

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

1. PURPOSE AND PRINCIPLE

- 1.1. This SOP describes the procedure for collecting patient specimens that are brought to the IDI core Laboratory.
- 1.2. Collecting blood and urine samples is an important component of patient care. Specimens are collected for laboratory analysis at the request of a qualified healthcare provider to help in the diagnosis and care of patients.
- 1.3. In order to provide a biologically representative blood specimen, it is necessary to obtain the blood specimens as skillfully as possible. In order to do this, it is each phlebotomist's responsibility to master phlebotomy procedures and become familiar with established policies.

2. SCOPE

- 2.1. This SOP applies to all IDI Core Laboratory client clinic and study staff responsible for the collection of blood, urine, and other specimens for which testing or processing is to be done at the IDI Core Laboratory.
- 2.2. The core lab regularly monitors quality of specimens received and may from time to time provide feedback to clients on quality of specimens collected with a view of improvement in phlebotomy practice.

3. RESPONSIBILITIES

- 3.1. It is the responsibility of the laboratory director or designee to review (at least once annually) and approve this SOP.
- 3.2. It is the responsibility of all Core Laboratory staff to follow this SOP that impacts the research/clinical activities they perform

4. PROCEDURE

4.1. General Procedure

4.1.1. Specimen collection training

- 4.1.1.1. It is the responsibility of respective studies and clinics to ensure and document that all personnel performing patient blood collection have been trained in collection techniques and in the proper selection and use of equipment/supplies.

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

4.1.2. Patient preparation

4.1.2.1. Depending on tests requested, patients should be appropriately and accordingly prepared or instructed ahead of the performance of specimen collection procedures. For example, when fasting glucose levels or creatinine clearance test are to be done, patients should be instructed and prepared appropriately before specimens are collected.

4.1.3. Collection Containers and Specimen amounts to be collected

4.1.3.1. Specimen collection containers ought to be appropriate for the purpose and quantity needed for testing for which specimen is required. Containers must be suitable for mode of transport and storage of the specimen(s) collected prior to testing.

4.1.3.2. All phlebotomy supplies and collection containers must be used within their expiry date and stored as per manufacturer's instructions.

4.1.4. Types and amounts of preservatives or anticoagulant

4.1.4.1. Blood specimen collection containers usually have color coded rubber stops to reflect the type of additive (preservative or anti-coagulant) contained. *Please see example - appendix 1 – BD Tube Guide chart for venous blood collection*

4.1.4.2. Blood should NEVER be poured out from one tube to another since the tubes may have different additives or coatings; or additionally, the appropriate blood to anticoagulant ratio may also be altered when anticoagulant tube contents are poured into another anticoagulant coated tube.

4.1.4.3. In order to obtain proper anticoagulant to blood ratio, tubes with anticoagulant should fill completely; but NEVER over filled. Vacutainer tubes will fill automatically to a premeasured amount depending on the vacuum.

4.1.4.4. When multiple blood collection tubes are to be filled in a single phlebotomy event, tubes need to be drawn in a specific order to prevent carryover of the additive from one tube to another. *Please see appendix 2 – BD Vacutainer order of draw for multiple tube collections guide.*

4.1.5. Special handling needs

4.1.5.1. Some specimen types may require special procedures for handling such as immediate refrigeration, transport on ice, expedited delivery, etc. As applicable, such needs should be clearly documented and appropriately communicated.

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

4.1.6. Patient Identification and specimen labeling

- 4.1.6.1. All primary specimen containers must be labeled with at least two identifiers (e.g., patient's Clinic/Study ID/name, date of birth, collection date, patients' initials, etc.) as a confirmation of the patient's identity. This information must be in agreement with the information documented in the Lab requisition form and other patient documentation.
- 4.1.6.2. Any specimen labeling discrepancies must be resolved prior to sending the patient's specimen to the lab. The lab will reject samples with illegible labels.
- 4.1.6.3. Correction of illegible or incorrect specimen labels by IDI core laboratory reception/processing staff is not permitted under any circumstances except in cases of very precious specimens such as cerebrospinal fluid where the lab management may contact the clinic to resolve the problem.

4.1.7. Need for appropriate data when indicated

As applicable, and when indicated, clinical information should be provided on test request forms in order to aid in the accurate interpretation of test results.

4.2. Venous Blood Collection

4.2.1. Purpose (Venipuncture)

- 4.2.1.1. Quality patient care and accurate specimen results are dependent upon proper **venipuncture** technique, timely specimen collection, and proper processing of patient specimens. Common collection errors encompass incorrect identification of the patient, hemolyzed specimens, and the use of an incorrect anticoagulant. This procedure establishes criteria for the proper collection of blood specimens by venipuncture.

4.2.2. Venipuncture Procedure

- 4.2.2.1. Wash your hands. Frequent hand washing is an important standard precaution to be done prior to and after contact with each patient.
- 4.2.2.2. Put on a pair of single-use disposable gloves to collect the specimens
- 4.2.2.3. Organize the type and number of tubes required. Refer to the Core lab Samples requirements to determine the tube type(s) and quantity required.
- 4.2.2.4. Assemble the needle to the vacutainer holder. Leave protective sheath covering needle intact. The most frequently used needle is 21 gauge. A 23 gauge needle can be used for very small veins. This smaller needle must be used carefully as it can easily cause specimen hemolysis.
- 4.2.2.5. Position patient's arm in a flat position with the wrist lower than the bend of the arm. Tie the tourniquet approximately 4 inches above the intended venipuncture site. The tourniquet should be looped so that it can be released with a gentle pull while holding both ends of the tourniquet.
- 4.2.2.6. Palpate patients arm for the best venipuncture site. Ask the patient to make a fist; this helps to distend the veins. Choose the vein that feels the fullest. A good vein is ALWAYS palpable. It springs back like a small rubber band. If you have any doubt about using the veins in one arm, check the other arm

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

before starting the venipuncture. (The vein may not be visible. Small surface veins are not always the best because they collapse with the pressure of the vacuum).

- 4.2.2.7. Apply disinfectant (e.g., alcohol prep) and clean potential venipuncture site, wiping with a circular motion from the center to the periphery. Dry venipuncture site with a Cotton pad one time only to assure the site is dry before performing the puncture. Have spare cotton readily available. If the venipuncture site proves difficult and the vein must be touched, the site must be cleansed again. *(If volatile or toxicology tests are being collected, DO NOT USE alcohol pads to clean the venipuncture site. Use a betadine swab to eliminate the potential of alcohol contamination in the specimen)*
- 4.2.2.8. Discard the protective sheath covering the needle. Visually inspect the needle tip to determine that it is free of hooks at the end of the point. Assure that the bevel of the needle is up. Hold the skin below the site taut by drawing the skin downward with your thumb. Stick the vein in the same direction as the vein runs. Start puncture at bend of arm or just below bend whenever possible. Make the puncture as smooth and quick as possible while holding the vacutainer barrel at approximately a 15 degree angle.

Note: NEVER PASS EQUIPMENT IN FRONT OF PATIENT'S FACE.

- 4.2.2.9. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper. Fill the tube until the vacuum is exhausted at which time blood flow will cease. Remove the tube from the holder. The shut off valve re-covers the point, stopping blood flow until the next tube is inserted into the holder.
- 4.2.2.10. Vacutainer tubes must be collected in the proper order based on the additive type and whether the tubes are glass or plastic. If using BD tubes - refer to the BD tube chart for the order of draw.
- 4.2.2.11. Any tubes containing **anticoagulants** (lavender top, blue top, clot activator tubes, and others) **should be gently inverted several times** immediately after collection to prevent specimen clot formation. These tubes **should be filled to capacity** to obtain the proper anticoagulant to blood ratio.
- 4.2.2.12. While the last tube is filling untie the tourniquet holding both ends and remove it from the patients arm. Remove the last vacutainer tube from the holder. Put a cotton pad over needle. Remove needle in a quick careful motion. Don't press down on cotton pad until the needle has been completely removed from the vein. Apply pressure to puncture site. (Patient can hold, if possible.) Activate the needle safety device (if applicable) and immediately

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

discard the needle in the sharps container to prevent reuse or accidental injury.
NEVER RECAP THE NEEDLE.

- 4.2.2.13. All specimens must be labeled in the presence of the patient before leaving collection site.
- 4.2.2.14. Observe the patient's venipuncture site to assure that bleeding has stopped. Cover site with a bandaid or cotton held in place with surgical tape.

Note: Specimen collection date/time and the collector's initials must be recorded in the appropriate areas of the lab requisition

4.3. Blood Collection by Butterfly Needle

4.3.1. Purpose (Butterfly Blood Collection)

- 4.3.1.1. The butterfly is a stainless steel beveled needle with attached plastic wings and tubing. The most common butterfly needles are 21 and 23 gauge. The butterfly is used in the collection of blood from patients who are difficult to stick by conventional methods or in pediatric patients.

4.3.2. Venipuncture Procedure With Butterfly

- 4.3.2.1. Identify patient as in 3.7.1. follow steps 4.2.1 to 4.2.3 (in Venipuncture Procedure)
- 4.3.2.2. Open the package of the butterfly needle device and unwind the coiled tubing. Attach the luer end of the butterfly to the vacutainer collection barrel.
- 4.3.2.3. Position patient's arm in a flat position with the wrist lower than the bend of the arm. Tie the tourniquet approximately 4 inches above the chosen venipuncture site. The tourniquet should be looped so that it can be released with a gentle pull.
- 4.3.2.4. Palpate patients arm for the best venipuncture site. Ask the patient to make a fist; this helps to distend the veins. Choose the vein that feels the fullest. A good vein is ALWAYS palpable. It springs back like a small rubber band. If you have any doubt about using the veins in one arm, check the other arm before starting the venipuncture. (The vein may not be visible. Small surface veins are not always the best because they collapse with the pressure of the vacuum).
- 4.3.2.5. The larger and fuller median cubital and cephalic veins are used most frequently, but wrist and hand veins are also acceptable. Determine the direction in which the vein runs. Open alcohol prep and clean potential venipuncture site, wiping with a circular motion from the center to the periphery. (If volatile or toxicology tests are being collected, DO NOT USE alcohol pads to clean the venipuncture site. Use a betadine swab to eliminate the potential of alcohol contamination in the specimen). Dry venipuncture site with cotton piece one time only to assure the site is dry before performing the puncture. Have spare cotton piece readily available. If the venipuncture site proves difficult and the vein must be touched, the site must be cleansed again.
- 4.3.2.6. Discard the protective sheath covering the butterfly needle. Hold the butterfly needle by its attached plastic wings. Visually inspect the needle tip to

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

determine that it is free of hooks at the end of the point. Hold the skin below the site taut by drawing the skin downward with your thumb. It is wise to tell the patient just prior to performing the skin puncture so that the patient is not frightened. Stick the vein in the same direction as the vein runs. Start puncture at bend of arm or just below bend whenever possible. Make the puncture as smooth and quick as possible.

- 4.3.2.7. Grasp the flange of the needle holder and push the tube forward until the butt end of the needle punctures the stopper. Fill the tube until the vacuum is exhausted at which time blood flow will cease. Remove the tube from the holder. The shut off valve re-covers the point, stopping blood flow until the next tube is inserted into the holder.
- 4.3.2.8. Vacutainer tubes must be collected in the proper order based on the additive type and whether the tubes are glass or plastic. Refer to the BD tube chart for the order of draw. Any tubes containing anticoagulants (lavender and blue tops, for example) should be inverted several times immediately after collection to prevent specimen clot formation. These tubes should be filled to capacity to obtain the proper anticoagulant to blood ratio.
- 4.3.2.9. While the last tube is filling untie the tourniquet holding both ends and remove it from the patients arm. Remove the last vacutainer tube from the holder. Put a cotton pad over needle. Remove needle in a quick careful motion. Don't press down on cotton pad until the needle has been completely removed from the vein. Apply pressure to puncture site. (Patient can hold, if possible.) Activate the needle safety device and immediately discard the needle in the sharps container to prevent reuse or accidental injury. **NEVER RECAP THE NEEDLE.**
- 4.3.2.10. All specimens must be labeled in the presence of the patient before leaving collection site. Place barcode labels with the printed specimen number adjacent to the stopper end.
- 4.3.2.11. Observe the patient's venipuncture site to assure that bleeding has stopped. Cover site with a band aid or cotton piece held in place with surgical tape.

4.4. Capillary Blood Collection (Finger-stick / Heel-stick)

4.4.1. Purpose (Capillary Blood Collection)

- 4.4.1.1. Blood specimens obtained by skin puncture are **especially important in pediatrics** because, with this technique, small but adequate amounts of blood for laboratory tests can be obtained. Capillary blood is obtained from the **finger** or from the plantar surface of the **heel of an infant**. Obtaining blood by venipuncture from infants may be difficult. Obtaining large quantities of blood, especially from premature infants, may result in anemia. At times, it is also advantageous to obtain skin-puncture blood specimens from **adult**

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

patients who are severely burned, extremely obese, or geriatric patients whose superficial veins are very fragile.

4.4.2. Capillary Blood Collection Procedure

4.4.2.1. Wash your hands. Frequent hand washing is a mandatory standard precaution to be done prior to and after contact with each patient, and put on a pair of single-use disposable gloves and collect the specimens according to the venipuncture procedure.

4.4.3. **Fingerstick Blood Collection (for adults and small children)**

4.4.3.1. Patient should be comfortably positioned.

4.4.3.2. Select an appropriate puncture site. The lateral part of the end of the middle or ring finger is most often used to obtain the specimen. If the patient's finger is cold, it can be warmed by rubbing it or placing it in warm water. Dry site with cotton pad.

4.4.3.3. Cleanse the potential fingerstick site with an alcohol pad. The site must be thoroughly dried with a cotton pad before being punctured because residual alcohol causes rapid hemolysis and discomfort. **Betadine should not be used to disinfect a fingerstick site because it falsely elevates levels of potassium, phosphorus, and uric acid.**

4.4.3.4. Open the sterile lancet without contaminating the unit.

4.4.3.5. Without touching the disinfected puncture site, firmly grasp the patient's finger.

4.4.3.6. Hold lancet on site with moderate pressure, plunger oriented upward.

4.4.3.7. Depress plunger with index finger to make puncture.

4.4.3.8. Immediately release plunger while holding lancet on site. Remove lancet from the puncture site.

4.4.3.9. Wipe off the first droplet of blood with a pad.

4.4.3.10. Hold microtainer tube at a 30 to 45 degree angle from the surface of the puncture site.

4.4.3.11. Touch the collector end of the reservoir to the drop of blood.

4.4.3.12. Blood will flow down the wall of the reservoir to the bottom of the tube, filling the microtainer. Occasionally tap the lavender microtainer to ensure that blood flows to the bottom of the tube and mixes with the anticoagulant. This

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

will help prevent clotting. *Fill lavender microtainers between the 250 to 500 ul mark.*

- 4.4.3.13. Replace the microguard closure by twisting and pushing the cap downward until it snaps. Mix the lavender samples by inverting the tube a minimum of 10 times

4.5. Heel stick Blood Collection (infants)

- 4.5.1. The infant should be firmly held by seated adult.
- 4.5.2. Select the appropriate puncture site on the infant's heel. The puncture shall not be through a previous puncture site which may be infected, nor shall it be at the curvature of the heel. Warming of the infants heel prior to disinfection increases blood flow to the area. The shaded area illustrates the recommended site for the heel stick.
- 4.5.3. Cleanse the potential heel stick site with an alcohol pad. The site must be thoroughly dried with a cotton pad before being punctured because residual alcohol causes rapid hemolysis.
- 4.5.4. Open the sterile lancet without contaminating the unit.
- 4.5.5. Without touching the disinfected puncture site, firmly grasp the infants' heel.
- 4.5.6. Hold lancet on site with moderate pressure.
- 4.5.7. Depress plunger with index finger to make puncture.
- 4.5.8. Immediately release plunger while holding lancet on site, and remove lancet from the puncture site.
- 4.5.9. Proceed with specimen collection using the microtainer device. Follow the instructions in the finger stick procedure.

4.6. Urine Collection

- 4.6.1. Proper urine specimen collection and handling is an essential part of the overall process to obtain quality laboratory results. Adherence to policy is essential with any urine specimen collection whether it is a clean catch, random void, or a timed urine collection.

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

Variables such as the container, storage, collection technique, and transportation are significant because they affect the outcome of the analysis.

- 4.6.2. When both urine and pelvic specimens are to be collected from the same patient at the same clinic visit, *urine specimens should be collected first before any pelvic specimens are collected.*

4.6.3. Procedure:

- 4.6.3.1. Label the container with the patient's name &/or ID number. Have the patient verify that the ID on the container is their ID. Instruct the patient to do the following:

4.6.3.2. Collecting the Specimen - Female

- 4.6.3.2.1. Wash your hands with soap and water; rinse; dry with a paper towel.
4.6.3.2.2. Open container and place lid on counter with sterile side up.
4.6.3.2.3. Spread labia minora (genital folds)
4.6.3.2.4. Using wipes provided wipe front to back. Moving from above your urethra towards the anus.
4.6.3.2.5. Make three passes using a clean side of the wipe. Once down the left, then right side of the labia, and once down the center.
4.6.3.2.6. Continue holding the labia and start to urinate.
4.6.3.2.7. While still urinating place the sterile container under the urine stream. Collecting a mid-stream sample.
4.6.3.2.8. Replace cap securely on the specimen container (only touching the outside).
4.6.3.2.9. Wipe excess urine off the outside of the container.
4.6.3.2.10. Transport the specimen to the laboratory within 2 hours of collection.
4.6.3.2.11. If this is not possible, refrigerate and transport as soon as possible within 12 hours

4.6.3.3. Collecting the Specimen - Male

- 4.6.3.3.1. Wash your hands with soap and water; rinse; dry with a paper towel.
4.6.3.3.2. Open container and place lid on counter with sterile side up.
4.6.3.3.3. Hold penis with one hand and if uncircumcised, retract the foreskin before cleansing. Using a circular motion and an antiseptic swab, cleanse the urethral opening, moving from center to outside.
4.6.3.3.4. While continuing to retract foreskin, start to urinate into toilet.
4.6.3.3.5. While still urinating, place the sterile container in to the urine stream. Collecting a mid-stream sample.
4.6.3.3.6. Replace cap securely on the specimen container (only touching the outside).
4.6.3.3.7. Wipe excess urine off the outside of the container.
4.6.3.3.8. Transport the specimen to the laboratory within 2 hours of collection.
4.6.3.3.9. If this is not possible, refrigerate and transport as soon as possible within 12 hours

4.6.4.

- 4.6.4.1.1. Wash his/her hands, and dry them.
4.6.4.1.2. Open the urine collection container

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

**PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating
Procedure (SOP)**

- 4.6.4.1.3. Start to urinate (for a second) into the toilet, then collect the middle portion of the urine specimen in the container.
- 4.6.4.1.4. Tightly secure the lid on the container.
- 4.6.4.1.5. Wash his/her hands with soap and water.
- 4.6.4.1.6. Finally instruct the patient to return the collected urine to the phlebotomy station for relay to the laboratory.

4.6.4.2.

4.7. Stool Collection

- 4.7.1. Stool samples should be collected into clean, wide mouthed containers and should not be contaminated with urine. Stool samples collected from diaper/nappy residues are not acceptable as the liquid content of diarrheic stool will have been lost. Examining the liquid content of stool is important for the detection of motile protozoan parasites.
- 4.7.2. Liquid and diarrheic samples should be examined within 30 minutes of collection to detect motile protozoan trophozoites and therefore should be delivered to the laboratory IMMEDIATELY upon collection.
- 4.7.3. Semi formed specimens should be delivered to the laboratory within 45 minutes, while formed specimens should be kept at room temperature if delivery to the lab may occur after more than one hour following collection.

4.8. Vaginal Swab Specimen Collection for Wetmount/KOH Preparation

- 4.8.1. Vaginal secretions are obtained with a sterile swab from the lateral vaginal wall during a pelvic examination. *Please refer to respective clinic guidelines for detailed procedures for pelvic specimens' collection.*
- 4.8.2. Swabs are placed into tube with 6 drops of sterile physiological saline.
- 4.8.3. Collections are to be transported to the Stat Laboratory IMMEDIATELY upon collection to facilitate preparation and observation within 30 minutes after collection.
- 4.8.4. To preserve the motility of *T. vaginalis*, the specimen should not be refrigerated and should be examined as soon as possible following collection.

4.9. Vaginal Specimen swabs for *T. vaginalis* rapid testing:

- 4.9.1.1. A sterile rayon swab must be used when collecting specimens from the vaginal cavity. Swabs with cotton tips or wooden shafts are not recommended. Use of swabs supplied in the kit or BD BBL™ culture swab is recommended.
- 4.9.1.2. Transport the swabs to the processing laboratory as soon as possible after collecting the specimen.

INFECTIOUS DISEASES INSTITUTE CORE LABORATORY
New Mulago Hospital P.O.BOX 22418, Kampala,

PROS-012-rev#4-Core Lab Phlebotomy and Urine Collection Standard Operating Procedure (SOP)

4.10. Other Specimen types

- 4.10.1. The core lab may receive other specimen types for processing and/or storage but for which testing is not required or performed at the core lab.
- 4.10.2. Respective clinic and study protocols should be followed appropriately in the collection and transportation for those specimens.

4.11. Specimen handling/transportation

- 4.11.1. All specimens must be delivered immediately to the laboratory (e.g., with the next available runner).
- 4.11.2. All blood glucose specimens requiring blood glucose test but not collected in potassium oxalate or sodium fluoride coated bottles **MUST** be delivered to the laboratory as **IMMEDIATELY** in order to facilitate testing within 30 minutes of collection.
- 4.11.3. When handling blood & body fluids must follow BBP/Universal Precautions.

5. APPENDIXES

Appendix Title	Rev#	Rev#	Rev#	Rev#
PROS-012-rev#3-Core Lab Phlebotomy and Urine Collection Standard Operating Procedure-BD tube guide chart-rev#Jan10	Jan2010			
PROS-012-rev#3-Core Lab Phlebotomy and Urine Collection Standard Operating Procedure-BD tube order of draw chart-rev#Jan10	Jan2010			

6. REFERENCES

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