THE SURVEY OF THE HEALTH OF WISCONSIN


Version 2

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INTRODUCTION

This Manual of Operations addresses the SHOW standards applied to the collection of biospecimens from Survey Participants (SPs) and to the implementation of the SHOW Laboratory and Biorepository Operations. It is designed to standardize specimen collection and processing across the teams and sites to ensure the quality and biological integrity of the collected samples and to assure quality control measures are applied to this very important component of the survey.

Utilizing procedures in this manual, consented specimens 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. Quality control procedures are followed throughout.
A. SURVEY SITE PREPARATION (1)

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 survey site.

Laboratory technicians 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 Appendix, Bloodborne Pathogens (BBP) Exposure Control Plan.)

If designated survey site is a hotel room, site preparation includes the administrative team sending a letter in advance regarding protection against blood borne pathogens (Appendix, BBP Letter to Hotel).

a. Supply readiness/Stock inventory

At the end of each week, the lab technicians inventory the permanent and/or mobile laboratory stock to be sure that the following supplies are sufficient for handling the scheduled number or an approximate 25 subject's specimens in the following week.

Subject Kit contents:
- Gloves, 3 sizes of 2 types.
- 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 (1 box of each is the minimum)
- Lab requisition forms for the day with carbon paper when no copier available
- Freezer and refrigerator thermometers
- Sharps containers
- Dry ice box with straps
- Insulated specimen bag for transport of specimens when applicable
- Temperature checklist forms
- Unexpired collection tubes (extra tubes – need approximately 10-20 of each)
  - 1 Gold SST 5mL (gel barrier) (Marshfield Laboratories provides)
  - 2, 10mL Redtops
  - 2, 3mL lavender tubes (Marshfield Laboratories provides)
  - 2, 10mL lavender tubes
- Kit contents:
  - Holders and needles
  - Alcohol wipes (order when down to 2 boxes)
  - Gauze (order when down to 2 packages)
  - 1mL Cryovials for serum and plasma (order when down to 2000)
  - 2mL Cryovials for urine
o Cryovial freezer boxes
o Plastic pipette
o Band aids
o Tourniquets
o Sterile urine cups
o Butterfly needle kits

Kit contents are ordered on an as-needed basis.

b. Area preparation

1. Laboratory and phlebotomy counter preparation. At the start of each day, the survey technicians spray counters with a disinfecting solution, let it sit for 10 minutes, then wipe the counters dry. Because of the tight laboratory and storage conditions in the MEC and at survey sites, the use of commercial disinfectants that are EPA–registered sodium hypochlorite products are preferable because of their longer shelf life than the bleach solution that is prepared daily and discarded.

2. Restroom Preparation. At the start of each day, the survey technicians check the restroom for sufficient stock of paper towels. At the permanent survey 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. In the MEC, assure that water pump is turned on and water is flowing from tap.
A. PREPARATION (2)

2. SURVEY PARTICIPANT (SP) PREPARATION

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

The survey technicians review each day’s schedule of SPs in advance as well as the consent signature pages to determine any possible specimen collection exclusions. They review the QC sample selection and outline target subjects on the schedule.

The survey interviewers are responsible for welcoming the SPs to the Survey 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.

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

a. General instructions
   1. One of the surveyors greets and introduces him or her self by name and title or role.
   2. This surveyor 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. The surveyor shows the SP where to change and provides disposable scrubs in their size.
   5. If SP has indicated they will give samples, the surveyor confirms with other staff that draw tubes and cryovials can be labeled or personally does the labeling.
   6. If the SP’s wishes regarding biospecimens have changed since the consent, then the surveyor prepares to re-consent the SP prior to any further procedures.
   7. The surveyor 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.
   8. After the SP has changed and any needed re-consenting is done, the surveyor instructs the SP on the urine collection. After collecting the sample the surveyor escorts them to the phlebotomy area.
   9. The phlebotomist again confirms that informed consent for blood donations has been obtained before drawing blood.
   10. The phlebotomist confirms the SP’s SPID#, name with the name printed on the schedule and that correct SPID # is on the labels.
   11. The SP is seated while the venipuncture is performed.
   12. The SP spends approximately 5 minutes in the laboratory area.

b. Subject preparation for urine specimen
   - Whenever possible and feasible collect the urine sample at the beginning of the survey center visit. Right after the SP has changed into disposable scrubs and before venipuncture is probably the best time.
   - IF SP is unable to urinate at the beginning of survey visit, collect urine whenever
possible. Encourage participants to stay hydrated even if they fasted for the visit. However, do not collect samples after acute fluid load (>24 ounces) or after participant exertion. Participants who have difficulty producing a urine specimen may be offered a glass of water, and subsequent urine specimens may be collected later in the visit to bring the volume up to the required amount.

- 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 Subject: Subjects are given a brown paper bag which has, inside, 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 rest room 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 towlette packs.
3. **WOMEN**: Stand in a squatting position, using the towlette, 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 towlette, 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 the attached Appendices.

c. **Subject preparation for blood specimen collection**

- Seven 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.

- 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 also build rapport for the later interviews and exams. 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 the blood drawing 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." 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 go on with another part of the visit and return later for the blood draw.

- Assisting participants who look or feel faint.
  o Technicians have the SP remain in the chair and sit, if necessary, with head between knees until his/her color returns and he/she feels better.
  o A basin is provided if the SP feels nauseated.
  o A cold wet cloth may be placed on the back of the neck.
  o If the SP faints, staff will help them to the floor, removing anything in the way that may cause harm to the participant. The staff member sits with the SP until he or she regains consciousness.
  o If the person continues to feel ill, the Field Team Manager is consulted.
  o If SP does not regain consciousness, 911 is called and an incident report form is completed.
B. BIOLOGICAL SPECIMENS COLLECTION AND PROCESSING (1)

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
- Cleansing wipes
- Sterile urine collection container
- 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 Tests Form (see Appendix)
- Laboratory Inventory and Activity Form (see Appendix)

c. Forms and labeling

- The Laboratory Tests 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 subject’s SPID labels on the collection container prior to collection and on the 2 mL cryovials just prior to filling.

d. Preparation of SP for urine collection

Whenever possible and feasible, collect the urine sample at the beginning of the survey center visit. Right after the SP has changed into disposable scrubs and before venipuncture is probably the best time. The subject is handed a bag which is labeled with instructions for obtaining the sample.
Review these instructions with the subject.

- IF SP is unable to urinate at the beginning of survey visit, collect urine whenever possible. Encourage participants to stay hydrated even if they fasted for the visit. However, do not collect samples after acute fluid load (>24 ounces) or after participant exertion. Participants who have difficulty producing a urine specimen may be offered a glass of water, and subsequent urine specimens may be collected later in the visit to bring the volume up to the required amount.

- 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 Subject (THESE ARE FOUND ON THE BAG THAT CONTAINS NEEDED SUPPLIES FOR SUBJECTS):

  1. Wash hands thoroughly.
  2. Remove the lid of the container being careful not to touch the inside: open the towlette packs.
  3. **WOMEN**: stand in a squatting position, using the towlette, 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 towlette, 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.

- Urine collection.
  - Containers for routine specimens are chemically clean, and hold about 50 mL in volume, and must have a tight-fitting lid.
  - Orient the participant to the restroom and the location of supplies (antiseptic wipes, collection container), and explain the procedure and show the SP where the detailed directions are on the brown bag.
  - Show the SP how to lock the door for privacy. The participant’s privacy should be assured. Always knock on the door before trying to enter to be sure another participant is not using the restroom.

  See Appendix for specific Urine Collection Instruction Sheet for female participants.

**e. Urine processing**
• (This paragraph also repeated under C.2. BLOOD SPECIMENS, d. Survey Participant Labels.) SHOW HQ supplies each field center with sheets of identification number (ID) barcode labels to use for labeling draw tubes, working tubes, cryovials and freezer boxes. There can be up to 92 labels (some of which will be used for urine collection and processing).
  o 18 labels for the various urine container, tubes and vials
  o 7 labels for the draw tubes
  o 2 labels for the cryovials freezer box
  o 55 cryovial labels
  o 10 extra labels

• Verify the label is on the urine container and the SP is identified. Label up to 15, 2mL cryovials with SPs SPID # and collection date. Refer to the Reference Chart that will estimate number of cryovials by volume of urine donated to determine the number of cryovials to prepare (see Appendix).

• Pour equal amounts of urine into two 15 ml conical centrifuge tubes and centrifuge it for 5 minutes at 400 rcf (RCF = 1.118*10^5 * radius in centimeters * RPM^2). 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.

• Pipette 1.5 mL of the centrifuged urine into each previously prepared 2mL cryovial, up to 15 or if less, until there is less than 1.5 mL remaining, 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. 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 SP’s labeled cryovial box at -20 C for later transfer to the biorepository’s -80° C freezer.

f. Forms completion
• See the Laboratory Test Form: Urine Sample Line. Complete first column (status) as to whether samples was:
  o (D) donated
  o (R) refused; SP refused to give urine sample
  o (F) failed to provide; SP was unable to provide sample when attempted
  o (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 the right hand column.

Make note of urine cryovials on the Laboratory Inventory and Activity Form.

Refer to Section, D. BIOSPECIMEN DISTRIBUTUION for protocols on packaging and shipping urine (and blood) samples to SHOW headquarters and eventually to the biorepository.
B. BIOLOGICAL SPECIMENS COLLECTION AND PROCESSING (2)

2. BLOOD SPECIMENS

Blood samples (a maximum of 51 mL per SP) collected and processed by the SHOW lab technicians 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.

There are designated field team members assigned to the procedure of biospecimen collection and processing according to protocol. The Program Manager and Field Team Manager are responsible for monitoring for 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
- BBP Exposure Control Plan
- Blood Processing Protocol Outline
- Laboratory Tests Form
- Laboratory Inventory and Activity Form

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) and human immunodeficiency virus (HIV).
- **Contaminated sharps** means 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 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.

b. Equipment and supplies
This equipment is available at the permanent SHOW sites and is mobile to designated survey sites throughout the state. The majority of the Laboratory supplies will be stored in a mobile wheeled cart, with the larger pieces of equipment safely stored in a tote. The necessary supplies and equipment include the following:
- Lab coats and gloves
- Phlebotomy chair
- Plastic cart with wheels (or plastic tray with compartments) for supplies
- Blood tube racks
- Basin (just in case)
• Washcloths and towels
• 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
• Surgical tape/paper tape
• "Bandaids"
• Gauze (2x2-inch)
• Blood spill kit
• Biohazards waste container
• Needle/sharps container
• 10% bleach solution or approved biohazard disinfectant

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 lab staff is trained to follow the standard laboratory safety protocol as outlined below. Also see the BBP Exposure Control Plan, Appendix.

• 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 1:10 bleach solution or other EPA-approved disinfectant.

• Hepatitis B vaccine is offered to all unvaccinated technicians who handle blood. Documentation of vaccination, or technician'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 in each phlebotomy site which includes where and how injury occurred, type and brand of device involved.
d. Survey participant labels

1. SHOW HQ supplies each field center with sheets of identification number (ID) barcode labels to use for labeling draw tubes, working tubes, cryovials and freezer boxes. There can be up to 92 labels (some of which will be used for urine collection and processing).
   - 18 labels for the various urine container, tubes and vials
   - 7 labels for the draw tubes
   - 2 labels for the cryovials freezer box
   - 55 cryovial labels
   - 10 extra labels

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. The third subject of each survey site who yields a minimum of 15 cryovials of each biospecimen type is selected as the subject from whom 2 cryovials of serum/plasma/urine will be stored as quality control specimens. These quality control samples will be labeled as per routine protocol but will be stored in separate cryovials boxes for future quality control testing.

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

5. Once the SP confirms that they will have a blood draw, select a sheet of MLab accession labels and scan the identification number into the computer to link it to the SP.

6. Once SP arrives and confirms they are willing to donate blood specimens, pre-label the collection tubes with the SP’s SPID #.

7. Cross-check the labels with each participant’s ID number prior to the phlebotomy.

8. Labeled blood collection tubes are placed in a tube holder system. This tray is 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 Tests 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, and DNA samples. In addition, the Laboratory Tests Form facilitates the monitoring of phlebotomy and other quality assurance parameters and provides information critical to the interpretation of the assay results.

2. The Laboratory Inventory and Activity Form documents the sample aliquots for entry into the biorepository data system, documents samples selected for quality control (QC) and links real SPID of the donor to the QC SPID. Transfer of samples to Prevention Genetics is also noted on this form.
f. Venipuncture procedure

Preparation

1. Blood drawing is completed with 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 HG A1C
   - Tube 8: when designated and SP amenable, one additional
   Also reference the Blood Processing Protocol Outline, Appendix

3. Blood drawing is standardized for the *sitting position*. Technicians may have participants clench their fists (moderately) during phlebotomy for up to two minutes. Venipuncture is performed with 21 or 22 gauge needles and, typically, with butterfly needles.

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

5. Technician *applies tourniquet*.

6. Technician examines participant's arms for the *best site for venipuncture*. Release *tourniquet*.

7. Technician cleanses venipuncture site by *wiping with alcohol prep pad in a circular motion from center to periphery*. Allow area to dry.

**Venipuncture**

8. **Tourniquet is re-applied and timer started.** Technician 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, technician 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 technician 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.

**Blood collection**

12. The technician 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.)*

13. The technician 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 technician will then move the needle slightly without causing discomfort to the participant.

14. If the collection tube does not fill, the technician 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 Test Form.)*

15. 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.

16. 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 vein. Tubes less than half full are not acceptable, but those for MLab should be sent if no full tube can be obtained.

**Completion of blood draw**

17. When the blood flow ceases, the technician 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).

18. Lavenders are to be gently inverted several times after being drawn *(see 23. Mixing, Clotting Time, etc. below).*

19. Tourniquet is released, if still applied.

20. 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. The participant is asked to hold the gauze pad firmly for one to two
minutes to prevent formation of a hematoma. Needle is discarded into a puncture-proof sharps container.

21. Technician records on Laboratory Tests Form the start and stop time of the venipuncture.

22. If the participant continues to bleed, the technician applies pressure to the site with a gauze pad. The arm is to be kept elevated until the bleeding stops. If necessary, tightly wrap a gauze bandage around the pad and leave in place for at least 15 minutes.

Post-draw handling
23. Mixing, Clotting Time, Centrifuge and Storage Time.
   o Tube 1 (Gold - SST) is inverted once, and left to clot for 40 minutes at room temperature and then centrifuged 10 minutes.
   o Tube 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 same time.
   o 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.
   o Tubes 6 and 7 (Lavender – 3 ml Plasma) are gently inverted 5-6 times before being placed in biohazard specimen bag and refrigerated immediately.

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

25. The technician completes remainder of the Laboratory Tests Form and, if applicable, the Quality Control Lab 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 only chooses to have only certain tests completed, the SHOW phlebotomist notes this on the Laboratory Tests Form and only draws and processes those specimens that are indicated. Please note that for those refusing DNA donations, SHOW still draws all the tubes including the large lavenders, but after removing the plasma for the biobank, the technician discards the remainder of the large lavender tubes that were meant for DNA extraction. SPs who refuse to give certain samples will not be asked for a QC sample of that specific kind of sample. Careful review of the original or revised consent will guide the phlebotomist in who should be asked for QC samples.

2. Difficult 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 Tests Form that the labs were not drawn and the reason. If the SP agrees to another venipuncture, a second attempt may be made. No more than three attempts are made to obtain a blood sample and only if the participant agrees. A different phlebotomist should attempt the third draw.

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 technician may move needle slightly in hope of entering 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 technician 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 technician 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 Tests 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. Special considerations for quality control specimens

Labeling. The phlebotomist verifies that there are SHOW SPID labels on each of the tubes and cryovials that are obtained and processed, one on each of the forms: the Laboratory Test Form, the Marshfield Laboratory Requisition Log, and the Laboratory Inventory and Activity Form for each SP. The small lavenders and the small red and the MLab serum vial are labeled with the Marshfield Laboratories Requisition Number.

1. Laboratory Tests Form. The phlebotomist indicates on the SHOW Laboratory Test form the date and time the specimens were obtained and indicate for each specimen tube whether the tube was obtained (check “yes” or “no”) and what if any problems occurred. If the SP gave a QC sample, the QC box will be marked with an “x” next to the specimen that was duplicated.

2. No Specimens obtained. If no specimens are obtained because the SP was a hard draw or refused the blood draw, the technician indicates this on the Laboratory Tests form documenting the reason, or problem.
   o Notes: Any problems with the venipuncture or deviations from the protocol are noted in the Notes section on the Laboratory Tests Form.

i. Processing blood specimens

1. Technicians 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 the Appendix, Blood Processing
Protocol Outline. The instructions are by the priority of draw. Note: serum/plasma should be removed from cell pellet within 5 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 min.), then centrifuged for 10 minutes at 3000 rpm.
   - Serum is pipetted or poured off the tube and the serum transferred to the provided transfer tube. It is labeled with SPID and MLab barcode labels. 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 3000 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 maintainuffy coat separation.
   - Serum is pipetted off and deposited in 0.5mL quantities into 1mL cryovials labeled with SHOW SPID barcode labels. Note that cryovials are only half full.
   - Re-centrifuge samples for 10 min if needed ("gelled-clot" has formed).

   **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 and sent to Prevention Genetics for DNA extraction and storage. Please note: **These tubes are drawn on all blood donors even if the Survey Participant has refused to donate DNA.** The processing technician 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 inverted gently 10 times and centrifuged immediately for 10 minutes at 3000 rpm.
   - Plasma is pipette off and deposited into 0.5mL quantities into 1ml cryovials labeled with SHOW SPID barcode labels.
   - Remaining buffy coat and red cells are frozen at -20 (Milw) or placed on dry ice (MEC) until can be moved to -80°C freezer: In MEC, within 2-3 days, In Milwaukee, within days-4 weeks, dependent on timing of visits by FT, FTM, etc.

5. **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, placed in specimen biohazard bags and refrigerated for pickup by the MLab courier.
show laboratory and biorepository manual

j. Aliquoting

1. Aliquoting 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. When aliquoting serum and plasma, care is taken not to disturb the top of the cell pellet with the pipette tip, as this will result in platelet, white cell, and red cell contamination.

3. A new pipette tip is used for each draw tube.

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

5. If any tubes are accidentally mixed during pipetting, so that plasma is contaminated with red cells, they may be re-centrifuged.

6. There is no minimum or maximum number of cryovials. SHOW takes as many as possible (AMAP). When there is less than 0.5mL left of either plasma or serum, it is discarded.

7. Collection and draw tubes are discarded after pooling and aliquoting are completed.

8. Description of aliquots

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Type</th>
<th>Number of Cryovials</th>
<th>Color Code</th>
<th>Volume per Cryovial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5mL Gold</td>
<td>1</td>
<td>Clear</td>
<td>5mL</td>
</tr>
<tr>
<td>2&amp;3</td>
<td>10mL Red</td>
<td>AMAP/1mL</td>
<td>Yellow Cap</td>
<td>0.5mL Serum</td>
</tr>
<tr>
<td>4&amp;5</td>
<td>10mL Lavender</td>
<td>AMAP/1mL</td>
<td>Clear</td>
<td>0.5ml Plasma</td>
</tr>
<tr>
<td>6&amp;7</td>
<td>3mL Lavender</td>
<td>0</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

AMAP, as many as possible.

9. EDTA plasma cryovials. Aliquot, by volumes specified in the table above, into white-topped, clear cryovials. Cryovials are frozen in an upright position at -20 OR -70°C.

10. Serum cryovials. Pool and aliquot serum at room temperature. Carefully pipette 0.5 mL of pooled serum into each of yellow-capped cryovials 28–44. Cryovials are frozen in an upright position at -70° C.

k. Special circumstances for processing and aliquoting

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 technician on the Laboratory Tests Form.
2. If serum and plasma cryovials cannot be frozen at -20°C within 10 minutes of aliquoting, the technician does it as soon as possible after that – with notation on the Laboratory Tests 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 Tests Form.

I. Processing completion

1. The Laboratory Tests Form is part of each SP’s data file and must be kept with that file and be forwarded with all other paper-assisted participant interviews (PAPi’s) to SHOW HQ. The Marshfield Laboratories Requisition Form 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. The Laboratory Inventory and Activity Form is retained by the lab technician for biospecimen data entry.

2. Completed, frozen cryovials from each participant are packed into one freezer box. A participant label is attached to the front cover of the freezer box. Frozen 2 mL urine tubes are placed in remaining space in freezer box. Where sample volume allows, the freezer boxes can be divided in half to allow two SP’s sample sets to be stored in one box. In such cases, two SPID labels will be placed on the cover of each box.

3. Previously, the QC SPID frozen specimen box was utilized throughout the week to collect the duplicate specimens from different SPs. Now, QC samples are stored in the same box as SPs non-QC samples.

4. All work areas are wiped down with 10% bleach solution or approved biohazard disinfectant.

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

6. Data from the laboratory forms is entered into the database (see section F. BIOREPOSITORY, 2. OVERVIEW OF STORAGE METHOD). This process records/sends information both to data management as well as the biorepository storage record system.
B. BIOLOGICAL SPECIMENS COLLECTION AND PROCESSING (3)

3. SALIVA SPECIMENS

This is to detail the procedure for saliva collection. 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 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 total volume of the two EDTA lavender top tubes for DNA is estimated to be < 5 mL (e.g., only the red top tubes were filled and no lavender top tubes were filled or only the first 10 mL lavender top tube was filled and it was less than half full, etc.).

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 to ensure they are correctly coded. Collection containers are to be labeled prior to collection.

c. Preparation of participants for saliva collection

- The surveyor verifies that the participant has had nothing to eat, drink, smoke or chew 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 a 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 big cap.

e. Forms completion
   - Complete the Laboratory Tests Form noting the collection time and the reason the sample is being done. Make notation on the Laboratory Inventory and Activity Form that saliva was collected.

f. Saliva processing
   - Place the labeled saliva collection tube in the saliva sample bin provided to each lab.
C. BIOSPECIMEN DISTRIBUTION (1)

1. SHIPPING SAFETY

SHOW policy regarding shipping of biospecimens obtained from SHOW research subjects (including storage/shipping using dry ice) requires that all staff involved in the storage/shipping of biospecimens must complete the Bio HazMat Shipping Certificate program provided by the University of Wisconsin.

a. Biosafety courses

The Office of Biological Safety (OBS) provides a training program for shipping infectious substances and other biological materials with an emphasis on laboratories and research groups (Biosafety Courses 205, 206, 207). This concentrated infectious & biological hazardous materials shipping training is offered as a service only to University of Wisconsin System and Campus employees, staff, faculty and students.

Among the topics covered are: Federal regulatory definitions of infectious substances, patient specimens and biological products; use of the Dangerous Goods Table to locate proper shipping instructions; requirements for shipping biologicals with dry ice; proper packaging; and correct completion of the shipping documentation.

Instructions for completion of this training can be found at this website: http://www.ehs.wisc.edu/bio-biotraining-biohazmatshiptraininginfopage.htm

After completion of the training, individuals will receive a Bio HazMat Shipping Certificate of Training which is valid for 2 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 10 days of completion of required training elements. Date of the certificate will reflect the date training is completed, not date the email is issued.
C. BIOSPECIMEN DISTRIBUTION (2)

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 and Field Staff.
- 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 3 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 70-80 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. Personnel must never stick one's head into the chest to obtain the dry ice.

3. **Explosions:** Placing dry ice into a tightly sealed container can permit sufficient 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 build-up 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.

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:
   a. Letting the unused portion sublime (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);
   b. NEVER dispose of dry ice in a sink, toilet or other drain (such action can destroy the structure because of the temperature difference);
   c. NEVER dispose of dry ice in the trash or garbage; and
   d. 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 or 2) dry ice blocks (approx. 10 lb blocks)
   - Note: dry ice blocks will last longer before dissipating

2. **After-hours or when in urgent need**, dry ice can be located in the **dry ice bin in the K4/5 core area of UW Hospital and Clinics**

See **Dry Ice Procurement, Appendix**.
C. BIOSPECIMEN DISTRIBUTION (3)

3. SPECIMEN DISTRIBUTION

This procedure details the per-subject and QC duplicate distribution of biosamples.

a. Location of samples

1. To SHOW Biorepository via SHOW Headquarters:
   - As much as possible (AMAP) frozen 1 mL aliquots serum and plasma
   - AMAP frozen 2ml aliquots urine

2. To Central Laboratory (Marshfield Laboratories):
   - 2 refrigerated 3mL lavender tubes
   - 1 - 5mL cryovial of serum

3. To Prevention Genetics/Via SHOW Headquarters:
   - 2 frozen 10mL lavender tubes with buffy coat intact and most plasma removed

b. Transport to SHOW Biorepository

   - Specimens for SHOW Biorepository will be temporarily stored at the permanent sites, transferred to the headquarters in Middleton -80°C freezer and transported frozen in batches to long term storage site for the SHOW biorepository (five miles away in Madison) by local transport.

c. Transport to Marshfield Laboratories

   - 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. PM 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 all supplies for transporting those specimens, including biohazard bags, labels, absorbent cloth, requisitions and ziplock transport bags.

   - Alternative drop off spots for Marshfield Labs exist around the Middleton Survey site.

d. Transport to Prevention Genetics

   - Specimens for Prevention Genetics will be temporarily stored at the permanent sites, transferred to the headquarters -80°C freezer and batch shipped or transported frozen on dry ice to Prevention Genetics in Marshfield. Specimens for Prevention Genetics will be delivered in the MEC or by car on a trip north or shipped via FedEx or DHL in dry ice shipping containers for next day delivery.

e. Packaging instructions for transport in MEC or Car

   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 with security straps is to be 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 Processing Form for that shipment.

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 are 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. Seal the top of the Styrofoam cooler with tape or cinch the carrying straps so that cooler lid cannot come off in an accident.

9. If transporting to biorepositories, place the listing of all samples included in that particular Styrofoam cooler, on the top in an envelope and tape it to the cooler.
D. BIOSPECIMEN TESTING (1)

1. LABORATORY TESTS AND ALERT VALUES

Within 48 hours, Marshfield Laboratories completes the following chemistry tests on serum drawn in 1 Gold-top 5 ml plastic SST tube: Serum Creatinine, Glucose, Total Cholesterol and Total HDL-Cholesterol.

Marshfield Laboratories completes the following tests on plasma drawn in 2, 3ml plastic lavender top tubes containing the anticoagulant, ethylenediaminetetraacetic acid (EDTA): complete blood count (CBC) and Hemoglobin A1C (HbA1c).

g. 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 Manual of Operations – Survey Methods). 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>Test</th>
<th>Units</th>
<th>Reference Range for Women</th>
<th>Reference Range for Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Cell Count</td>
<td>x10^9/L</td>
<td>4.1-10.9</td>
<td>4.1-10.9</td>
</tr>
<tr>
<td>Red Cell Count</td>
<td>x10^12/L</td>
<td>3.85-5.05</td>
<td>4.15-5.55</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/dL</td>
<td>11.7-15.5</td>
<td>12.9-17.3</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>%</td>
<td>35-46</td>
<td>38-51</td>
</tr>
<tr>
<td>Platlet Count</td>
<td>x10^9/L</td>
<td>175-450</td>
<td>175-450</td>
</tr>
<tr>
<td>Glucose</td>
<td>mg/dL</td>
<td></td>
<td>70-99</td>
</tr>
<tr>
<td>Glycohemoglobin (A1c)</td>
<td>%</td>
<td></td>
<td>4.0-6.0</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>mg/dL</td>
<td></td>
<td>100-200</td>
</tr>
<tr>
<td>HDL</td>
<td>mg/dL</td>
<td></td>
<td>40-59</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>mg/dL</td>
<td></td>
<td>0.7-1.3</td>
</tr>
</tbody>
</table>
D. BIOSPECIMEN TESTING (2)

2. GENETIC TESTING

No genetic testing is done at this time, though DNA samples are gathered and stored. SHOW obtains permission from participants to do research on genetic material with an approval from the University's Institutional Review Board.
E. BIOREPOSITORY (1)

1. OVERVIEW

Following specimen collection, processing and handling for immediate testing, the below steps are taken:

1. Complete an individual lab record for each subject/specimen.

2. Complete a daily record of tally of specimens collected.

3. Assure safe storage and transport of specimens to the Middleton office (holding) freezer prior to transport to the SHOW Biorepository. This includes secure maintenance of the portable coolers and paper documentation.

4. Place biorepository specimens in the -80 degree holding freezer in designated shelf for incoming samples.

5. Assure data entry of required information into the database.

6. Assure timely transport of DNA specimens to the Prevention Genetics facility. Maintain log of samples to be shipped, samples shipped and assurance of receipt of these samples at Prevention Genetics.

7. Assign a storage space for the specimens and transfer to the Biorepository freezers. Record this storage location.

8. Log samples into system to assure availability, safety and viability of samples for future research.

At the end of each session of working with the biospecimens the staff also:

1. Assure that the freezer is locked and alarm systems are re-activated.

2. Assure that the freezer location is locked/secured.

3. Assure that records are closed/secured and not accessible to any staff other than those with authorized access.

Quality control steps: To assure accurate recording biospecimen handling, 10% of samples collected on an annual basis will be physically checked for the following:

1. Consent is complete

2. Accurate recording of subject ID number on box.

3. Accurate recording of number of cryovials contained in the box for each of these subjects.

4. Accurate recording of location of storage.

5. Data record completion (i.e. all elements of record for these specimens are completed.
E. BIOREPOSITORY (2)

2. OVERVIEW OF STORAGE METHOD

The following describes the organizational system for storing biospecimens for the SHOW Biorepository. The Medical Lab Technician on staff who conducts the phlebotomy/processing of the sample will oversee the organization of the freezers and samples.

Related Documents
- Laboratory Inventory and Activity Form
- Lab Record for Time 3
- Excel spreadsheets:
  - S:\Biorepository\SHOW BIOREPOSITORY FREEZER LIST\FREEZER 1 BIOREPOSITORY.xlsx
  - S:\Biorepository\SHOW BIOREPOSITORY FREEZER LIST\FREEZER 2 BIOREPOSITORY.xlsx
  - S:\Biorepository\SHOW BIOREPOSITORY FREEZER LIST\FREEZER 3 BIOREPOSITORY.xlsx
  - S:\Biorepository\SPECIMENS\complete list of samples\BIOREPOSITORY 2008-2012.xlsx

a. Equipment and supplies
- Freezer boxes – 84 well separators
- Bar code reader
- Freezer labels

b. Description of storage system
1. Samples are stored on -80 freezer shelves in freezer boxes.

2. The plasma/serum/urine from one subject typically takes up ½ the space in a box, so two subjects can be stored in each box with loose cryovials placed on top of the ones that are in the well of the box.

3. Subjects providing urine only are combined into one box so there may be 4 subjects in a storage box that has urine only.

4. The outside of the box, on each side, will be clearly marked with the SPID# for the samples that are stored inside the box.

5. The freezer shelves hold four boxes deep, four boxes high and four boxes wide. Therefore, on any given shelf of a freezer there are a minimum of 64 boxes holding 128 subject samples.

6. Freezers are labeled on the outside with the date that corresponds to the samples that are housed in that freezer:
   - Freezer 1: 2008
   - Freezer 3: 2010/2011

7. If there is a combination of years, every effort will be made to segregate the year of the samples by shelves within the freezer.
c. Data entry
From information on the Laboratory Inventory and Activity Form, samples are permanently recorded in the biorepository spreadsheet:

S:\Biorepository\SPECIMENS\complete list of samples\ BIOREPOSITORY 2008-2012.xls.

Box contents (SPID#) and location are noted on the appropriate (Freezer number) spreadsheet:

S:\Biorepository\SHOW BIOREPOSITORY FREEZER LIST\FREEZER# BIOREPOSITORY.xlsx

This spreadsheet will maintain some basic information about the SP (SPID#, date of collection) as well as the volume of aliquots of serum/plasma and urine that are available for each subject.

The database stands as the record of samples and has the capability to serve as a mechanism for sample selection (requested by collaborating researchers) and the subsequent revised volume of sample remaining per subject.

d. Steps for data management
1. Data are entered onto source documents (paper copies; Laboratory Inventory and Activity Form, see Appendix) and then entered into excel spreadsheets at the time of the Survey Center week.

2. Data entry includes bar-code reading the SPID labels attached to the storage boxes, verifying the number of cryovials for all specimen types and confirming date of specimen collection.

3. The excel spreadsheet can be accessed off-site on the computers used by the Laboratory staff using UW Wisconsin VPN to connect.

4. At the completion of each block group, connecting to the S: drive updates the ACCESS database for subject samples.

5. Data entered from the source document is subsequently populated into the Biorepository spreadsheet with portions of this data simultaneously transferred to the main oracle server for use by the scientific team.

6. At the end of each quarter, 10% of the source documents will be selected for double data entry to serve as quality control.
E. BIOREPOSITORY (3)

3. SAMPLE SELECTION PROCESS
The following describes the system/steps for responding to a request for Biorepository samples.

a. Overview
- The Biorepository Manager receives, confirms and finalizes requests for samples.
- The SHOW Scientist works with the requesting researcher to identify the sample (i.e. males/females, location, date, disease type). From this interaction a selection of SPIDs will result in a list of SPID numbers.
- The Data Manager will create a list, using the Biorepository data base that will result in a listing of SPIDs in conjunction with the location that the sample can be found.
- The Data Manager will forward this list to the technician who will be responsible for the pull.
- The Technician will complete the pull in the timeline specified and create a list of samples that are pulled.
- The samples pulled list will be returned to the Data Manager to confirm that the specimen volume is confirmed with the reduction of samples.

b. Equipment and supplies
- Biospecimen request process completed
- Samples identified and sample list compiled
- Dry ice for use during pull process
- Computer/barcode reader
- Extra sample boxes for placing selected cryovials

c. Procedures
1. The Biorepository Manager receives, confirms and finalizes requests for samples. The biospecimen request process is completed as detailed in the Biorepository request form.

2. The SHOW Scientist works with the requesting researcher to identify the sample. The selection process, based on subject specification (i.e. males/females, location, date, disease type), is confirmed with the Scientist who is able to ascertain whether there is sufficient sample to meet the needs of the researcher. This selection process includes verifying availability of sample. A list of SPIDS is generated that finalizes the SPIDs from whom samples will be pulled.

3. The Data Manager will take the list of SPIDs and using the biorepository spread sheet, will expand the list of SPIDs with the column that indicates the location of the sample (i.e., which freezer, which shelf, which column).

The Data Manager will also complete and deliver to the Technician for pulling the SHOW Biorepository Specimen Selection Instructions Form (see Appendix) which indicates:
- Type and volume (# of cryovials) of sample.
- Anticipated date for completion of the sample pull.
- Information about sample transfer to the researcher (e.g. will the sample be picked up from SHOW or will the researcher meet the Technician at some location).
• Additional info about delivery (e.g. are all samples to be delivered at one time, is there a schedule for incremental delivery of samples).

4. The Technician will:
   • Obtain dry ice and coolers as needed to complete the work.
   • Use the computer and barcode reader to complete the project.
   • Select the subject box, withdraw the designated sample and transfer to a cryovial box that will house the samples being selected.
   • Assure that timing is such in the selection process that the temperature of the freezer and the integrity of the samples are maintained.
   • Return SHOW samples to the freezer in the order in which they need to be maintained.
   • Barcode read the selected sample cryovial and then store the withdrawn sample boxes in a location specific for the selection process.
   • Upon completion of the selection process or as designated by the Data Manager, deliver the sample to the requesting investigator.
   • Forward the list of samples selected to the Data Manager to assure that a reduction in remaining volume of sample is completed on the Biorepository spread sheet

5. The Data Manager
   • Takes the listing of selected samples pulled and compiles this into the Biorepository data list to assure that the volume of the remaining sample is accurate.
   • The Data Manager maintains the list of pulled samples in the database to maintain a historical record of samples used.
   • The purpose for which the sample will be used / protocol / investigating researcher will be maintained for historical records of the SHOW Biorepository.
E. BIOREPOSITORY (4)

4. BIOREPOSITORY -80°C FREEZERS

SHOW -80°C Biorepository Freezers are located in the Middleton office (1) and the University of Wisconsin School of Medicine and Public Health’s Wisconsin Institute for Medical Research (WIMR, East Room 2030) Freezer Farm (2).

The following describes the safety precautions necessary for staff and for assuring the maintenance of the integrity of the biospecimen samples. The Medical Lab Tech (MLT) for SHOW has significant responsibility for the integrity of the sample storage system. Anyone needing access to these samples must either work with the MLT for SHOW – or be instructed by this person prior to performing independent functions in these freezers. Additionally, the Field Team Manager (FTM) must give approval for access to these freezers along with the use of the time of the MLT for the work with the freezers.

Individuals working in the freezers must have read the Dry Ice Shipping and Handling protocols (section D.2. of this Manual) and the protocols on Freezer Security and Alarms Systems (for instructions on how to interact with alarm system, section F.4.c. of this Manual, below).

a. Equipment and supplies

- Biospecimen request form or definition of work to be conducted
- Dry ice for use during pull process
- Coolers for transfer of cryovial boxes
- Computer/barcode reader
- Extra sample boxes to place the selected cryovials
- Protective gloves for working in -80°C freezers

b. Procedures for working in the -80°C freezers

- Freezers typically have five shelves. Within each shelf of the first four shelves, boxes are stacked in four columns that are four boxes high and four boxes deep. The fifth shelf holds four columns each five boxes high.
- Prior to working with the freezer and opening it for more than a couple minutes at a time, the alarm system must be put on hold for the expected duration of work in the freezer.
- The person must have coolers available for storage of the samples during movement.
- The person must place approximately 3-4 inches of dry ice on the bottom of each cooler.
- Dry ice is available on the fourth floor of the WIMR (take only what is needed for the work being conducted at that time). Take the cooler to location of dry ice in order to transfer directly into the cooler.
- The person working with the boxes/shelves needs to wear the specified -80 °C freezer gloves in order to protect their hands. These are especially important when handling the dry ice. However, modified hand coverings may be needed in order to conduct work with the cryovials (i.e. fine finger function).
- In order to locate a specific specimen, it is typical that:
  - One column of boxes needs to be removed from the freezer and placed in the cooler that has the dry ice.
  - Each box is then examined in order to locate the specific specimen being sought.
  - When four boxes have been examined they are placed back in the freezer.
• At all times, the staff member working in the freezer needs to be aware of the freezer’s ability to regulate the interior temp. If the temp consistently falls above 60 degrees and takes more than several minutes to recover, it is necessary to halt operations within the freezer until the temperature is regulated.
• Recovered samples need to be stored at a designated location within the freezer, or if they are being immediately prepared for delivery to a researcher, these should be packaged as directed.

c. Monitoring the -80°C freezers
• The -80°C freezer in the Middleton SHOW office has its own log and is changed monthly. Logs are kept in a black binder in the Middleton office in Room 109, Suite 3.
• Overall monitoring is done with freezer alarms which is detailed fully in the Freezer Security and Alarm System MOP.
E. BIOREPOSITORY (4)

5. FREEZER SECURITY AND ALARM SYSTEMS
The following is to provide clear instructions on emergency procedures to be used in the event of -80°C freezer failure for any of the SHOW freezers. Appropriate storage of biospecimens is a core requirement for the operation of a successful biorepository. On occasion equipment may fail. The following procedures are in place to ensure that loss and damage to the collection is avoided and procedures that should be followed when samples must be transferred to back up equipment.

Note: This is not intended to cover detailed safety procedures for handling biospecimens. Personnel must follow the specified guidelines, including those documented in the BBP Exposure Control plan (Appendix) for handling biological specimens.

In the case of -80°C freezer failure, appropriate action should be taken to:

- Respond to the alarm.
- Assess the level of the problem.
- Implement a plan of action if there is a freezer failure.
- Transfer samples to back-up storage without damage to samples or loss of sample identity and tracking, if called for.

Follow all standard guidelines and universal precautions for working with biohazardous materials. Use gloves, eye protection, coats or gowns and other appropriate apparel for protection from exposure to blood borne pathogens or other potentially infectious materials.

Related documents:
- Dry Ice Procurement
- Rees Series II Summary Update
- Rees Series II Call List

a. Alarm and response
For more detailed information, see the Biorepository Freezer and Security Alarm System Document.

1. SHOW has 1 -80°C freezer in the Middleton office and 4 -80°C freezers in the WIMR, (2030 Repository, 2nd floor WIMR1111 Highland Ave). Alarm systems at the two sites are described below.

2. The Middleton freezer is wired to an alarm that triggers a system within the University of Wisconsin Campus Police Department. The campus police are provided phone numbers of staff to call (see call list, below) until they connect with staff who will take the responsibility to determine the problem with the freezer.

3. The freezers housed in WIMR freezers are outfitted with Rees Scientific probe/alarm systems. These systems connect to an automated telephone system that when triggered, follows a phone tree that continues to ring through until a staff member is reached who can appropriately deal with the problem. The three -80°C freezers within the WIMR location are connected to the Rees Series II alarm system (see Rees Series II Summary Update, Appendix). If for any reason the temperature begins to rise above the set alarm point for greater than 30 minutes, the Rees system will begin to call the TSB group call list.
4. The call list (Rees Series II Call List, Appendix) consists of the home phone #’s of the SHOW administrative staff. The Campus Police and the Rees Series II will systematically go from one number to the next until it has successfully contacted an individual who can reply to the alarm message.

5. Details of the procedure for response to this phone alarm are contained in Appendix.

b. Back-up freezer identification
1. If determined that a freezer is failing, a back-up freezer is designated for transferring all of the stored specimens until the failing freezer can be serviced.

2. Backup-freezers include the:
   - Middleton SHOW office freezer.
   - -20°F Freezers at SHOW field sites.

3. Initial response to a freezer failure is to secure dry ice (see below) for the coolers that will be used to transfer samples.

c. Sample transfer
1. The initial responder to the freezer failure assesses the freezer failure and whether samples need to be transferred (dependent on current freezer temperature, time of day, weekend/weekday, advice from Service Engineer, etc.)

2. Notify other personnel that a sample transfer has to be performed and keep freezer doors shut until other personnel arrive.

3. Using the proper equipment/supplies, samples are quickly transferred from the failing freezer to the back-up freezer.

4. Fill insulated containers with dry ice, available in dry ice bin in the K4/5 core area or purchased from local retailers (see Appendix, Dry Ice Procurement). Remove sample boxes from malfunctioning freezer and place them on dry ice.

5. Immediately move samples to back-up freezer.

6. Boxes are labeled with original shelf rack, box location to ensure correct return of samples to assigned locations.

d. Service call
1. Notify UW Physical Plant to perform a service call on the failing freezer.
F. BIOHAZARDOUS WASTE DISPOSAL

Biohazardous waste, materials used for blood sample collection and processing, are handled according to the University of Wisconsin Madison Bloodborne Pathogen Exposure Control Plan. For more information see http://www.ehs.wisc.edu/occ-research-bloodbornepathogens.htm.

Biohazard waste containers are utilized for used needles and other contaminated supplies or products. Used needles are disposed of in a sharps container. The sharp container lid is closed and snapped in place when full or once a month depending on which occurs first. The container is then placed in the biohazard waste bin until pickup by MERI. Biohazard bags for non-sharp contaminated materials are twisted closed and placed in hard containers at the end of each survey site or survey center until transferred to larger MERI bins upon pick up. MERI is contacted for pickup of the biohazard material when necessary, typically once a month dependent upon the exam schedule..

For pick up, notify:
MERI (Madison Environmental Resources Inc. 1310 Badger Rd, Madison WI. Phone: 608-257-7652).
G. APPENDICES

SHOW BIOREPOSITORY SPECIMEN SELECTION INSTRUCTIONS:

Instructions prepared by ________________________________

Sample pull completed by ________________________________ ________________________________ ________________________________ ________________________________ ________________________________

Total Number of Specimens to be pulled ________

Type of Specimen:
Serum ____ yes ____ no  # of cryovials 1 2 3 4 ______
Plasma ____ yes ____ no  # of cryovials 1 2 3 4 ______
Urine ______ yes ____ no  # of cryovials 1 2 3 4 ______

Date by which samples should be pulled:
All samples pulled by (date) ________________________________

Split sample pull:
____ # to be pulled by (date) __________
____ # to be pulled by (date) __________
____ # to be pulled by (date) __________
____ # to be pulled by (date) __________

Sample Delivery
Arrangements for sample delivery are as follows:
Samples to be delivered packed in this manner:
Dry ice _____ yes ___ no
Ice ____________ yes ____ no
Room Temperature _____ yes ___ no

SAMPLES will be ______ picked up at this location ________________________________
____ to be delivered to this location ________________________________
### BLOOD PROCESSING PROTOCOL OUTLINE

<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>5mL</td>
<td>once</td>
<td>40 (60 mins max)</td>
<td>5-10 min, re-centrifuge if needed for 10 min</td>
<td>3000</td>
<td>Serum transfer tube</td>
<td>SPID &amp; MLab</td>
<td>bag &amp; refrigerate</td>
<td>MLab</td>
<td>Win 24hrs</td>
</tr>
<tr>
<td>2</td>
<td>2, 3</td>
<td>Red Top</td>
<td>10mL</td>
<td>NONE</td>
<td>40</td>
<td>10 mins</td>
<td>3000</td>
<td>0.5 mL serum to as many 1mL cryovials as possible, discard red cells</td>
<td>SPID ONLY</td>
<td>box &amp; freeze</td>
<td>SHOW</td>
<td>Immediate</td>
</tr>
<tr>
<td>3a</td>
<td>4, 5</td>
<td>Lavender</td>
<td>10mL</td>
<td>10 times</td>
<td>Centrifuge immediately</td>
<td>10 mins</td>
<td>3000</td>
<td>0.5mL plasma to as many 1mL cryovials as possible</td>
<td>SPID ONLY</td>
<td>box &amp; freeze</td>
<td>SHOW</td>
<td>Immediate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Buffy coat and red cells remain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6, 7</td>
<td>Lavender</td>
<td>3mL</td>
<td>5-6 times</td>
<td>Immediately label, bag and refrigerate</td>
<td></td>
<td></td>
<td>2 tubes to biohazard specimen bag</td>
<td>SPID &amp; MLab</td>
<td>bag &amp; refrigerate</td>
<td>MLab</td>
<td>Win 24hrs</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Quality Control Duplicate of any set above will be processed the same as the original specimen. Only one set per subject will be duplicated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ALTERNATIVE DNA COLLECTION -- Instead of Tubes 4 and 6**

| 3b | Oragene Tube | Oragene DNA Self-Collection Kit | 5 times | NA | NA | NA | Throw out the funnel and big cap | SPID ONLY | Ambient or -20 or -80 | SHOW & PG | Immediate |
| 3c | Oragene Tube | Quality Control Duplicate of Saliva sample will be processed the same as the original specimen. | | | | | QC SPID Only | Ambient or -20 or -80 | SHOW & PG | Immediate |
BLOODBORNE PATHOGEN LETTER TO HOTEL

The attached letter is sent to Hotels/Motels that would be used for conducting the Survey Site exams. The purpose is to explain our Bloodborne Pathogen (BBP) polices and assure the site of our responsibility to follow these policies.

1. Responsibilities
The Administrative team is responsible for sending this letter at the time that a Survey Site is secured.

2. Related Documents

3. Definitions
- **Bloodborne Pathogens** means 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) and human immunodeficiency virus (HIV).

- **Clinical Laboratory** means a workplace where diagnostic or other screening procedures are performed on blood or other potentially infectious materials.

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

- **Contaminated Laundry** means laundry which has been soiled with blood or other potentially infectious materials or may contain sharps.

- **Contaminated Sharps** means 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.

4. Procedural Steps
- Send letter at time of securing Survey Site location
- Annual review is required for all staff who have the potential for exposure to blood or bodily fluids.
Hello,

Thank you for hosting the examination component (we call it Time 3) of the research study that is being conducted by the University of Wisconsin School of Medicine and Public Health. The study is called SHOW (Survey Health of Wisconsin). We are grateful for your help in this important research study and would like to inform you of what we will be doing in your facility and the steps we will take to protect your facility.

What is Show?

SHOW is a project conducted by the University of Wisconsin School of Medicine and Public Health. The goal of SHOW is to present a comprehensive picture of the Wisconsin health residents; we hope to identify health needs and target health resources in the state of Wisconsin. SHOW is funded by the Partnership Fund for a Healthy Future and is directed by Dr. Javier Nieto and the University of Wisconsin School of Medicine and Public Health.

Before Our Visit

SHOW staff will have met with your facility to assure that we can safely and adequately complete the study in the space available. The staff will be working in your community recruiting participants over two weeks prior to us using your facility for "Time 3". At any time, we will be more than happy to answer any questions or concerns you may have about the study or the procedures.

Staff Certifications

All University of Wisconsin Staff have been professionally trained and certified to perform the aspects of “Time 3" which includes obtaining blood samples. Our procedures are standardized and the methods of conducting these procedures are in accordance with all OSHA standards (29 CFR 1910.1030) our staff, as professionals, are willing to discuss all details of our procedures with any appropriate personnel. If you have questions regarding our visit, our staff or our research including the laboratory portions of the study, please feel free to contact Jen Tratnyek (Office Manager) at 608-821-1242 or you can visit our website at www.show.wisc.edu.

Precautions

SHOW staff is trained and certified in the regulations of protecting themselves from all exposure to bodily fluid, and thus, this means that they are to protect against all exposure of themselves or their environment to a blood spill. Steps to assure this protection and appropriate study conduct include:

- Thick plastic rug/floor covering protection
- Clean-up kits are available to staff at all times
- Portable refrigerator and freezing units for storage of samples
- Portable equipment including a work table and examination equipment.
• Divider systems for confidentiality of participants

We would like to again thank you for the use of your facility in this important research study. If you would like more information (or a complete copy of this procedure) please call the SHOW hotline at (888)-433-SHOW or for general information visit our website at www.show.wisc.edu. We would be more than happy to answer any of your questions. Together we can build a healthier Wisconsin and look forward to a brighter future.

Sincerely,

SHOW Personnel
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 Helgesen Drive
Madison, WI 53718
Phone: (608) 223-0275
FAX: (608) 223-0331
http://www.continentalcarbonic.com/dry-ice-madison-wisconsin.html

**SHOW customer number:** #10189

- Request pounds required in 1) nuggets or 2) dry ice blocks (approx. 10 lb blocks)
- Note: dry ice blocks will last longer before dissipating
UW Hospital and Clinics, K4/5 core area: After-hours or in urgent need, dry ice can be located in the dry ice bin in the K4/5 core area of UW Hospital and Clinics (http://www.uwhealth.org/patient-guides/uw-hospital/getting-around-uw-hospital/10149)
LABORATORY INVENTORY AND ACTIVITY FORM

This form is to be used in the following steps:

- Form is completed by the lab processing staff in the field.
- Data entry into the biorepository data system is completed by designated lab personnel using these forms.
- Recording of samples for transfer to Prevention Genetics.
- An annual collection of the forms will be stored with the research records in a file labeled for that year.

Completing this form:

1. Top of page: Indicate the number of the block group for which this list is recording samples. (BG # ____________)

2. Top of page: Indicate the field team managing this survey site; i.e from Middleton, Milwaukee or the Marshfield team. (Circle one: Midd Mke Mfld)

3. 1st column: Insert the date of the blood draw and processing.

4. 2nd column: FOR MILWAUKE ONLY – Insert the date that the sample was put into the -20 freezer.

5. 3rd column: Insert the date that the sample was placed into a -80 degree freezer.

6. 4th column: Insert whether subject is a male or female.

7. 5th column: Place the barcode label that corresponds with the label that is placed on the cryovials box. This label also has the Survey Participant (SP) ID.

8. 6th column: Enter the corresponding number of processed cryovials for plasma, serum and urine.

9. 7th column: Enter the barcode label that corresponds to the barcode label placed on the samples being shipped to Prevention Genetics.

10. 8th column: Enter the number of DNA tubes (10 ml lavender tubes) that are packaged for shipment to Prevention Genetics (PG) storage. If there are no lavender tubes for DNA, indicate whether there is a saliva tube (SAL) being sent to PG. If DNA sample is refused, check the DNA refused box.
11. **9th column:** Place initials of persons drawing blood and processing the samples.

12. **10th column:** Comments, note any issues with tubes, with quality or stability of sample. Also note which samples (by checking ( ) qc) were set aside for Quality Control. Note date sample was sent to Marshfield labs.

<table>
<thead>
<tr>
<th>Survey Site Lab Inventory and Activity Log</th>
<th>BG #</th>
<th>/ Midd</th>
<th>Mke</th>
<th>Mfd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of blood draw / processing</td>
<td>Date in -20 freezer (MKE only)</td>
<td>Date in -80</td>
<td>M or F</td>
<td>Box SPID Label</td>
</tr>
<tr>
<td>□ PL</td>
<td>□ SR</td>
<td>□ UR</td>
<td>□ Tube</td>
<td>□ SAL</td>
</tr>
<tr>
<td>□ PL</td>
<td>□ SR</td>
<td>□ UR</td>
<td>□ Tube</td>
<td>□ SAL</td>
</tr>
<tr>
<td>□ PL</td>
<td>□ SR</td>
<td>□ UR</td>
<td>□ Tube</td>
<td>□ SAL</td>
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<td>□ PL</td>
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<td>□ UR</td>
<td>□ Tube</td>
<td>□ SAL</td>
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<tr>
<td>□ PL</td>
<td>□ SR</td>
<td>□ UR</td>
<td>□ Tube</td>
<td>□ SAL</td>
</tr>
<tr>
<td>□ PL</td>
<td>□ SR</td>
<td>□ UR</td>
<td>□ Tube</td>
<td>□ SAL</td>
</tr>
</tbody>
</table>
Laboratory Tests Form

SPID: _______________________________ SP initials: __________

DATE of draw ___/___/____ labdate Phlebotomist(#s): _______ lab020 Processor (#s):

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

- Mastectomy
- Hematoma
- Burns, Scars, Tattoos
- Damaged Veins
- Shunt, Fistula or Graft
- Recent IV
- Cast
- Edema
- Obesity
- Skin sores

Last ate: lab040@m

___/___/____ (date)

____:____ (time, military)

Subject had only water during past 8 hours ___yes ___no

lab040@_2

Tube status
D=Done
F=Failed
R=Refuse
d
NA=not attempted

<table>
<thead>
<tr>
<th>Lab #/Label/Barcode</th>
<th>TYPE and SIZE OF TUBE</th>
<th>COMMENTS (Why failed, why not attempted, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D=Done</td>
<td>TYPE and SIZE OF TUBE</td>
<td>Comments (Why failed, why not attempted, etc.)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BLOOD DRAWS</th>
<th>1st attempt time <strong><strong>:</strong></strong></th>
<th>Draw time <strong><strong>:</strong></strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>lab060</td>
<td>(ML Label) 5mL SST Gold top for ML</td>
<td></td>
</tr>
<tr>
<td>lab070</td>
<td>(SPID. Label) 10 mL Redtop for Repository</td>
<td></td>
</tr>
<tr>
<td>lab080</td>
<td>(SPID. Label) 10 mL Redtop for Repository</td>
<td></td>
</tr>
<tr>
<td>lab090</td>
<td>(SPID. Label) 10 mL Lavender for DNA</td>
<td>☐No DNA authorized lab090@a2</td>
</tr>
<tr>
<td>lab100</td>
<td>(SPID. Label) 10 mL Lavender for DNA</td>
<td>☐No DNA authorized lab100@a2</td>
</tr>
<tr>
<td>lab110</td>
<td>(ML Label)</td>
<td>3mL Lavender for ML</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>lab120</td>
<td>(ML Label)</td>
<td>3mL Lavender for ML</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>BLOOD DRAW</strong></td>
<td>End draw time</td>
<td>Centrifuge start time</td>
</tr>
<tr>
<td></td>
<td>lab140</td>
<td>_<strong><strong>:</strong></strong></td>
</tr>
<tr>
<td># of Attempted Sticks</td>
<td>lab130</td>
<td># of plasma vials</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lab160</td>
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<tr>
<td></td>
<td></td>
<td>(0.5mL in each 1mL cryovial)</td>
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<tr>
<td></td>
<td></td>
<td># of serum vials</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lab170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.5mL in each 1mL cryovial)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood Freezer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>entry time:</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em><strong><strong>:</strong></strong></em> lab180@h</td>
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<tr>
<td><strong>URINE SAMPLE</strong></td>
<td>Collection time</td>
<td>Centrifuge Time:</td>
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<tr>
<td></td>
<td>lab190@_1</td>
<td><em><strong><strong>:</strong></strong></em></td>
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<tr>
<td>lab200</td>
<td>(SPID. Label)</td>
<td>Urine Sample</td>
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<td>Done</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Failed</td>
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<tr>
<td></td>
<td></td>
<td>Insufficient</td>
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<tr>
<td><strong>SALIVA SAMPLE</strong></td>
<td>Collection time:</td>
<td>Freezer Time:</td>
</tr>
<tr>
<td></td>
<td>lab240@a</td>
<td><strong><strong>:</strong></strong></td>
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<tr>
<td>lab240@a</td>
<td>(SPID. Label)</td>
<td>Blood draw</td>
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<td></td>
<td></td>
<td>Refused</td>
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<tr>
<td></td>
<td></td>
<td>Inadequate</td>
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<tr>
<td>Willing to be rescheduled</td>
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</tbody>
</table>

**PROCESSING PROBLEMS/COMMENTS**

lab250
Clean-Catch Urine Collection Procedure 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
Clean-Catch Urine Collection Procedure 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 inside of 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 the toilet for a few seconds.

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

7) Urinate the remainder of 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 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.
Likely Number of Urine Specimens based on Volume of Urine Collected

<table>
<thead>
<tr>
<th>mL Urine</th>
<th># of 2mL Cryovials with 1.5 mL of Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
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