FUNGAL BLOOD CULTURE PROCEDURE

A. Purpose: To detect yeast or fungi in blood.

B. Principle: The Du Pont 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:

Preferred:
1. one Du Pont isolator 10 ml tube;
2. needles and syringes;
3. alcohol and iodine preps.

Suitable:
1. one Du Pont isolator 10 ml tube;
2. one BD 6.0 ml heparinized vacutainer tube
3. needles and syringes
4. alcohol and iodine preps.
5. BD Vacutainer Eclipse Blood Collection Needle
6. BD Hub

D. Specimen Collection:

CAUTION: Isolator tubes should be at room temperature before collection.

Preferred – Isolator Tube:

1. Scrub the black rubber stopper of the BD 6.0 ml heparinized vacutainer tube 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. 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 patient's name, chart number, time, and date of collection, and a completed requisition (including time of collection) should be transported immediately to the Microbiology Laboratory.

Suitable – Heparinized vacutainer:

1. Scrub the black rubber stopper of the BD 6.0 ml heparinized vacutainer tube 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 BD 6.0 ml heparinized vacutainer tube to the Microbiology department for processing.
E. Rejection Criteria:

1. Isolator tubes 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 Dupont Isolator 10 ml tube, which would not allow processing of the sample within 16 hours from the collection time, is not acceptable.

3. An extended delay in specimen transport >7 days of the heparinized green top collection tube.

F. Materials for Processing:

<table>
<thead>
<tr>
<th>Beckman TJ-6 centrifuge</th>
<th>Alcohol preps</th>
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<tbody>
<tr>
<td>Isostat rack</td>
<td>Isostat supernatant pipet</td>
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<tr>
<td>Isostat cap</td>
<td>Isostat concentrate pipet</td>
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<tr>
<td>Isostat press</td>
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<tr>
<td>2 - SAB dextrose plates</td>
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<tr>
<td>1 - LJ slant and 1 MGIT tube (if AFB ordered)</td>
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</table>

G. Processing:

1. Blood samples
   a. 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 Dupont Isolator 10 ml tube should never exceed 16 hours.

   b. Heparinized tubes should be processed as soon as possible upon receipt in the laboratory. The time from collection to processing of heparinized tubes should never exceed 16 hours.

   Upon receipt in the Microbiology Department, transfer the contents of the heparinized vial (green top) to a Du Pont isolator 10 ml tube, using the BD Vacutainer Eclipse Blood Collection Needle and the BD hub.

   Perform all further manipulations in the Biological Safety Cabinet.

   - Prior to specimen transfer disinfect the stoppers (Dupont 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 1**.
   - Insert unit (heparinized vial, BD hub and Eclipse Needle) into the Dupont Isolator 10 ml tube. The vacuum within the Dupont 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 30 minutes contact time to allow for lysis of the red blood cells, prior to the centrifugation process.

2. 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). In order to prevent breakage of the tube, 3000 g must not be exceeded. DO NOT USE THE CENTRIFUGE BRAKE.

   a. If sample is for Fungus (FBC) and Mycobacteria (ABC) culture, DO NOT process Isolator tube for up to 1 hour from time of collection. The one hour contact time allows for lysis of the red blood cells, prior to the centrifugation process. The extended (1 hour) lysis step is required for mycobacteria culture only.

3. 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.
4. Disinfect the stopper with an alcohol wipe. DO NOT allow the alcohol to pool in the stopper cavity. Allow to dry for one minute.

5. 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 concentrate equally among the agar media. Use two SAB plates for fungal culture. If a mycobacteria culture is requested, add one Lowenstein Jensen slant and a MGIT tube. Keeping the lids of the plates as low as possible, dispense the concentrate in a straight line along the surface of the agar. Keep the inoculum away from the edge of the plate.
    b) Using a sterile inoculating loop, streak through the concentrate, making about 15 to 20 streaks perpendicular to the original inoculum line. Streak lines should be kept away from the edges of the plate.

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

13. Wrap the fungal plates with fungal tape. Incubate at 25 C for 8 days. Check plates daily.
14. Incubate fungal media according to fungal incubation procedures. Report positive fungal or mycobacteria blood cultures following the same protocol as for bacterial isolate.

H. REFERENCES:


DESCRIPTION OF DOCUMENT

Rationale for New or Revised Document: Updated – Current Practice and References, current director

Note all previous revision dates: Created 3/5/97; Updated 10/9/09, 12/17/12

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List any related documents/forms/processes that are affected. Prepare additional Doc. Control Forms as needed. N/A

Revision affects the following:

MIBH ___ GENERAL LAB ___ LAB ADMIN
Specific MIBH Lab sections: ___ CLP ___ Chem X Micro ___ Heme ___ Blood Bank ___ Cytology
___ Surgical Path ___ POC ___ LIS ___ Phlebotomy

Regional Sites:
___ CRH ___ Hamilton ___ Herkimer ___ OCH ___ Oneonta ___ OSS Dialysis ___ TRH ___ POC

Is process validation affected? ______ YES X _____ NO

If so, why?

SIGNATURE APPROVAL

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Issue Date for Training/Review: NA

Effective Date for Use: NA

COMMUNICATION PLAN

_____ Section Meeting Date: ____________________________

X E-mail to Micro Dept. Date: ____________________________

_____ Lab Manual on website Date: ____________________________

_____ Memo to Date: ____________________________