UnityPoint Health METHODIST	Page 1 of 19	Section: UPM MICRO	Policy #: 03.003
	Approved by: see signature block at end of document		Date: 2/26/18 Review by: 2/26/20
MICROBIOLOGY LABORATORY	Supersedes: 11/20/17, Date Revised: 6/24/11, 5/5/12, 9/19/12, 6/10/14, 10/1/14, 2/4/16, 7/20/16, 11/6/17, 11/20/17, 2/26/18		
	Primary Responsible Parties: Marsha Bishoff Secondary Responsible Parties: Audrey Davis		
	CAP Standard: NA		
SUBJECT: BLOOD CULTURE INSTRUMENT PROCEDURE BD BACTEC FX			

I. PRINCIPLE

When microorganisms are present in culture vials, they metabolize nutrients in the culture medium, releasing carbon dioxide into the medium. A dye in the sensor at the bottom of the vial reacts with CO2. This modulates the amount of light that is absorbed by a fluorescent material in the sensor. A photo detector at each station measures the level of fluorescence, which corresponds to the amount of CO2 released by organisms. Then the measurement is interpreted by the system according to pre-programmed positivity parameters.

At system startup, the onboard computer performs self-diagnostics and downloads operating instructions to the drawer rows. Then the instrument(s) automatically begin testing. Light Emitting Diodes (LEDs) behind the vials illuminate the rows, activating the vials' fluorescent sensors. After a warm-up period, the instrument's photo detectors then take the readings. A test cycle of all rows is completed every ten minutes. Positive cultures are immediately flagged by an indicator light on the front of the instrument, an audible alarm, and are displayed on the LCD display.

When positive vials are identified, the lab technologist pulls them from the instrument for confirmation of results, and for isolation and identification of the organism.

II. CLINICAL SIGNIFICANCE

Blood cultures are essential in the diagnosis and treatment of the etiologic agents of sepsis. Bacterial sepsis constitutes one of the most serious infectious diseases and; therefore, the expeditious detection and identification of blood borne bacterial

pathogens is an important function of the diagnostic microbiology laboratory.

III. POLICY SCOPE

The scope of this policy applies to all Laboratory staff that prepares or performs testing on laboratory specimens at UnityPoint Methodist.

IV. SPECIMEN

Biosafety Level 2
Order: Blood Culture-Routine; Blood Culture Fungus; Blood Culture AFB\

V. COLLECTION

a. SITE SELECTION
    a. Select a different body site for each culture drawn.
    b. Avoid drawing blood through indwelling intravascular catheters unless blood can not be obtained by venipuncture. Blood collected from intravascular catheters should be done with the knowledge that contamination may be an issue.

b. SITE PREPARATION **(ChloraPrep® One-Step Frepp®Applicator)**
    a. Pinch the wings on the applicator to break the ampule and release the antiseptic. Do not touch sponge.
    b. Wet the sponge by repeatedly pressing and releasing the sponge against treatment area until liquid is visible on the skin
    c. Use repeated back-and-forth strokes of the applicator for approximately 30 seconds. Completely wet the treatment area with antiseptic.
    d. Allow the area to air dry for approximately 30 seconds
    e. Do not blot or wipe away.

c. DISINFECTING BLOOD CULTURE VIALS-
    a. Remove the flip-off caps from **BACTEC** culture vials.
    b. Wipe top of each vial with a separate 70% isopropyl alcohol pad and allow to dry.
    c. **Do not use iodine to disinfect tops of vials.**

d. VENIPUNCTURE
    a. Avoid touching the venipuncture site. If it is necessary to touch the site after it has been cleaned, wipe your fingers with povidone iodine before touching the site.
    b. **When using the Blood Collection Set ("butterfly"), the phlebotomist MUST carefully monitor the volume collected by using the 5 mL graduation marks on the vial label. If the volume is not monitored, the stated maximum amount collected may be exceeded.**
    c. If using a needle and syringe, typically a 20 mL syringe is used for adults. Draw 16 to 20 mL of blood for one blood culture set (aerobic and anaerobic). Aseptically inject 8 to 10 mL of specimen into each vial. Aseptically inject 3 to 5 mL into the MYCO/F LYTIC vial.

d. For pediatric patients, a 3 mL syringe is frequently used. Draw 1 to 3 mL of blood and transfer the entire amount into **BACTEC**™ PEDS PLUS/F vial.
e. Continue to care for the venipuncture site following guidelines recommended by your institution.
f. **The inoculated BACTEC vials should be transported as quickly as possible to the laboratory.**

e. CENTRAL VENOUS CATHETER (CVC) CULTURES:
a. Draw one set of Blood Cultures from the CVC (MD may order cultures from each lumen of the CVC) and one set from a Peripheral site.
b. Clean CVC cap with alcohol swab for 3 seconds.
c. Draw 20ml blood from the CVC lumen(s). (Do not draw "waste" blood or flush the lumen when drawing cultures, the heparin / saline will not affect the cultures.)
d. **The inoculated BACTEC vials should be transported as quickly as possible to the laboratory.**

VI. VOLUME

The volume of blood cultures is critical because the number of organisms per mL of blood in most cases of bacteremia is low, especially if the patient is on antimicrobial therapy. In infants and children, the number of organisms per mL of blood during bacteremia is higher than adults, so less blood is required for culture.[6]

a. Children: 1 to 5 mL of blood per venipuncture. Transfer the entire amount to a **BACTEC**™ Peds Plus/F vial.
b. Adult: 16 to 20 mL of blood per venipuncture. If it is impossible to draw the required amount, aliquot as follows:

Amount per Venipuncture	Amount in BACTEC Plus Aerobic Vial	Amount in BACTEC Plus Anaerobic Vial
16 – 20 mL	Split equally between aerobic and anaerobic vials	
13 – 16 mL	8 mL	5 – 8 mL
10 – 12 mL	5 – 7 mL	5 mL
5 – 9 mL	entire blood amount	0

NOTE: Optimum recovery of isolates will be achieved by adding 8 to 10 mL of blood (**BACTEC** Peds Plus/F: 1 – 3 mL; **BACTEC** Myco/F Lytic: 3 – 5 mL). The use of lower or higher volumes may adversely affect recovery and/or detection times.

VII. BLOOD VOLUME MONITORING

To monitor appropriate blood volumes from blood culture collection a quarterly review is performed as follows:

A. A report from the Bactec can be generated under reports. Choose blood volume monitoring reports, blood volume summary, choose the correct date range. Choose all for hospital location, then print.

B. Feedback will be provided to areas where average collection amounts are significantly below the optimal 10 mls.

VIII. SPECIMEN LABELING

A. Each vial should be labeled with the appropriate patient information:
   1. Patient's name
   2. Hospital number (Patient ID)
   3. Date and time of collection
   4. Collector's initials
   5. Site of venipuncture
   6. Or other information as per facility

IX. NUMBERING AND TIMING

Most cases of bacteremia are detected using two to three sets of separately collected blood cultures. More than three sets of blood cultures yield little additional information. Conversely, a single blood culture may miss intermittently occurring bacteremia and make it difficult to interpret the clinical significance of certain isolated organisms.[6]

X. REAGENTS

A. MEDIA

1. BACTEC™ Plus Aerobic /F

   Contains resins for antibiotic neutralization. Recommended for use in adult populations due to higher blood volume capacity and resins. Recommended for 3.0 to 10.0 mL (8.0 to 10.0 mL optimal) blood volume.

2. BACTEC™ Peds Plus

   Contains resins for antibiotic neutralization. Optimized to detect organisms associated with pediatric septicemia and for low blood volumes (1.0 – 3.0 mL optimal; 0.5 to 5.0 mL recommended).

3. BACTEC™ Lytic/10 Anaerobic /F

   Non-resin medium containing the blood lysing agent saponin. Provides better time-to-detection and recovery than standard anaerobic media. The lysis of red cells provides additional nutrients for microbial growth and reduced blood background. The lysis of white cells releases phagocytized organisms. Recommended for 3.0 to 10.0 mL (8.0 to 10.0 mL optimal) blood volume.

4. BACTEC™ Myco/F Lytic

   Specialized media for the detection of fungi and mycobacteria from whole blood and sterile body fluids. Recommended for 1.0 to 5.0 mL (3.0 to 5.0 mL optimal) blood volume. A supplement may be required for use with non-blood specimens.

B. OTHER MATERIALS
   1. Vacutainer™ Safety-Lok™ Blood Collection Set OR 20 mL Luer-Lok™ syringe with a 21 gauge needle, 3 mL Luer-Lok syringe with a 23 gauge needle
   2. 9.5 ml yellow top (SPS) tube or Bactec Myco/F lytic bottle for AFB Cultures only
   3. Blood Transfer Device
   4. CHLORAPREP® One-Step Frepp®Applicator,
   5. 70% isopropyl alcohol (alcohol pads)

XI. INSTRUMENTATION/EQUIPMENT

A. Bactec™ FX

Microorganisms, if present in the blood samples, metabolize nutrients in the **BACTEC** culture vial and release CO_2 into the medium or utilize the oxygen in the medium. The instrument monitors the fluorescence of the vial sensor which increases as CO_2 is produced or oxygen is utilized. Analysis of the rate and amount of CO_2 produced or $O_{2\ utilized}$ enables the instrument to determine if the vial is positive; i.e., the presumptive presence of viable organisms.

B. Overview BACTEC FX:

Modular instrument design permits flexibility to accommodate laboratory needs

   1. Sliding drawers provide increased vial density, thus saving laboratory floor space.
   2. Graphical user interface with color display and touch screen provides ease of use.
   3. Real-time vial presence sensors located in each vial station on the rows provide immediate feedback on vial insertion and removal from stations.
   4. Agitation provides additional enhancement of organism growth and detection.
   5. The ability to mix bacterial, fungal, and mycobacterial cultures within a module or system is accomplished by varying the medium type.
   6. Can be connected to a BD EpiCenter workstation for enhanced instrument reporting and data management capabilities. Alternatively, the BD™ BACTEC™ FX System can be connected to a compatible Laboratory Information System (LIS).

C. Measurement Subsystem

The measurement subsystem activates the sensor in the bottom of a media vial optically. The interrogation consists of illuminating the sensor with an LED and collecting fluorescent light back from the sensor with a photo detector. The collected data is processed, normalized and compensated for thermal variation. Measurement is performed and processed by the Row Board.

D. Vial Presence Sensing

Each station has a vial presence sensor that immediately detects the insertion or removal of vials. This allows users to place vials in any location, or to assign stations through Vial Entry. Station indicators immediately reflect the changed status. Vial presence sensing is performed by the Row Board

E. Station Indicators
LED indicators (shaped like crescents) located above vial stations indicate vial status and are illuminated when a drawer is opened. Station indicators are controlled by the Row Board.

F. LCD and Touchscreen
The display is a 6.4" diagonal color Liquid Crystal Display. It is covered by a touchscreen that enables you to perform actions and operations simply by touching buttons and fields shown in the screen.

G. McKesson unidirectional interface
The Bactec FX is configured with a unidirectional interface. Information, including patient demographics and accession numbers, is down loaded to the Bactec FX once it is logged into Microbiology LIS. The barcode and patient demographics are linked to the appropriate blood cultures when the vials are scanned into the instrument. The Epicenter middleware sits between the unidirectional interface and the Bactec instruments.

H. A complete User's Manual
"Bactec Fluorescent Series User's Manual" is available at the bench for technologists to use as a reference guide. This guide includes introduction, installation, controls and standards, operation, reference, maintenance, and trouble-shooting information.

I. Biological Safety Cabinet
Microscope; materials for staining; slides and sub-culturing supplies

XII. QUALITY CONTROL

A. Media
Blood culture media have been classified as exempt from additional end-user quality control testing per CLSI document M22. Records of lot numbers and date of receipt are retained for 2 years in our laboratory according to CAP regulations.
DO NOT USE culture vials past their expiration date.
DO NOT USE culture vials that exhibit any cracks or defects; discard the vial in the appropriate manner.
Each case of media has a Quality Control certificate indicating the organisms tested and the acceptability of those tests. These are filed and kept for 2 years.

XIII. INSTRUMENT MAINTENANCE

A. **Bactec FX**:
Each day several simple maintenance procedures should be performed. The best time to perform maintenance is first thing in the morning, but it may be done at any time you find convenient.
The following procedures should be performed:
   a. Check the paper supply to the printer. If the paper supply is low or exhausted, replace the paper as explained in the operating manual furnished separately.
   b. Tap the “maintenance” tab. The Test display appears.

c. Open drawer A. Then tap the “red” button to illuminate the red station indicators. Make a note of any station that does not illuminate red.
d. Next tap the “green” button to illuminate the green station indicators. Make a note of any station that does not illuminate green.
e. Repeat Steps 3 - 4 for each of the drawers in the system.
f. Close the drawer.
g. Tap the “alarm” button to verify that the audible alarm is functioning.
h. Finally, tap the “status” button to illuminate the system status indicators on the mullions. Both sides of all the indicators (amber, red, and green) should illuminate. If any indicator does not light, contact your local BD representative for service.
i. Check the temperature on the temperature vial(s).
j. Each filter (2 per instrument) are checked and cleaned weekly.
k. Information is recorded on the Maintenance QC Report.

XIV. PROCEDURE

A. General Safety Considerations

Pathogenic microorganisms, including Hepatitis viruses and Human Immunodeficiency Virus, may be present in specimens. “Standard and institutional guidelines should be followed in handling all items contaminated with blood or other body fluids.

1. Wear gloves while handling inoculated vials.
2. Perform all blood culture processing in a biological safety cabinet.
3. Properly dispose of all contaminated materials. Place Bactec Myco/F lytic bottles,, syringes, needles, and other sharp contaminated materials in a puncture proof container.
4. All other blood culture vials can be disposed of in a red biohazard bag since they are plastic.

WARNING: Never attempt to recap a needle.

B. Processing New Blood Cultures

1. Drawn blood cultures are received into the lab via LIS.
2. Blood cultures are received drawn in blood culture vials. (Refer to "Specimen" section of procedure part B, "Volume of Blood".)
   Exception - Blood, AFB: The two yellow tops (SPS) or a Bactec MYCO/F Lytic bottle are sent to ARUP for inoculation and testing. Complete ARUP paper work, bag specimen, and deliver to Referral Specialist.
3. Make sure barcoded Microbiology LIS labels are affixed to blood culture vials. These should be attached vertically and should not cover the barcode present on the blood culture vial media label. (Both must be scanned when entering vials into the instrument.)

C. Entering Data And Loading Instrument

To enter vials in the instrument, select a drawer where there are available stations. (The number of available stations is shown below the “vial entry” icon on the Status display.)

Then follow one of the two methods described below.

1. Method 1 (Vial Activated)
    a. Select a drawer that has available stations, and open that drawer
    b. The barcode scanner turns on
    c. Scan a vial sequence barcode label
    d. The Vial Entry display appears and the Sequence, Media, and default Protocol are automatically entered
    e. If you did not scan the Accession, scan or enter it now
    f. To change the protocol tap the “modify” button, then tap the up arrow to increase or down arrow to decrease the protocol length
    g. Place the vial into an available station (solid green indicator)

2. Method 2 (Icon Activated)
    a. Select a drawer that has available stations, and open that drawer
    b. Tap the “vial entry” button on the Status display
    c. The Vial Entry display appears and the barcode scanner turns on
    d. Scan the vial sequence barcode label
    e. The Sequence, Media, and default Protocol are automatically entered
    f. If you did not scan the Accession, scan or enter it now
    g. To change the protocol tap the “modify” button, then tap the up arrow to increase or down arrow to decrease the protocol length
    h. Place the vial into an available station (solid green indicator).
    i. When a vial is placed into the last available station in a drawer, the Activity Complete tone sounds (3 beeps).
    j. To continue entering vials, select another drawer with available stations.

D. Inserting Vials in the Instrument
Before inserting vials into the stations, visually inspect all vials for positives. Evidence of microbial growth includes hemolysis, turbidity, and excess gas pressure (causing the vial septum to bulge outward). All such vials should be treated as positives; they should be stained and subcultured.

After all vials have been inspected and inserted in stations, close the drawer.

A vial presence sensor immediately senses the insertion of a vial in a station and the instrument updates the station LED indication and the status shown on the LCD.

Once vials are placed in their stations, you should avoid moving them to other stations unnecessarily.

Avoid opening the drawer unnecessarily. Drawers should not remain open longer than 10 minutes.
Make sure all vials are fully inserted in the stations before closing the drawer.

WARNING

VIALS SHOULD BE HANDLED WITH EXTREME CARE AT ALL TIMES. VIAL NECKS ARE SUSCEPTIBLE TO BREAKAGE IF THEY ARE STRUCK AGAINST ANOTHER OBJECT.

E. Anonymous Vial Entry
Vials can be placed into available (GREEN indicator) stations without being scanned into the instrument. Vials that are not scanned into the instrument are called “anonymous” vials. Anonymous vials are recognized by the instrument when they are placed in stations, but are assigned an “unknown” medium type and default protocol of 5 days. Anonymous vials are evaluated with general positivity criteria. They cannot use the specific positivity criteria tied to the characteristics of the medium since the instrument does not know the medium type.

We recommend that at some point you identify these anonymous vials to the system using the ID(entify) Anonymous vials activity. The instrument is able to apply medium specific positivity criteria when the medium type is known, and can apply these specific criteria to collected test readings. In addition, the protocol is adjusted (if necessary) to the default for that medium type once the vial is identified.

NOTE: Once an anonymous vial has been placed in the instrument, do not remove the vial and reenter it without identifying it (ID Anonymous activity). All test readings are discarded if you remove the vial without identifying it.

F. Positive and Negative Vials
1. Notification of positive and negative vials
   a. The system notifies you of new positive cultures in several ways:
2. Positive Vial audible alarm sounds
   a. Station Indicators: FLASHING RED or FLASHING AMBER / RED (alternating) -Anonymous Positive
   b. Message box appears on screen
   c. Positive vial system indicator for that drawer illuminates
   d. On the Status display, the “positives” icon is active (color is red, not grayed out) and the number of positive vials in the drawer is shown
   e. Out-of-Protocol Negatives are indicated by the following:
      i. Negative vial system indicator for that drawer illuminates

ii. On the Status display, the “negatives” icon is active and the number of negative vials in the drawer is shown
iii. Station indicators: FLASHING GREEN

3. Removing positive vials
   a. Select a drawer that has positive stations, and open the drawer by pulling it out.
   b. The barcode scanner turns on.
   c. All positive, final negative, available, and anonymous (all variations) are indicated by the appropriate lit or flashing station indicators.
   d. Tap the “remove positives” button on the Status display, OR
   e. Remove a vial from a FLASHING RED (positive) or FLASHING AMBER / FLASHING RED (anonymous-positive) station
   f. The Positive Removal display appears. (If an anonymous positive vial was removed, the ID Anonymous display appears. Scan the sequence and accession for the anonymous positive vial and tap the “Save” button. Then tap the “Exit” button to return to the Positive Removal display.)
   g. If the Show Related Vials function is enabled in configuration, the LEDs of vials with the same accession number illuminate GREEN (in the current drawer), and the Culture – Specimen display shows the related vials in the Vial Window (not applicable to Positive / Anonymous vials).
   h. Remove any related vials if desired, and either confirm or scan the sequence number (depending on the system prompt). When you have finished removing related vials, tap the “exit” key to return to the Positive Removal display
4. Removing negative vials
   a. Select a drawer that has negative stations, and open the drawer by pulling it out.
   b. The barcode scanner turns on. All positive, final negative, and anonymous (all variations) are indicated by the appropriate flashing station indicators.
   c. For Single Vial Removal
      i. Tap the “remove negatives” button on the Status display, OR
      ii. Remove a vial from a FLASHING GREEN (negative) station and scan it.
      iii. The Negative Removal display appears.
      iv. Remove and scan all the negative vials. (If any vial sequence numbers were entered manually, the system asks you to verify that the sequence number is correct. You must manually confirm that the sequence number on the vial is the same as the one shown on the screen, and tap the “Verified” button.)
   d. For Batch Vial Removal
      i. Remove the negative vials from the FLASHING GREEN station
      ii. These vials do not have to be scanned (and the scanner does not turn on). Any vials left in the instrument remain in the database as negatives.

iii. Counters on the display are updated dynamically as vials are removed.
iv. When all negatives are removed from the drawer, the “activity complete” tone sounds.

5. Processing Positive Vials
   a. Preparation of Gram stain
      i. Obtain a glass slide and record the accession number and media type on frosted end.
      ii. Reprint a workcard label in Micro Set Up in Sunquest.
      iii. Mix blood culture vial
      iv. Insert needle of 3 mL smart tip syringe into blood culture vial with attached syringe into rubber septum of vial and withdraw approximately 0.5 ml.
      v. Place 1-2 drops of blood/media mixture on slide and spread to make a thin film.
      vi. Heat fix on slide warmer until completely dry.
      vii. Stain smear using Gram stain procedure (UPMMICRO 10.001).
6. Record results of Gram Stain.
   1. Positive smears
      i. Gram stain showing gram positive cocci or gram negative bacilli should have FilmArray Biofire testing. See procedure 04.017 Biofire Blood Culture (BCID panel) for complete instructions. The Biofire result and gram stain can be phoned at the same time.
      ii. Phone results to physician or nursing unit. Positive blood cultures are considered "critical" results.
      iii. Subculture all morphology types to a BAP, Chocolate, MaConkey and a CDC ANA plate. Gram stains that show yeast should also be subcultured to a SABS plate.
   2. Enter gram stain and phoned information in Micro Result Entry.
      i. Place plates in incubator in the appropriate atmosphere and leave blood culture vial at the processing bench or place at routine bench.
7. Perform Susceptibility testing
   a. MicroScan Neg Combo panels are inoculated directly from the culture medium for Gram-negative bacilli only, using the B-D vacutainer serum separation tube:
      i. Invert the Bactec vial to mix the contents and aseptically remove and transfer 10 ml into the serum separator tube.
         a) Centrifuge for 10 minutes at 3100 rpm. After centrifugation, most of the red cells remain underneath the silicon gel barrier and the supernatant can be poured off, leaving behind a layer of organisms that is concentrated on the top of the gel material.
         b) Re-suspend the microbial concentrate in the residual broth and transfer a drop or two to approximately 3ml sterile saline, adjusting to a 0.5 McFarland standard.
         c) Pipet 100 µl of this suspension into 25 ml of MicroScan inoculum

water and inoculate the appropriate panel according to our routine procedure.

b. When the culture is positive for Gram positive cocci or Gram-positive bacilli, the susceptibility is set up from the subculture plates (later the same day or the next morning), **NOT** directly from vial.
c. Alternative susceptibility procedures may be necessary, depending on the ID of the organism recovered. (Anaerobes and Haemophilus are referred for testing.)

8. Workup of multiple positives on same patient. If all sets have the same Gram stain morphology, subculture all vials to appropriate media. Perform biochemicals and susceptibilities on one set of vials. If the colony morphology is the same the next day for all the cultures, refer those without specific biochemical and susceptibilities to the set that was worked up completely. If colony morphologies are different, biochemicals and susceptibilities must be performed on all isolates. Positive cultures within a 7 day period may be referred to previous susceptibility results if identification is the same. After that period of time, susceptibility testing should be repeated or repeated if specifically requested by the physician. Communication with the PharmD or physician is important with these situations. If there are 2 cultures that both have SCN please set up a rapid PID on both cultures to make sure they are so same organism. If so, work up the first culture and refer the second. If the isolates do not identify as the same organism, report as contaminants unless culture is a line draw. For 2 cultures that both have the same gram negative bacilli work up the first culture and refer the 2nd if the morphology looks the same.
9. All isolates of *N. meningitidis* should be additionally subcultured to a chocolate agar slant and referred to the IDPH for typing.
10. Negative smear
    a. Perform blind subcultures to a BAP, Chocolate, MaConkey and a CDC ANA plate.
    b. In aerobic media, *S. pneumoniae* will typically be visually and instrument positive, but in some cases no organisms will be seen on Gram stain or recovered on routine subculture. If an anaerobic vial was also inoculated, the organism can usually be recovered by performing an aerobic subculture of the anaerobic vial, since this organism has been reported to grow well under anaerobic conditions.[8]
    c. Return "Smear negative" positive vial to instrument within 5 hours of removal from the instrument to retain Status information for vial.

XV. SPECIAL REQUESTS AND CIRCUMSTANCES

1. Extension of incubation past 5 day protocol - If a physician requests that blood cultures be held for longer than 5 days, this can be changed at any time using the touch screen.
    a. Touch Culture tab
    b. Enter Accession, Patient ID, or Patient name.

c. Touch Protocol number to access number pad.
d. Change protocol length. (Protocol length is 1-42 days.)
e. Save

2. Brucellosis requests - The aerobic vial is held 30 days to include the isolation of *Brucella sp.* Change protocol length to 30 days. In addition, subcultures should be made to BAPS at day 7, day 14, and day 21. All subcultures should be incubated at 35ºC in 10% CO_2. (Parafilm all subcultures so that they do not dry out and keep until culture is finalized.) Gram stain vial before discarding at day 30. Make note of subculture dates in Result Entry.
3. Subculturing for Fastidious Organisms - Culture with positive smears that yield no growth on routine subculture should be handled as follows:
   a. Gram-positive cocci
      i. Check for nutritionally deficient Strep - Streak inoculum over the entire agar surface of a BAP. Place a Staph streak down the middle of the agar surface and incubate overnight at 37ºC in the CO_2 incubator. The next day, observe for tiny colonies growing next to the Staph streak.
      ii. Extend incubation of the anaerobic plate - Incubate the anaerobic pouch 48-72 hours before opening.
   b. Gram-negative bacilli
      i. Extend incubation of the anaerobic plate - Incubate the anaerobic pouch 48-72 hours before opening.
   c. Gram-positive bacilli
      i. Check for AFB or *Nocardia* - Subculture to SABS and incubate at 37ºC.
      ii. Extend incubation of the anaerobic plate - Incubate the anaerobic pouch 48-72 hours before opening.

   NOTE: Hold all plates for 5 days before discarding. If you are still unable to recover organisms you may subculture 0.1 to 0.2 ml of the positive culture into a fresh vial and test according to the routine procedure. This may dilute out any inhibitors that may be present in the original culture vial.
4. Requests for Fungal isolation
   a. 5 mls of blood is drawn into a Mycolytic Blood culture vial.
   b. Fungus vial is held for 30 days.
   c. Positive cultures:
      1) Prepare one blood smear and perform a Gram stain.
      2) If Gram stain is positive for bacteria, subculture vial to appropriate media, compare growth with routine culture, workup organisms that are different. Blind subculture vial to inhibitory and non-inhibitory fungus media weekly. Hold fungus plates for 30 days before finalizing. If Gram stain is positive for yeast or hyphae, subculture to fungus media and document in the LIS.

XVI. REPORTING RESULTS

A. Reporting Positives
   1. Panic Value
      a. Positive blood culture results are considered panic values on all shifts.

These results must be phoned and documented on the report. Include the individual receiving the report (nurse or physician), the date and time the report was phoned, and the individual phoning the report.

2. Reporting in Micro Result Entry Positive blood cultures are reported using the following format:
   a. Day positive culture is detected
      i. Positive at _____ hours. Determine this by printing the "Current Positives" report. If vial has been removed the "Unloaded Positives" report can be printed to find the age of culture.
      ii. For___. Description of organisms observed in Gram stain.
      iii. Phoned to_____. Nurse or Physician
      iv. By_____. Tech that placed call.
      v. At_____. Date and time of call.
   b. On next available observation line for ISO1, ISO2, or ISO3 enter gram stain morphology using appropriate phrases. These are accessible using the drop down menu in Result Entry.
   c. Biofire FilmArray testing should be reported in the miscellaneous test tab in Micro Result Entry. If yeast is not seen in the original gram stain but is detected by Biofire testing, do NOT report the yeast. Flood a SABS plate with specimen from the positive blood culture bottle. Hold the SABS sub for 5 days before finalizing culture.
   d. Next day
      i. Identification of organism(s) unless definitive identification given from the Biofire Film Array (see Biofire Blood Culture procedure 04.017 for more information).
      ii. Susceptibilities set up if appropriate.
3. Algorithms for detecting and reporting contaminated blood cultures:
   a. The following microorganisms are considered likely contaminants when isolated from blood cultures:
      1) Coagulase negative *Staphylococci*
      2) Aerobic and Anaerobic Diphtheroids
      3) *Micrococcus spp*.
      4) *Bacillus spp*.

      NOTE: Report the number of positive cultures/total cultures drawn. (Each culture being a separate draw within a 48 hour period.)
   b. If only a **single blood culture** is obtained and it grows one of the likely contaminants listed above, the isolate is reported with the statement: "Isolate reported is of indeterminate significance. The physician is advised to notify lab if further identification and susceptibilities are required." (**Phrase: SBC) If the physician contacts the laboratory and requests a full work-up, then the previous phrase is removed from the report, susceptibilities are performed and reported.** (If the isolate is one that we don't fully identify, then we will report as normal or refer if identification required. If isolate is one that we can only report MICs because there is no interpretation, then we will only report the MICs.)

c. If **two or more blood cultures** are obtained and **only one is positive** for one of the likely contaminants listed above, the isolate is reported as a probable contaminant and susceptibility testing is not performed. (**Phrase: TBC or THBC) If the physician calls and asks for a complete work-up, then susceptibilities are performed and reported.**
d. If **two or more blood cultures** are obtained and **two cultures are positive within a 24 hour period** with a likely contaminating organism then a full work-up is done. Let the physician determine significance of isolate based on patient clinical data.

XVII. Reporting Negatives (Clock Time Defaults)

1. Blood Fungal cultures
   a. Negative prelims will be issued at the bench when fungal cultures are read.
   b. Negative final is issued at Day 30.
2. Routine Blood Cultures
   a. Negative Preliminaries are issued at Days 1, 2, 3, & 4.
   b. Negative finals are issued at day 5.

XVIII. PROCEDURAL NOTES/PROBLEM-SOLVING TIPS

A. Contamination
   1. Care must be taken to prevent contamination of the sample during collection and inoculation into the BACTEC™ vials. A contaminated sample will give a positive reading, but this does not indicate a clinically significant result. Such a determination must be made by the user, based on such factors as type of organisms recovered, occurrence of the same organism in multiple cultures, patient history, etc.

B. Recovery of SPS Sensitive and Fastidious Organisms from Blood Samples
   1. Because blood can neutralize the toxicity of SPS toward organisms sensitive to SPS (such as some *Neisseria* species), the presence of optimum volumes of blood, based on media type, benefits the recovery of these organisms.
   2. Some fastidious organisms, such as certain *Haemophilus* species, require growth factors, such as NAD, or factor V, which are provided by the blood specimen. If the blood specimen volume is 3.0 mL or less for **BACTEC**™ Plus Aerobic/F and Anaerobic/F or 0.5 mL or less for **BACTEC**™ Peds Plus/F, an appropriate supplement may be required for recovery of these organisms. **BACTEC FOS**™ Fastidious Organism Supplement (Catalog # 442153) or whole human blood may be used as nutritional supplements.

C. Non-viable Organisms

   A Gram-stained smear from a culture medium may contain small numbers of non-viable organisms derived from medium constituents, staining reagents, immersion oil, glass slides, and specimens used for inoculation. In addition, the patient specimen may contain organisms that will not grow in the culture medium or on media used for subculture. Such specimens should be subcultured to special media as appropriate. [10]

D. Antimicrobial Activity
Neutralization of the antimicrobial activity by resins varies depending on dosage level and timing of specimen collection.

E. Recovery of Streptococcus pneumoniae
In aerobic media, *S. pneumoniae* will typically be visually and instrument positive, but in some cases no organisms will be seen on Gram stain or recovered on routine subculture. If an anaerobic vial was also inoculated, the organism can usually be recovered by performing an aerobic subculture of the anaerobic vial, since this organism has been reported to grow well under anaerobic conditions.[8]

F. General Considerations
Optimum recovery of isolates will be achieved by adding the appropriate volume of blood for the type of vial inoculated. Use of lower or higher volumes may adversely affect recovery and/or detection times. Blood may contain antimicrobials or other inhibitors which may slow or prevent the growth of microorganisms. False negative readings may result when certain organisms do not produce enough CO2 to be detected by the system or if significant growth has occurred before placing the vial into the system. False positivity may occur when the white blood cell count is high.

It is recommended that related vials remain out of the instrument for no more than 10 minutes to minimize the possibility of the vial becoming a “false” positive vial.

1. **BACTEC Myco/F Lytic** vials are not selective and will support the growth of other aerobic organisms besides mycobacteria, yeast and fungi. Positive vials may contain one or more species of mycobacteria and/or other non-mycobacterial species. If present, fast growing organisms may mask the detection of slower growing mycobacteria, yeast and fungi. Subculture and additional procedures are required. The consistency of microscopic morphology in **BACTEC** Myco/F Lytic has not been established.

2. Inoculation of blood volumes of 1 to 5 mL are acceptable; but optimum recovery is obtained with 3 to 5 mL. During internal studies with less than 3 mL of blood, *M. intracellulare*, *M. malmoense*, *M. haemophilum* and *M. xenopi* exhibited detection delays and/or compromised recovery with **BACTEC** Myco/F Lytic. False positivity most likely will increase when the blood volume is above 5 mL.

3. Blood may contain antimicrobials or other inhibitors which may slow or prevent the growth of microorganisms.

4. *Penicillium purpurescens* and *Blastomyces dermatitidis* were not detectable in the **BACTEC** Myco/F Lytic culture medium. *Hansenula anomala*, *Exophila jeamselmei*, *Actinomyces bovis*, *Rhodotorula rubra* and *Mucor ramosissimus*

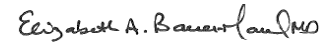
exhibited inconsistent results at low inoculum levels (<10 CFU/vial) with seeded culture studies. Recovery of such organisms may require additional culture methods.

Any vial assigned to a new station (i.e., in the event of a bad station) should be subcultured immediately prior to placing in the new station.

XIX. REFERENCES

A. **BACTEC™** Plus Aerobic/F and Plus Anaerobic/F Culture Vials Insert. Rev. PP-088 (2008/01) BD Diagnostics.
B. **BACTEC™** Peds Plus/F Culture Vials Insert.Rev. PP-091(2008/01) . BD Diagnostics.
C. **BACTEC** Myco/F Lytic Culture Vials Insert.Rev. PP-162 (2008/01). BD Diagnostics.
D. **BACTEC** Fluorescent Series User's Manual. Document Number MA - 0074. BD Diagnostics.
E. **BACTEC FX** System User's Manual. Document Number 8005110 (2008/04). BD Diagnostics.
F. **BACTEC** Blood Culture Procedural Trays. Document Number L-001810 (A). BD Diagnostics.
G. Clinical and Laboratory Standards Institute. 2004. Approved Standard M22-A3. Quality control of commercially prepared microbiological culture media, 3rd ed. CLSI, Wayne, Pa.
H. Jorgensen, James H, Michael A. Pfaller, and Karen C. Carroll. *Manual of Clinical Microbiology*. Washington: ASM Press, 2015. Print
I. Leber, Amy L. *Clinical Microbiology Procedures Handbook*. Washington, DC: ASM Press, 2016.

POLICY CREATION :		
Author:	***Terry A. Smith***	***June 24, 2011***
Medical Director:	***Douglas McGrady, M.D.***	***June 24, 2011***

MEDICAL DIRECTOR		
DATE	NAME	SIGNATURE
February12, 2017	Elizabeth A. Bauer-Marsh, M.D.	
SECTION MEDICAL DIRECTOR		
August 24, 2015	Lori Racsa, DO	

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Changed processing vials to include transfer device, LIS changes	T Smith	4/23/12
2	Added "blood culture vials" to the General Safety Considerations on page 7 number 3. Must place glass vials in puncture proof container.	T. Smith	9/17/12
3	Minor monthly QC update to include filter cleaning that is currently being performed and updated LIS interface section	J Corpus	9/10/14
4	Update to include blood volume monitoring bi-annually	J Corpus	9/30/14
5	Formally procedure 8B	T. Lanan	9/7/15
6	Removed HLAB order number and how to enter results in HLAB	T Nuese	2/2/16
7	Changed method for blood culture vial volume monitoring	T. Nuese	7/20/16
8	Changes in culture workup and susceptibility testing	T. Nuese	11/06/17
9	Change in subculturing of positive vials.	T Nuese	11/27/17
10.	Grammar correction and addition of performing Biofire testing on positive bottles. Changed blood culture volume review to quarterly. Updated LIS input information. General reformatting. Updated references.	A Davis	02/15/18

Reviewed by

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
			6/24/11		6/24/11
			4/23/12		5/5/12
			9/17/12		9/19/12
			6/6/14		6/10/14
			9/30/14		10/1/14
			2/1/16		2/4/16
			7/11/16		7/20/16
			10/25/17		11/6/17
Marsha Bishoff	11/20/17		11/17/17		11/20/17
Marsha Bishoff	2/21/18		2/15/18		2/26/18