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**Manual: Microbiology Manual II**

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**Department of Pathology and Laboratory Medicine**

IU Health Morgan Hospital Laboratory

Martinsville, IN 46151

**BLOOD CULTURE**

**By**

**BACTEC 9120 INSTRUMENT**

1. **PRINCIPLE/PURPOSE**

Blood cultures are an essential tool in detecting sepsis when bacteria or fungi overcome the host’s normal defense mechanisms and enter the blood stream through the lymphatics or from extravascular sites. The procedure describes the operation of the Bactec 9120 instrument and the protocol for processing and reporting positive and negative blood culture vials.

1. **SPECIMEN REQUIREMENTS**
   1. **PATIENT PREPARATION**

N/A

* 1. **SPECIMEN TYPE**

Blood samples drawn by aseptic technique and put into a BC bottle/vial.

* 1. **SPECIMEN VOLUME**

Adults: 10 ml (8 – 10 ml optimal) of blood per bottle (aerobic & anaerobic)

Pediatric: 3 ml (1 – 3 ml optimal) blood per bottle

* 1. **SPECIMEN HANDLING and STORAGE**
     1. Blood culture bottles are stored at room temperature until use. Once inoculated, immediately transport the bottles to the Microbiology department.
     2. If delay in transport occurs, the bottles may be left at room temperature for up to 4 hrs before loading into the Bactec.
  2. **SPECIMEN REJECTION CRITERIA**
     1. Blood bottles received unlabeled.
     2. Do not process bottles when cracked or broken.

1. **EQUIPMENT/REAGENTS/SUPPLIES** 
   1. **EQUIPMENT**
      1. BACTEC 9120
   2. **REAGENTS** 
      1. Sterile syringe
      2. Alcohol preps
      3. Gram stain reagents
      4. Anaerobic biobags/anaeropouch
      5. Bactec bottles aerobic & anaerobic (stored at 15-30°C)
      6. Bactec Pediatric bottles (stored at 15-30°C)
      7. Microscan panels
      8. Prompt™ Inoculation System (stored at 15-30°C)
      9. Glass microscope slides
      10. Culture media (BAP, CHOC, CDC BAP & MAC)
2. **ASSAY PROCEDURE**
   1. **DAILY MAINTANENCE**
      1. Check the paper supply and re-stock if necessary
      2. Check the temperature read outs of each rack in the Bactec 9120 cabinet. Verify that each rack is 35°C +/- 1.5 °C and cabinet air is 30°C +/- 1.0 °C.
      3. Open the instrument cabinet doors
      4. Check the temperature of the thermometer located in the vial station. Verify that the station temperature is 35°C +/- 1.5 °C.
      5. Take the instrument's bar code wand and scan the menu option: “Illuminate green indicators”. The green lamp at each station should illuminate. If any lamp does not, the station should be removed from service. From the main menu press (Maintenance); from the maintenance menu click on the station and an (X) will appear in the circle. The station is now blocked. If there is a vial currently in progress in the station then refer to Bactec Procedure Manual to resolve the error.
      6. Repeat step 5, except scan menu option: “Illuminate red indicators”.
      7. Next scan the selection: “Illuminate front panel indicators. All four of the indicator lamps should illuminate together, then one at a time. If any lamp does not illuminate, refer to the Bactec Procedure Manual for instructions to replace the bulb.
      8. Scan the selection: “Audible alarm test”. The instrument's alarm will sound three times. If the alarm does not sound, contact the Field Service Representative.
3. **ENTERING DATA**
   * 1. Take the blood culture bottles to the Bactec computer. The monitor is equipped with a touch screen to allow for quick entry.
     2. From the Main Menu, press the culture tab at the bottom of the screen.
     3. Complete the following fields:
        1. *Patient ID*
        2. *Patient Name*
        3. *Accession number (starts with prefix "BC")*
     4. Check the collection time and change if necessary.
     5. With the bar code scanner wand, scan the vial's barcode label (not the patient bar code label). The sequence number, the media type, station and status fields are filled in automatically.
     6. Check the protocol field. If you want to change the length of the protocol, advance the cursor to this field and enter the desired value.
     7. If there is only one vial for this patient then press SAVE to save the entry.
     8. If there are additional vials for this specimen; then repeat step 5 and then press save to save the entry.
4. **LOADING THE BACTEC 9120 INSTRUMENT**
   * 1. Take the new blood culture bottles to the instrument. Open the instrument doors.
     2. Visually inspect all vials for evidence of microbial growth. Positive vials will show hemolysis, turbidity, and excess gas pressure. Hemolysis will cause the blood in the bottle to turn a deep chocolate or burnt red color. Turbidity will cause the liquid in the bottle to be cloudy or hazy in appearance. The septum on the Bactec bottle will be "bulging" if there is excessive gas pressure. All such vials should be treated as positives; they should be gram stained and subcultured.
     3. If the vials appear normal, scan the bottle's bar code label. Again listen for the beep indicating a good scan.
     4. Find the station with the illuminated green/red LED.
     5. Carefully insert the vial into the illuminated station
     6. Repeat the process of assigning stations and inserting vials for each of the new cultures.

*(Note: once the vial is seated in the station, it should not be twisted or turned. Vials should only be removed if they become positive or if they need to be reassigned to a different station.)*

* + 1. The package insert for the Bactec blood culture bottles recommends that bottles be loaded immediately after inoculation; however, inoculated bottles may be held at room temperature for up to 4 h prior to loading into the Bactec.

1. **PRINTING REPORTS**
   * 1. An unloaded positive, unloaded negatives and a quality control report will print out every morning at 0700 a.m. These reports can be reprinted. Refer to the Bactec Procedure Manual to perform this operation.
     2. Select the report menu and print a “current negatives” each day to identify vials that have completed a five day incubation period and are ready to be reported as final in Meditech.
2. **PERFORM SYSTEM BACKUP**
   * 1. Insert a formatted floppy disk (one made for each day of the week) into the disk drive.
     2. Close disk drive to begin backup.
     3. When the back-up is complete, a message appears on the screen.
     4. Perform backup each morning with the next day’s disk.
3. **REMOVING POSITIVE VIALS FROM THE INSTRUMENT**
   * 1. The system will notify you that there is a positive culture with an audible alarm and a red positive indicator lamp on the front of the Bactec monitor.
     2. To acknowledge the alarm press the red alarm bar on the Bactec monitor.
     3. Find the location of the positive vials by pressing the instrument status screen which will show the location of all current positives vials.
     4. On the instrument status screen press on the red positive vial to bring up the culture report screen which will display the patient and vial information.
     5. In the vial section of the culture report screen, the current status of each vial will be displayed as positive or ongoing.
     6. Convert the ongoing vial to positive by selecting the down arrow (under status section) and change vial status from **Ongoing** to **Manual Pos**.
     7. If more than one set of positive vials are displayed, repeat steps 4 - 6.
     8. After converting positive vial sets, **HIT SAVE**.
     9. **PRINT A CURRENT POSITIVE REPORT FROM THE REPORTS SCREEN.** (The report will be used to document the time the vial went positive, the gram stain results, and the critical call information.)
     10. Open the instrument doors.
     11. Take the instrument's bar code wand and scan the menu option: "Remove Positives".
     12. Find the station with the flashing green, flashing red LED's. Remove this vial from the instrument.
     13. Scan the “bottle” bar code label **not** the patient label.
     14. Repeat steps 13 - 14 to remove any additional positive vials.
     15. Label a slide for **EACH** vial with patient information and vial type.
     16. Label one Blood, Chocolate, CDC Anaerobic Blood agar for each set of positive vials. Add MacConkey agar if gram negative rods are seen in gram stain smear.
     17. Sterilize vial caps and insert syringe.
     18. Gently rotate vial to mix contents and remove 0.5 cc of blood.
     19. Inoculate **one** set media and both slides with one to two drops from each vial. (Be sure to place the CDC Anaerobic Blood agar plate into a pouch with an anaeropouch sachet and an ANO2 indicator strip.)
     20. Incubate the Blood and Chocolate/MacConkey agar plate in the CO2 incubator at 35° C.
     21. Incubate the CDC Anaerobic Blood agar plate in the Non-CO2 incubator at 35° C.
     22. As soon as the blood smear has dried, stain and report the results.
     23. All gram stains should be processed and stained immediately and results reported within **sixty minutes** to a physician or licensed caregiver.
     24. If the gram stain reveals "no organisms present;" return the vial to the Bactec by scanning the bottle label and inserting the bottle into location highlighted. (Once a positive vial is removed you have **two hours** to return the vial to its original location
4. **REMOVING NEGATIVE VIALS FROM THE INSTRUMENT**
   * 1. Open the instrument doors.
     2. Take the instrument's bar code wand and scan the menu option, "Remove Negatives."
     3. Find a station(s) with a flashing green LED and remove all vials indicated.
     4. Discard all bottles in biohazard waste container.
5. **REPORTING RESULTS**
   1. **REPORTING RESULTS ON NEGATIVE BLOOD CULTURES**
      1. When blood cultures are *received* the following preliminary report is automatically entered into Meditech and the EMR:

*"Blood cultures are continuously monitored for 5 days. When a culture becomes positive, the physician or nursing unit will be immediately notified with the gram stain morphology. Generally, identification and susceptibility testing results are available in 48 hours. No notification by the Microbiology Lab indicates a negative culture."*

* + 1. If there is no growth after five days incubation, the preliminary result listed above is replaced by *"No growth after 5 days incubation."*
    2. It is the microbiology technologist's responsibility to go into the specimen number and enter/verify the result.

1. **REPORTING RESULTS ON POSITIVE BLOOD CULTURES**
   * 1. **Microbiology technologists:** Issue a preliminary report in Meditech based on the gram stain and phone results to a physician, nurse or licensed caregiver of the patient. Be sure to document the date, time, person/credentials notified, your name and verbal read back of the information in the preliminary report (use RESM).

**Evening shift technologist:** Enter results into Meditech using RESM. If the results are “*questionable*” document gram stain results and call information on the “positives report” obtained from the Bactec computer and leave the report for a Microbiology technologist or supervisor to review.

* + 1. Single positive vials (from multiple draws) which grow microorganisms generally considered skin contaminants (Coagulase Negative Staphylococcus, Viridians group Streptococcus, Corynebacteria, *Propionibacterium*) perform only minimal identification and do not perform AST. Report “One set of two is positive. Isolate could be possible skin contaminant. Contact microbiology if further work up is indicated.”
    2. For Coagulase Negative Staphylococcus (CNS) isolated from one bottle only, do not perform complete work up if there are other blood cultures bottles collected and still negative in the instrument. Use the canned text comment: MBCCNS.

*"Coagulase Negative Staphylococcus species isolated from one culture vial only. Susceptibility testing is not routinely done. If sensitivity testing is indicated; please contact the Microbiology Lab."*

NOTE: IF ONLY ONE CULTURE VIAL IS DRAWN; THEN PROCEED WITH WORK UP TO CONFIRM Coagulase Negative Staph (CNS).

* + 1. Once identification and susceptibility testing are performed using the Microscan Walkaway and/or conventional biochemicals; Issue a final report making sure to speciate all Staph and Strep.

1. **ACTION TO BE TAKEN IF METHOD BECOMES INOPERABLE**

Blood cultures will be processed manually until the instrument is repaired or replaced. If necessary, blood culture bottles can be shipped to reference lab for processing.

1. **REFERENCES**
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***Signature Page***

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**XI. APPROVAL SIGNATURES**

Approved by:

Jim Hallagan, M(ASCP) 01-29-13

Technical Coordinator Date

Angela M. Alford, MSM, MT(ASCP) 01-29-13

Laboratory Director Date

Darin L. Wolfe, MD 01-29-13

Medical Director Date

Written by: Jim Hallagan, M(ASCP) Date: 01/10/2013

Reviewed/Revised Date

Reviewed/Revised Date

Reviewed/Revised Date

Reviewed/Revised Date

Reviewed/Revised Date

Reviewed/Revised Date

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