva (not bubbles) reaches the level shown in picture #1 of the Oragene® Donor Instructions. The yield of DNA is increased with increasing saliva volume so error on the side of more saliva rather than less. Note: Instruct the participant to not touch the inside of the tube.

3. If the participant is having a difficult time making enough saliva, suggest that they close their mouth and wiggle their tongue or rub their cheeks.

4. Take the tube from the participant, holding it upright, and screw the cap back on the tube. Again, be careful not to touch the inside of the tube or cap. Closing the cap will puncture the seal on the inside of the cap and release the Oragene® solution so make sure you close the tube tightly.

5. Hold the container upright and unscrew the funnel from the tube by twisting the funnel counterclockwise.

6. Close the tube with the small cap.

7. Invert the tube 5 times.

8. Throw out the funnel and cap.
e. Forms Completion

- Complete the Laboratory Form noting the collection time and the reason the sample is being done.

f. Saliva Processing

- Freeze the saliva collection tube on dry ice or in provided -20°C freezer for later transfer to the biorepository’s -80°C freezer.

C. BIOSPECIMEN DISTRIBUTION

1. Biosafety

The Office of Biological Safety (OBS) provides a training program for handling biological materials with an emphasis on laboratories and research groups. All staff who will handle biological materials must complete the required trainings:

- Biosafety Required Training
- Biosafety 102: Bloodborne Pathogens for Laboratory and Research
- Biosafety 107: Centrifuge Safety
- Biosafety 205: Hazardous Materials Shipping – Infectious and Biological Substances
- Biosafety 206: Bio HazMat Shipping Packaging Workshop

Biosafety Required Training, Biosafety 102, Biosafety 107, and Biosafety 205 are completed online through UW’s Canvas System. Biosafety 206 is conducted in person. Instructions for registration and completion of this training can be found at https://ehs.wisc.edu/biosafety-training/.

After completion of the online trainings, individuals will be eligible for addition to the SHOW Biosafety Protocol. After completion of Biosafety 206, individuals will receive a Bio HazMat Shipping Certificate of Training which is valid for two years. These individuals are eligible to be certified by their employers to ship these substances in compliance with the DOT and IATA training requirements. Training Certificates will be sent via a pdf email attachment within ten days of completion of required training elements. The date of the certificate will reflect the date training is completed, not the date the email is issued.

2. Dry Ice Shipping and Handling

The following procedures document the safe storage, usage, and handling of dry ice. The main hazards of dry ice include burns and asphyxiation. Insulated gloves must be worn when handling dry ice. Use of dry ice in poorly ventilated areas can result in depletion of the oxygen level resulting in asphyxiation.

a. Definitions/Uses

- Dry ice is the solid form of carbon dioxide, non-combustible, available in flakes, pellets, or block form. Dry ice will sublime (vaporizes directly to the gas state) at a temperature of -78.5°C (-109.3°F) or higher.
Dry ice is commonly purchased from a commercial manufacturer. Dry ice is commonly used for short-term storage or to ship biological specimens. Material Safety Data Sheet for dry ice: http://www.safety.rochester.edu/restricted/msds.html

b. Personnel Affected

- SHOW lab personnel.
- Administrative staff working with the Biorepository.

c. Responsibilities

All SHOW personnel must follow the safe storage, usage, and handling of dry ice (see below). SHOW employees responsible for shipping packages containing dry ice must be properly trained in United States Department of Transportation (USDOT) shipping requirements and authorized by their employer (their department) to ship such packages. Training and recertification in HazMat Shipping and Handling is every two years.

d. Procedures

1. Dry ice is to be stored in a well-ventilated location and placed in a Styrofoam chest, insulated cooler, or a special cooler designed for the storage of dry ice.

2. Because of the thermal expansion of dry ice (one pound of dry ice produces about 250 liters of gaseous carbon dioxide), sufficient gaseous carbon dioxide can be released in a sealed container to cause an explosion. Dry ice is NEVER to be stored in any type of tightly sealed devices such as an ultra-low freezer or plastic/glass container.

3. Dry ice will sublimate about five to ten pounds every 24 hours (blocks last longer) in a typical storage cooler. Plan on purchasing dry ice as close as possible to the time needed. Typical order is for 30-80 lbs., dependent on length of time dry ice is needed.

4. Normal air is composed of 78% nitrogen, 21% oxygen, and only 0.04% carbon dioxide. Concentrations greater than 0.5% (5000 ppm) can become dangerous. Therefore, handle dry ice in well-ventilated locations.

e. Hazards and Precautions

1. **Burns/frostbite**: Dry ice can cause burns to the skin in short periods of times. Thermal gloves are to be used if it is necessary to handle dry ice.

2. **Suffocation**: Carbon dioxide is a simple asphyxiant. Always store dry ice in a well-ventilated area to minimize the buildup of carbon dioxide. Personnel must use caution should dry ice be stored in a deep cooler. Do not lean into the chest to obtain the dry ice.

3. **Explosions**: Placing dry ice into a tightly sealed container can permit enough gas build up to cause an explosion. Never place dry ice inside an ultra-low freezer or other enclosed space!

4. Placement of dry ice in rooms with little or no ventilation can result in a buildup of the carbon dioxide in the area. **Do not store dry ice in a confined area** such as walk-in coolers, refrigerators, freezers, closets, or cars/vans. A car window should be slightly open during
transport of samples.

5. The **Material Safety Data Sheet** for dry ice is available at: [http://www.safety.rochester.edu/restricted/msds.html](http://www.safety.rochester.edu/restricted/msds.html).


7. When using dry ice to ship materials, the **shipper must abide to all applicable shipping regulations**.

8. **Disposal of unneeded dry ice** is accomplished by:
   - Letting the unused portion sublimate (recommended for well-ventilated locations because it will occur over a period of several days and the ventilation will take care of the gas liberated);
   - NEVER dispose of dry ice in a sink, toilet or other drain (such action can destroy the structure because of the temperature difference);
   - NEVER dispose of dry ice in the trash or garbage; and
   - NEVER place unneeded dry ice in corridors (some corridors may not be well ventilated and the oxygen level can be reduced to low levels).

**f. Dry Ice Procurement**

1. Dry ice can be purchased by contacting:
   - **Continental Carbonics**
     4502 Helgesen Drive
     Madison, WI 53718
     Phone: (608) 223-0275
     FAX: (608) 223-0331
   
   - Request pounds required in (1) nuggets/pellets or (2) dry ice blocks (approximately 10 lb blocks). Pellets are typically requested.
   - Note: dry ice blocks will last longer before dissipating.

1. After-hours or when in urgent need, dry ice can be located in the **dry ice bin in the K4/S core area of UW Hospital and Clinics**.

   See **Dry Ice Procurement, Appendix 2**

3. **Specimen Distribution**

   This procedure details the per-subject distribution of bio samples.

   a. **Location of Samples**

   To **SHOW Biorepository via SHOW Headquarters**:
   Frozen 0.5 mL aliquots serum and plasma
Frozen 1.5ml aliquots urine
2 frozen 10mL lavender tube with buffy coat intact and most plasma removed
1 frozen PAXgene tube

To Central Laboratory (Marshfield Laboratories):
2 refrigerated 3mL lavender tubes
serum extracted from SST tubes

To Prevention Genetics/Via SHOW Headquarters:
1 frozen 10mL lavender tube with buffy coat intact and most plasma removed

b. Transport to SHOW Biorepository

- Specimens for SHOW Biorepository will be temporarily stored in -20°C freezer or on dry ice and transported frozen to long-term in the SHOW Biorepository at the University of Wisconsin-Madison.
- Prior to transport, all samples planned for transport must be audited and listed on a sample transport tracking form, found at S:\Biorepository\Sample transport.
- In SHOW Biorepository, samples are placed in freezer 21, McArdle building, room 507. The Sample transport tracking form can be left on the lab bench by the freezer or put into SHOW Biorepository Director in MSC, room 1083.

c. Transport to Marshfield Laboratories

- Samples for Marshfield Lab must be packaged for pick-up by courier within 24 hours of sample collection.
- Marshfield Lab will pick up blood samples every day but Sunday from permanent sites and prearranged remote sites. Blood samples drawn after courier pickup will be picked up the next pickup day at that site or from one of the permanent SHOW sites. Afternoon draws on Saturdays will require transferring specimens to Marshfield Lab in Waukesha, Marshfield, or Madison depending on location of operation that Saturday.
- Marshfield Laboratories will provide labels and requisitions. SHOW provides biohazard bags, absorbent cloth, and Ziplock transport bags.
- Note: It is very important to pack the Marshfield samples correctly in the cooler to minimize the likelihood of samples freezing. Place a barrier between the sample bag and the freezer packs and check the thermometer regularly.

d. Transport to Prevention Genetics

Specimens for Prevention Genetics will be temporarily stored in the SHOW Biobank -80°C freezer and batch shipped or transported frozen on dry ice to Prevention Genetics in Marshfield.
e. **Packaging Instructions for transport in vehicle**

All transport of specimens will follow the governing regulations promulgated by the International Air Transport Association’s Dangerous Goods Regulations-Packaging Instructions 650 and 904.

1. Styrofoam cooler is used to transport specimens to HQ and biorepositories.
2. Place approximately about 5-10 pounds of dry ice on the bottom of the cooler.
3. Place a layer of absorbent material on top of the dry ice, so that it will be between the dry ice and the freezer boxes containing the samples. This could be cardboard or other absorbent or cushioning material.
4. Collect the freezer boxes containing samples to be shipped and check the sample ID numbers against the Sample transport tracking from.
5. Put a rubber band around each cardboard freezer box containing samples before placing each box on top of the absorbent in the cooler. The rubber band helps prevent cryovial spill; the absorbent material is protective.
6. Place another layer of absorbent material on top of the sample freezer boxes.
7. Place the remaining dry ice on top of this last layer of absorbent material.
8. Place a layer of cardboard or other material on top of dry ice to prevent sublimation.
9. Secure the top of the Styrofoam cooler with tape or cinch the carrying straps so that the cooler lid cannot come off in an accident. Make sure that the top is NOT airtight sealed.
10. If transporting to biorepositories, place the Sample transport tracking from listing on the top in an envelope and tape it to the cooler.

D. **BIOSPECIMEN TESTING**

1. **Laboratory Tests and Alert Values**

Within 48 hours, Marshfield Laboratories completes the following chemistry tests on serum drawn in one gold top 5 ml plastic SST tube: **Serum Creatinine, Glucose, Triglycerides, Total Cholesterol, and Total HDL-Cholesterol**.

Marshfield Laboratories completes the following tests on plasma drawn in two, 3 ml plastic lavender top tubes containing the anticoagulant, ethylenediaminetetraacetic acid (EDTA): **complete blood count (CBC) and Hemoglobin A1C (HbA1c)**.

a. **Laboratory Alert Values**

No blood test results will be immediately available when an SP is in the Exam Center. All of the immediately reportable labs will be reported back to SHOW headquarters by Marshfield Laboratories where they will be reviewed by qualified SHOW staff. SHOW will then prepare reports to SPs to be sent.
back to them using the results reporting template (see **Survey Methods Manual of Operations**). Any blood test that is out of range will be flagged in the report. A written report will be sent to all SPs with these values so that the SPs may take them to their health care provider if they so choose. SHOW will not be providing reports directly to health care providers.

<table>
<thead>
<tr>
<th></th>
<th>Units</th>
<th>Reference Range for Women</th>
<th>Reference Range for Men</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White Cell Count</strong></td>
<td>x10⁹/L</td>
<td>4.1-10.9</td>
<td>4.1-10.9</td>
</tr>
<tr>
<td><strong>Red Cell Count</strong></td>
<td>x10¹²/L</td>
<td>3.85-5.05</td>
<td>4.15-5.55</td>
</tr>
<tr>
<td><strong>Hemoglobin</strong></td>
<td>g/dL</td>
<td>11.7-15.5</td>
<td>12.9-17.3</td>
</tr>
<tr>
<td><strong>Hematocrit</strong></td>
<td>%</td>
<td>35-46</td>
<td>38-51</td>
</tr>
<tr>
<td><strong>Platelet Count</strong></td>
<td>x10⁹/L</td>
<td>150-450</td>
<td>175-450</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td>mg/dL</td>
<td>70-99</td>
<td></td>
</tr>
<tr>
<td><strong>Glycohemoglobin (A1c)</strong></td>
<td>%</td>
<td>4.0-6.0</td>
<td></td>
</tr>
<tr>
<td><strong>Total Cholesterol</strong></td>
<td>mg/dL</td>
<td>100-200</td>
<td></td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td>mg/dL</td>
<td>40-59</td>
<td></td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>mg/dL</td>
<td>20.0-150.0</td>
<td></td>
</tr>
<tr>
<td><strong>Serum Creatinine</strong></td>
<td>mg/dL</td>
<td>0.7-1.3</td>
<td></td>
</tr>
</tbody>
</table>

b. **Extreme Values**

If Marshfield Labs staff identify an extreme value that requires immediate attention, SHOW staff are contacted by phone to follow-up. Staff obtain as much information as possible and work with the SHOW Medical Director to communicate findings to participants.

As of April 2019, this procedure is being updated. The most up to date procedure can be found at S:\MOO-MOPs\Critical values.

2. **Genetic Testing**

SHOW obtains permission from participants to do research on genetic material with an approval from the University’s Institutional Review Board. Internal and external scientists may analyze SHOW DNA samples to answer research questions.
# APPENDIX 1
## SHOW BIOSPECIMEN OVERVIEW

### SHOW BIOSPECIMEN PROCESSING OUTLINE 2018

<table>
<thead>
<tr>
<th>Priority of Draw</th>
<th>Tube #</th>
<th>Color</th>
<th>Size</th>
<th>Inversion</th>
<th>Clot Time</th>
<th>Centrifuge Time</th>
<th>Speed RPMS</th>
<th>Transfer what to what</th>
<th>Label</th>
<th>Storage Temp</th>
<th>Where To</th>
<th>When</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Gold SST</td>
<td>5 ml</td>
<td>Once</td>
<td>40 (60 min max)</td>
<td>10 min; re-centrifuge if needed for 10 min</td>
<td>3200</td>
<td>Serum transfer tube, then biohazard specimen bag</td>
<td>MLab SPID Date/Time</td>
<td>Bag and refrigerate</td>
<td>MLab</td>
<td>W/in 24 hours</td>
</tr>
<tr>
<td>2</td>
<td>2, 3</td>
<td>Red Top</td>
<td>10 ml</td>
<td>NONE</td>
<td>40 min</td>
<td>10 min</td>
<td>3200</td>
<td>Transfer to 15 ml tube and mix by inversion, then aliquot into 1 ml yellow-cap cryovials: 10 x 0.5 ml serum discard red cells</td>
<td>Label has: SPID Serum volume</td>
<td>Box and freeze</td>
<td>SHOW</td>
<td>Immediate</td>
</tr>
<tr>
<td>3a</td>
<td>4, 5</td>
<td>Lavender</td>
<td>10 ml</td>
<td>10 times</td>
<td>Centrifuge immediately</td>
<td>10 min</td>
<td>3200</td>
<td>Transfer to 15 ml tube and mix by inversion, then aliquot into 1 ml red-cap cryovials: 12 x 0.5 ml plasma</td>
<td>Label has: SPID Plasma volume</td>
<td>Box and freeze</td>
<td>SHOW</td>
<td>Immediate</td>
</tr>
<tr>
<td>4</td>
<td>6, 7</td>
<td>Lavender</td>
<td>3 ml</td>
<td>5-6 times</td>
<td>Immediately label, bag and refrigerate</td>
<td>2 tubes to biohazard specimen bag</td>
<td>SPID Date/Time</td>
<td>Freeze and bag</td>
<td>SHOW &amp; PG</td>
<td>Immediate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>PAXgene Red Top</td>
<td>2.5 ml</td>
<td>8-10 times</td>
<td>Sit upright at room temperature for 2-24 hours</td>
<td>SPID only</td>
<td>Freeze and bag</td>
<td>SHOW</td>
<td>W/in 24 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### ALTERNATIVE BLOOD COLLECTION - DRIED BLOOD SPOTS

| 3b | Blood spots | Blood spot card | Dry (4-24 hours) and store in Bitran bag | SPID only | Bag and freeze | SHOW | W/in 24 hours |

### ALTERNATIVE DNA COLLECTION instead of tubes 4,5 - SALIVA

| 3c | Oragen tube | Oragene DNA Self-Collectio n Tube | 5 times | NA | NA | Throw out the funnel and big cap | SPID only | Bag and freeze | SHOW & PG | Immediate |

### URINE

| Clean-catch urine | Urine cup | Centrifuge immediately | 5 min | 3200 | Transfer to 50 ml tube and mix by inversion, then aliquot into 2 ml blue cap cryovials: 12 x 1.5 ml urine | Label with: SPID 2D code Urine | Box and freeze | SHOW | Immediate |
APPENDIX 2:
DRY ICE PROCUREMENT

Dry ice can be procured by: (1) contacting Continental Carbonics or by (2) going to the K4/5 core area at UW Hospital and Clinics.

**Continental Carbonics**
4502 Helgeson Drive
Madison, WI 53718
Phone: (608) 223-0275
FAX: (608) 223-0331

SHOW customer number: **#10189**

- Request pounds required in (1) nuggets or (2) dry ice blocks (approximately 10 lb blocks)
- Note: dry ice blocks will last longer before dissipating
In Milwaukee area

Continental Carbonic Products, Inc.
4250 S Nevada Ave
St. Francis, WI 53235
Phone (414) 294-4330
Fax (414) 294-4335

https://www.continentalcarbonic.com/dry-ice-milwaukee-wisconsin.html
**APPENDIX 3:**
**CURRENT LABORATORY FORM**

**2018 LABORATORY FORM**

<table>
<thead>
<tr>
<th>ML Label: __________________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPID: ____________________________</td>
</tr>
<tr>
<td>____________________________________</td>
</tr>
</tbody>
</table>

Lab Date _____/_____/_______ labdate | Phlebotomist(#s):_________ lab020 |

| ___ Complete informed consent review with subject (Review bio-sample collection items, ensure the participant still consents to the procedures) |

| □ Provide t-shirt(s) to participant | SP decides which size they would prefer, selects sizes for their participating children |
| Confirmed Driver? part020 | Round Trip Mileage: ______ part030 | Does the participant need to be compensated for childcare? part040 |

| □ Yes | □ No | [ ]One Child ($12) |
| [ ]Three or more children ($19.50) |
| [ ]No Compensation |

| Other expenses: part050 |

| □ Bus | □ Parking | □ Taxi | □ None |
| Total Amount: $______ part060 |

| □ Yes | □ No |
| Have you returned your questionnaire booklet(s)? (SAQ/DHQ) forms? |

| □ Yes | □ No |
| Have you returned your actigraphs? |

Did the participant attempt to donate any samples for SHOW Core? [ ]Yes [ ]No lab410

Check any of the following that restricted choice of arm/vein lab010

| ___ Mastectomy/related | ___ Hematoma | ___ Burns, Scars, Tattoos | ___ Damaged Veins |
| ___ Shunt, Fistula or Graft | ___ Recent IV | ___ Cast | ___ Edema |
| ___ Obesity | ___ Skin sores |

When was the last time you ate or drank anything other than plain water? ______ lab040 <d> don’t know or <r> refused

Last ate: [ ] Yesterday | [ ] Today At what time? ____:____ (time, military) lab045 lab040@h
<table>
<thead>
<tr>
<th>BLOOD DRAWS</th>
<th>1st attempt time</th>
<th>Draw time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong><strong>:</strong></strong></td>
<td>lab050@_m1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TYPE and SIZE OF TUBE</th>
<th>Lab #/Label/Barcode</th>
<th>Tube status:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D=Done</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F=Failed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P=Partial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R=Refused</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NA=not</td>
</tr>
<tr>
<td></td>
<td></td>
<td>attempted</td>
</tr>
</tbody>
</table>

- **Reasons for partial, failure, not attempted:** poor veins, dehydration, loss suction on tubes etc.

<table>
<thead>
<tr>
<th>5mL SST Gold top for ML</th>
<th>ML Label Barcode SPID label SPID/date&amp;time label</th>
<th>lab060</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL Redtop for Repository</td>
<td>Barcode SPID label</td>
<td>lab070</td>
</tr>
<tr>
<td>10 mL Redtop for Repository</td>
<td>Barcode SPID label</td>
<td>lab080</td>
</tr>
<tr>
<td>10 mL Lavender for DNA</td>
<td>Barcode SPID label SPID/date&amp;time label</td>
<td>lab090</td>
</tr>
<tr>
<td>10 mL Lavender for DNA</td>
<td>Barcode SPID label SPID/date&amp;time label</td>
<td>lab100</td>
</tr>
<tr>
<td>3 mL Lavender for ML</td>
<td>ML Label Barcode SPID label SPID/date&amp;time label</td>
<td>lab110</td>
</tr>
<tr>
<td>3 mL Lavender for ML</td>
<td>ML Label Barcode SPID label SPID/date&amp;time label</td>
<td>lab120</td>
</tr>
<tr>
<td>2.5 mL Paxgene Redtop (mRNA)</td>
<td>Barcode SPID label SPID/date&amp;time label</td>
<td>lab125</td>
</tr>
</tbody>
</table>

| # of Attempted Sticks | ____ | lab130 |
| End draw time | ____:____ | lab140 |

| Plasma Centrifuge start time | ____:____ | lab150 |
| Serum Centrifuge start time | ____:____ | lab155 |

| Comments | _______ | lab160 |
|_________ | _______ | lab170 |

- **Reasons for partial, failure, not attempted:** poor veins, dehydration, loss suction on tubes etc.

<table>
<thead>
<tr>
<th>URINE SAMPLE</th>
<th>Lab200@a</th>
</tr>
</thead>
</table>

| Collection time | ____:____ | lab190@_h1 |
| Centrifuge Time | ____:____ | lab190@_h2 |

<table>
<thead>
<tr>
<th>(Barcode SPID label)</th>
<th>lmL urine centrifuged</th>
<th>Urine freezer time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Done</td>
<td>Refused</td>
<td>Failed</td>
</tr>
<tr>
<td>____</td>
<td>____</td>
<td>____</td>
</tr>
</tbody>
</table>

| ____ | ____ | lab210 |
| ____ | ____ | lab220 |

| ____ | ____ | lab230@h1 |

# of plasma vials (0.5 mL in each 1mL cryovial): | lab160 |

| ____ # of serum vials (0.5 mL in each 1mL cryovial): | lab170 |

| Plasma Freezer (or Dry Ice) entry time: | ____:____ | lab180@h |
| Serum Freezer (or Dry Ice) entry time: | ____:____ | lab185@h |
### COMPLETE IF BLOOD DRAW REFUSED OR INADEQUATE:

<table>
<thead>
<tr>
<th>BLOOD SPOTS</th>
<th>Collection time:</th>
<th>Freezer Time:</th>
</tr>
</thead>
<tbody>
<tr>
<td>lab235@a</td>
<td><em><strong>:</strong></em></td>
<td>___ :___</td>
</tr>
<tr>
<td>lab235@fim1</td>
<td></td>
<td>lab235@fim2</td>
</tr>
</tbody>
</table>

* **Barcode SPID label**
  * Number of spots completed on card: __________

<table>
<thead>
<tr>
<th></th>
<th>Done</th>
<th>Refused</th>
<th>Failed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not Attempted-blood draw completed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comments for failed: lab236</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SALIVA SAMPLE</th>
<th>Collection time:</th>
<th>Freezer Time:</th>
</tr>
</thead>
<tbody>
<tr>
<td>lab240@a</td>
<td><em><strong>:</strong></em></td>
<td>___ :___</td>
</tr>
<tr>
<td>lab240@h2</td>
<td></td>
<td>lab240@h3</td>
</tr>
</tbody>
</table>

* **Barcode SPID label**
  * (SPID/date & time label)

<table>
<thead>
<tr>
<th></th>
<th>Done</th>
<th>Refused</th>
<th>Failed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not Attempted-blood draw completed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PROBLEMS/COMMENTS</th>
<th>lab250</th>
</tr>
</thead>
</table>

If blood draw was refused, failed or inadequate, is SP willing to be rescheduled?

<table>
<thead>
<tr>
<th></th>
<th>Not applicable. Blood draw/blood spot complete.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>□ YES, when/comments:</td>
</tr>
</tbody>
</table>

______________

Thank SP for participating and inform them that the check for this study will come about 4 weeks after returning all materials

Complete paperwork

<table>
<thead>
<tr>
<th></th>
<th>Marshfield Labs requisition form, 2 copies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>Sample Collection Activity Log</td>
</tr>
<tr>
<td></td>
<td>□</td>
</tr>
<tr>
<td></td>
<td>Entered lab form into CASES _______________ (date)</td>
</tr>
</tbody>
</table>

**DATE/TIME PAXGENE TUBE FROZEN:**

<table>
<thead>
<tr>
<th>PAX Freeze Date</th>
<th>PAX Freeze time</th>
<th>N/A (not collected)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><strong>/</strong></em>/______</td>
<td><em><strong>:</strong></em></td>
<td>□</td>
</tr>
<tr>
<td>(lab420@m,d,y)</td>
<td>(lab420@h,mi)</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 4:
CLEAN-CATCH URINE COLLECTION PROCEDURES FOR MEN

1. Wash hands thoroughly.

2. Remove the lid of the container, being careful not to touch the inside of the cover or the container.

3. Wash the end of the penis with the wipes provided using circular motion to clean all areas (the foreskin of an uncircumcised male must first be retracted). Repeat the procedure with a clean wipe. Discard used wipes in the trash can.

4. Begin urinating into toilet.

5. Touching only the outside of the container and without letting container touch the penis, bring the urine container into the urine stream until a sufficient amount of urine (30-100mL = approximately 1-4 oz) is collected.

6. Urinate the remaining urine into the toilet.

7. Cover the specimen container with the lid provided, touching only the outside surfaces of the lid and container.

8. Clean any urine spilled on the outside of the container with an antiseptic wipe.

9. Wash hands.

10. Hand specimen container to phlebotomist or place where instructed if labeled.
APPENDIX 5:
CLEAN-CATCH URINE COLLECTION PROCEDURES FOR WOMEN

1. Wash hands thoroughly.

2. Remove the lid of the container, being careful not to touch the inside of the cover or the container.

3. Stand in a squatting position over the toilet.

4. Cleanse the area around the urethra (opening where urine comes out) on either side and around the opening with the special wipes, wiping from front to back. Discard used wipes in the trash can.

5. Begin urinating into toilet.

6. Touching only the outside of the container, bring the urine container into the urine stream until a sufficient amount of urine (30-100mL = approximately 1-4 oz) is collected.

7. Urinate the remaining urine into the toilet.

8. Cover the specimen container with the lid provided, touching only the outside surfaces of the lid and container.

9. Clean any urine spilled on the outside of the container with an antiseptic wipe.

10. Wash hands.

11. Hand specimen container to phlebotomist or place where instructed if labeled.
A. **INTRODUCTION**

SHOW collects dried blood spots from participants who refused or failed the venipuncture portion of the study. Analysis of these samples has not yet been determined and could include standard SHOW biological measures or more specialized analyses.

B. **DEFINITIONS**

1. **Dried Blood Spot**

   A. Dried blood spots are drops of blood collected onto filter paper and allowed to dry. They are placed in zip-lock bags with drying pouches and stored in a freezer. Dried blood spots have been analyzed for many different measures, including blood chemistry, cell counts, and environmental contaminants.

C. **BLOOD SPOT OPERATIONS**

1. **Preparing blood spot supplies for the field**

   B. Dried blood spot collection supplies are compiled into a kit for each participant to facilitate efficient data collection in the field.

   C. **Kit contents:**

      1. One alcohol wipe
      2. One single-use, permanently-retractable lancet
      3. Gas-impermeable storage bag
      4. Desiccant pack
      5. Humidity indicator cards
      6. Whatman 903 card
      7. One gauze pad

   D. **Additional supplies:**

      1. Waterproof marker (e.g. Sharpie fine point)
      2. Gloves
      3. SPID labels
      4. Whatman card drying rack

   E. Phlebotomists should have 5-10 blood spot kits with them for each data collection week to ensure enough supplies are available for all participants eligible for dried blood spot collection

2. **Blood spot collection**
With gloved hand, label card with patient ID. Disinfect selected site on the finger (see figure 1) and prick using a lancet.

Figure 1. Sample site selection.*

![Sample site selection](image)

Wipe away the first small drop of blood with the sterile gauze pad.

Uniformly saturate each circle by quickly and gently touching the drop of blood to the card. Do not press the puncture site to the filter paper or touch the Whatman Protein Saver 903 Card at any stage of collection. Do not touch the DBS circle once blood is applied. Make sure the circle is completely filled.

Figure 2. Sample collection.*

![Sample collection](image)

Fill each blood spot card from left to right:
Example of invalid sample:

Incomplete samples due to poor absorption cannot be used because the quantity of blood is insufficient for testing.

After collecting 4 dried blood spots, clean the site and leave it un-bandaged.

Using drying racks, allow the blood spot to air dry without the card flap covering the spots in a clean, dry place (protected from rodents, insects and direct sunlight) for at least 4 hours. Overnight drying may be necessary in areas with higher humidity. Do not heat, stack or allow DBS to touch other surfaces during the drying process.
Once dry, tuck in the flap on the card and store the card in a gas-permeable Ziplock bag with desiccant and humidity indicator.

Storing Blood Spots:
After spots are dried, place blood spots between two sheets of glassine paper (weighing paper) or wrap sheets of weighing paper around spots. Label low gas permeable zip-closure Bitran bag with 'Bitran bag' participant label. Store spots at -20°C, in low gas permeable zip-closure Bitran bag with several desiccant packs. Add a humidity indicator card to the bag or use desiccant packs with humidity indicator. The desiccant packs should be checked often and changed when the indicator turns pink. Initially, desiccant packs may need changing frequently. After a few changes, the cards will be drier, and the desiccant packs will need less changing. Note: the build-up of humidity can damage the quality of the sample.

Store both Ziplock bags with other frozen samples while in the field. Once at headquarters, store one bag in -20 and one in -80.

*Figures from IMPAACT Network: http://impaactnetwork.org/*