

Policy/Procedure Title: Blood Culture using BD BACTEC™ FX System

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Applicable Standards	
Standard	Organization
MIC.22630	CAP
MIC.22640	CAP
Related Documents	

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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. MATERIAL

A. MEDIA

Several media are available for use with the BD™ BACTEC™ FX system. Bottles in use at CHRISTUS Spohn Health System include:

BACTEC™ Plus Aerobic /F (glass or plastic)

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. *Default protocol length = 5 days*

BACTEC™ Peds Plus (glass)

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). *Default protocol length = 5 days*

BACTEC™ Lytic/10 Anaerobic /F (glass or plastic)

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. *Default protocol length = 5 days*

BACTEC™ Myco/F Lytic (glass)

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. *Default protocol is set to 28 days for fungus. AFB blood orders will be need to be manually changed to 42 days.*

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Each medium type has default test protocol duration. The default protocol can be overridden on each vial entered in the instrument.

B. MATERIALS REQUIRED BUT ONLY PROVIDED IN PROCEDURAL TRAY KITS

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, CHLORAPREP® One-Step Frepp® Applicator, ; 70% isopropyl alcohol (alcohol pads); Biological Safety Cabinet; ; venting units; ; microscope; materials for staining; slides and subculturing supplies.

C. INSTRUMENT

BACTEC™ FX

Microorganisms, if present in the blood samples, metabolize nutrients in the BACTEC culture vial and release CO₂ into the medium or utilize the oxygen in the medium. The instrument monitors the fluorescence of the vial sensor which increases as CO₂ is produced or oxygen is utilized. Analysis of the rate and amount of CO₂ produced or O₂ utilized enables the instrument to determine if the vial is positive; i.e., the presumptive presence of viable organisms.

D. Overview BACTEC FX:

Modular instrument design permits flexibility to accommodate laboratory needs

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

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.

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

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Entry. Station indicators immediately reflect the changed status. Vial presence sensing is performed by the Row Board

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.

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.

III. SPECIMEN

A. COLLECTION

1. 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.

2. SITE PREPARATION (ChloroPrep® 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.

3. 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.

4. VENIPUNCTURE

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- 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.**

B. VOLUME

The volume of blood cultured 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

1. Children: 1 to 5 mL of blood per venipuncture. Transfer the entire amount to a BACTEC™ Peds Plus/F vial.
2. 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 Anaerobic Lytic 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.

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C. SPECIMEN LABELING

1. Each vial should be labeled with the appropriate patient information:
 - a. Patient's name
 - b. Hospital number (Patient ID)
 - c. Patient's location (room and bed #)
 - d. Date and time of collection
 - e. Collector's initials
 - f. Site of venipuncture
2. Each request slip should also have all the information above.

D. NUMBER 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

IV. QUALITY CONTROL

A. MEDIA

Blood culture media have been classified as exempt from additional end-user quality control testing per CLSI document M22. However, quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

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.

B. INSTRUMENT MAINTENANCE

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:

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1. 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.
2. Tap the "maintenance" tab. The Test display appears.
3. 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.
4. Next tap the "green" button to illuminate the green station indicators. Make a note of any station that does not illuminate green.
5. Repeat Steps 3 - 4 for each of the drawers in the system.
6. Close the drawer.
7. Tap the "alarm" button to verify that the audible alarm is functioning.
8. 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.
9. Check the temperature on the temperature vial(s).
10. Information can be recorded on the Maintenance QC Report.

V. 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 syringes, needles, and other sharp contaminated materials in a puncture proof container.

WARNING: Never attempt to recap a needle.
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B. PROCESSING NEW BLOOD CULTURES

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.

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Method 1 (Vial Activated)

1. Select a drawer that has available stations, and open that drawer
2. The barcode scanner turns on
3. Scan a vial sequence barcode label
4. The Vial Entry display appears and the Sequence, Media, and default Protocol are automatically entered
5. If you did not scan the Accession, scan or enter it now
6. To change the protocol tap the "modify" button, then tap the up arrow to increase or down arrow to decrease the protocol length
7. Place the vial into an available station (solid green indicator)

Method 2 (Icon Activated)

1. Select a drawer that has available stations, and open that drawer
2. Tap the "vial entry" button on the Status display
3. The Vial Entry display appears and the barcode scanner turns on
4. Scan the vial sequence barcode label
5. The Sequence, Media, and default Protocol are automatically entered
6. If you did not scan the Accession, scan or enter it now
7. To change the protocol tap the "modify" button, then tap the up arrow to increase or down arrow to decrease the protocol length
8. Place the vial into an available station (solid green indicator)
9. When a vial is placed into the last available station in a drawer, the Activity Complete tone sounds (3 beeps).
10. To continue entering vials, select another drawer with available stations.

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.

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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.**

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.

VI. Positive and Negative Vials

A. Notification of positive and negative vials

1. The system notifies you of new positive cultures in several ways:
 - a. Positive Vial audible alarm sounds
 - b. Station Indicators: FLASHING RED or FLASHING AMBER / RED (alternating) - Anonymous Positive
 - c. Message box appears on screen
 - d. Positive vial system indicator for that drawer illuminates
 - e. 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

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2. Out-of-Protocol Negatives are indicated by the following:
 - a. Negative vial system indicator for that drawer illuminates
 - b. On the Status display, the “negatives” icon is active and the number of negative vials in the drawer is shown
 - c. Station indicators: FLASHING GREEN

B. Removing positive vials

1. Select a drawer that has positive stations, and open the drawer by pulling it out.
 - a. The barcode scanner turns on.
 - b. All positive, final negative, available, and anonymous (all variations) are indicated by the appropriate lit or flashing station indicators.
 - c. Tap the “remove positives” button on the Status display, OR
 - d. Remove a vial from a FLASHING RED (positive) or FLASHING AMBER / FLASHING RED (anonymous-positive) station
 - e. 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.)
 - f. 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).
 - 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

C. Removing negative vials

1. Select a drawer that has negative stations, and open the drawer by pulling it out.
 - a. The barcode scanner turns on. All positive, final negative, and anonymous (all variations) are indicated by the appropriate flashing station indicators.
 - b. For Single Vial Removal
 - Tap the “remove negatives” button on the Status display, OR
 - Remove a vial from a FLASHING GREEN (negative) station and scan it.
 - The Negative Removal display appears.

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- 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.)
- c. For Batch Vial Removal
- Remove the negative vials from the FLASHING GREEN station
 - 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.
 - Counters on the display are updated dynamically as vials are removed.
 - When all negatives are removed from the drawer, the “activity complete” tone sounds.

D. Processing Positive Vials

1. Remove the vial from the instrument and place in a biological safety cabinet.
2. Invert the vial to mix the contents.
3. Observe “**Universal Safety Precautions**”^{10, 11} to vent each presumptive positive blood culture vial. Use a venting needle.
4. Remove aliquot from the vial for stain preparations (Gram and/or AFB).
5. Subculture vials according to the Gram stain and/or AFB stain results.
6. Report preliminary results only after stain preparation.
7. Perform identification and susceptibility of organism(s) grown on solid media according to your laboratory protocol.

VII. LIMITATIONS

Contamination

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.

Recovery of SPS Sensitive and Fastidious Organisms from Blood Samples

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.

Non-viable Organisms

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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

Antimicrobial Activity

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

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

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.

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.

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.

Mycobacteria may vary in acid-fastness depending on strain, age of culture and other variables.

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

BACTEC Myco/F Lytic vials are incubated at 35°C potentially precluding the recovery of mycobacteria requiring other incubation temperatures such as *M. marinum*, *M. ulcerans*, or *M. haemophilum*. Recovery of such organisms requires additional culture methods.

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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.

VIII. REFERENCES

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