MYCOBACTERIA BLOOD CULTURE PROCEDURE

A. **Purpose:** To detect mycobacteria in blood.

B. **Principle:** The Wampole isolator tube contains agents which lyse the cellular components of blood and block coagulation. Proper use of the tube will provide a concentration of organisms, from a blood sample, to be inoculated directly onto agar surfaces.

C. **Materials for Collection:**
1. one Wampole isolator 10 ml tube or green top tube (sodium-heparin) 10 mL without gel
2. needles and syringes;
3. alcohol and iodine preps.

D. **Specimen Collection:** CAUTION: Isolator tubes or green top tube (sodium-heparin) should be at room temperature before collection.

**Preferred – Isolator Tube:**
1. The rubber stopper should be scrubbed with an alcohol, chlorhexidine or iodine (Betadine) solution. Avoid pooling of the solution on the rubber stopper. The iodine should then be allowed to dry.
2. The patient's arm should be disinfected appropriately for sterile venipuncture (see Blood Culture Collection procedure for skin preparation). The isolator tube should be filled as the “first” tube of a venipuncture draw, or alternatively, using a syringe. If a syringe is utilized to inoculate the tube, the specimen must not be forced into the vacutainer. Butterfly draws should not be used due to the potential for clotting of the sample prior to entry into the tube.
3. The amount of blood present is most critical. When the tube is nearly full, 7.5 ml will be present. Tubes with lesser amounts should not be processed.
4. Gently invert the tube four or five times immediately after collection of the blood.
5. The tube, properly labeled with the LIS Beaker label containing: patient's name, chart number, time, and date of collection should be transported immediately to the Microbiology Laboratory.

**Suitable – Heparinized vacutainer:**
1. Scrub the black rubber stopper of the heparinized vacutainer tube(s) with an iodine solution. Avoid pooling of the solution on the rubber stopper. Allow the iodine to dry.
2. Disinfect the patient's arm appropriately for sterile venipuncture (see Blood Culture Collection procedure for skin preparation). If a syringe is utilized to inoculate the tube, the specimen must not be forced into the vacutainer. Do NOT perform butterfly draws due to the potential for clotting of the sample prior to entry into the tube.
3. Gently invert the tube four or five times immediately after collection of the blood.
4. Immediately forward the heparinized vacutainer tube(s) to the Microbiology department for processing.

E. **Rejection Criteria:**
1. Isolator tubes or green tubes (sodium-heparin) which contain less than the required amount of sample (less than 7.5 ml) should not be processed.
2. An extended delay in specimen transport of the Wampole Isolator tube, which would not allow processing of the sample within 16 hours from the collection time, is not acceptable. If the sample will have a delay of >16 hours from collection to arrival at the Bassett Microbiology department, the green top (sodium-heparin) tube(s) should be utilized.

F. **Materials for Processing:**
Alcohol preps
Beckman TJ-6 centrifuge (O&P room)
Isolator tube(s) (C-cart back wall Microbiology department)
Isostat Products (Special’s room 2nd drawer down)
  - Isostat supernatant pipet
  - Isostat cap
  - Isostat concentrate pipet
Isostat press (Special’s room)
1 - LJ slant and 1 MGIT tube (Refrigerator #4, AFB room)

G. Processing:

1. Isolator tubes should be processed as soon as possible upon receipt in the laboratory. Batching of the specimens, when possible, should be performed to allow for maximum utilization of the technologist's time, but should provide maximum potential for the most rapid recovery of organisms. The time from collection to processing of Wampole Isolator 10 mL tube should never exceed 16 hours.

2. Heparinized tubes should be processed as soon as possible upon receipt in the laboratory. Green top heparinized tubes are acceptable for AFB and Fungal culture for up to 72 hours.

Upon receipt in the Microbiology Department, transfer the contents of the heparinized vial (green top) to a Wampole isolator 10 ml tube.
  - A BD Vacutainer Eclipse Blood Collection Needle and the BD hub may be used
  - OR
    - the sample can be transferred from the green top (sodium-haparin) to the Wampole isolator using a sterile pipette. Ensure rubber stopper is secure prior to centrifugation.

Perform all further manipulations in the Biological Safety Cabinet.

BD Vacutainer Eclipse Blood Collection Needle and the BD hub

  - Prior to specimen transfer disinfect the stoppers (Wampole Isolator 10 ml tube and BD 6.0 ml heparinized vacutainer tube) with alcohol. Allow adequate time for stoppers to dry.
  - **Insert BD Vacutainer Eclipse Blood collection needle into the BD 6.0 ml heparinized tube 1st**
  - Insert unit (heparinized vial, BD hub and Eclipse Needle) into the Wampole Isolator 10 ml tube. The vacuum within the Wampole Isolator tube will automatically draw in the contents of the BD 6.0 ml heparinized tube.
  - Gently invert the tube four or five times immediately after transfer of the blood. **Incubate for 60 minutes contact time to allow for lysis of the red blood cells, prior to the centrifugation process.**

3. Use the TJ-6 centrifuge in the Parasitology Room. Remove the rotor head currently on the centrifuge and replace with the rotor labeled Isolator Rotor. Place the tubes in the rotor using the adapters provided. Centrifuge the specimen for 30 minutes at 3000 g (the highest setting) or 3700 rpm’s. In order to prevent breakage of the tube, 3000 g must not be exceeded. **DO NOT USE THE CENTRIFUGE BRAKE.**

4. Following centrifugation, carefully remove each isolator tube from its centrifuge adapter and place in the Isostat rack. A slight clockwise twist will facilitate insertion of the tube into the rack. Be sure tubes are firmly seated and vertically aligned to avoid breakage during insertion of the cap.

5. Disinfect the stopper with an alcohol wipe. **DO NOT** allow the alcohol to pool in the stopper cavity. Allow to dry for one minute.

6. Place the rack on the base of the Isostat press.

Perform all further manipulations in the Biological Safety Cabinet.

6. Remove Isostat cap from the sterile package by pushing the base of the cap out through the paper wrapper and grasping the sides of the cap with your fingers.
7. Place a cap over the top stopper of each isolator tube. If more than one tube is being processed, position caps on all tubes in the rack before proceeding to the next step.

8. Position a tube with its cap under the press head. Gently pull the handle of the press down as far as possible. The spike will penetrate the stopper, and the cap will be firmly seated on the top of the tube. Return the handle to the upright position. If more than one tube is being processed, rotate the rack to position the next tube. Press the cap onto this tube, and continue until caps have been pressed onto each tube in the rack. Carefully move the rack from the press to the work area.

9. a) Open the heat seal at the top of a package of Isostat supernatant pipets (large size), then pull apart the zippered seal. Remove a supernatant pipet from the package.
   b) Squeeze the bulb of the Isostat supernatant pipet to collapse it and provide a vacuum for supernatant withdrawal. Do this before inserting the stem into the tube.
   c) Carefully insert the stem of the supernatant pipet into the isolator tube through the membrane in the Isostat cap while maintaining pressure on the bulb.
   d) Insert the pipet into the tube as far as possible; the base of the bulb should rest on the cap.
   e) Release the bulb and allow the supernatant fluid to be drawn into the pipet. Repeat this procedure with the remaining tubes in the rack.
   f) Confirm that air has entered the pipets, indicating that all the supernatant fluid has been withdrawn.
   g) When the supernatant fluid has been withdrawn from all Isolator tubes, remove and discard the pipets into an appropriate receptacle for contaminated waste.

10. a) Open the heat seal at the top of a package of Isostat concentrate pipets (small pipets), then pull apart the zippered seal. Remove a concentrate pipet from the package.
   b) Remove the first isolator tube from the rack and vigorously mix the contents for 5-10 seconds (using the vortex mixer at its highest setting) in order to achieve a homogenous emulsion.
   c) Squeeze the bulb of the concentrate pipet to collapse it and provide a vacuum for concentrate withdrawal. Do this before inserting the stem into the tube.
   d) Carefully insert the stem of the concentrate pipet into the isolator tube through the membrane in the Isostat cap while maintaining pressure on the bulb.
   e) Insert the pipet into the tube so that the tip reaches to the bottom of the tube. It may be necessary to manipulate both pipet and tube to properly orient the pipet tip.
   f) Gradually release pressure on the bulb and allow the concentrate to be drawn into the pipet. A slow controlled release of the bulb is necessary to achieve maximum recovery of concentrate.

11. a) Immediately remove the pipet and use it to distribute the among the AFB agar (MGIT tube and Lowenstein Jensen agar slant).
   b) Using a sterile sterile pipette, add 500 ul or ½ ml of Wampole Isolator cell button to the MGIT broth and 4 to 5 drops of Isolator cell button to the Lowenstein Jensen agar.

12. Discard used pipets and isolator tubes into an appropriate receptacle for contaminated waste.

13. Incubate mycobacteria media according to AFB procedures. Report positive fungal or mycobacteria blood cultures following the same protocol as for bacterial isolate.

H. REFERENCES:


Delayed growth of M. tuberculosis and M. avium in liquid media after addition of saponin, polypropylene glycol and sodium polyanetholesulphonate. 18th European Congress of Clinical Microbiology and Infectious Disease, Barcelona, Spain, April 2008.

