Blood Culture/Brucella Culture - Bactec™ FX

Purpose

This procedure provides instruction for Blood and Brucella Culture in the Microbiology Lab.

Principal and Clinical Significance

Blood cultures are essential in the diagnosis and treatment of the etiologic agents of sepsis. The bacterial detection of microorganisms in a patient's blood has diagnostic and prognostic importance. 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.

The Bactec™ FX is designed for the rapid detection of microorganisms in clinical specimens. The sample to be tested is inoculated into the vial, which is entered into the Bactec™ instrument for incubation and periodic reading.

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 positivist 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.

Policy Statements

This procedure applies to Microbiologists who perform culture set-up and plate reading.

Test Code

BC, BRCL

Materials

Reagents	Supplies	Equipment	Media
• Gram stain reagents	• 70% isopropyl alcohol wipes • BD™ Blood Transfer Device • 1 cc syringe • Snap cap tubes • Glass slides • Sterile transfer pipettes • Palladox catalysts • Inoculating loop	• BACTEC™ FX - Analyses of the rate and amount of CO2 produced or O2 utilized that enables the instrument to determine if the vial is positive. • Computer and Peripherals • The system computer stores all the system software, including the application software which controls instrument operations and the user interface, which enables the user to enter patient information, view results,	• Bactec™ Peds Plus/F Culture Vial1 (pink bottle): Optimum blood volume for each vial is 1 to 3 mL; 0.5 to 5 mL of blood is acceptable. Each vial contains: • 40 mL Enriched Soybean-Casein Digest Broth • 0.02% SPS • Resins • CO2 • O2 • Sensor for the detection of fluorescence

			print reports, identify errors, etc • Barcode Scanner- located at the front of each drawer. • CO2 incubator 35°C • Anaerobic chamber 35°C • Ambient air incubator 35°C • Incinerator • Microscope • Anoxomat	• Bactec™ Lytic/ 10 Anaerobic/F Culture Vial2 (purple bottle): Optimum blood volume for each vial is 8 to 10 mL; 3 to 10 mL of blood is acceptable. Each vial contains: • 25 mL Enriched Soybean-Casein Digest Broth • 0.05% SPS • CO2 and Nitrogen Gas • Sensor for the detection of fluorescence • Chocolate Agar (CHOC) • Sheep Blood Agar (BAP) • CDC Anaerobe Agar (ASB2) • CNA Agar (CNA) • MacConkey Agar (MAC) • Sabouraud Dextrose Agar, Emmons (SAB) • Candida Chromagar (CCAN)

Specimen

A. Blood
- 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.[4]
- For pediatric patients; 4-11 mL of blood is drawn per blood culture set. Inject 1 to 3 mL into the aerobic bottle and 3-8 ml into the anaerobic bottle using the following guidelines:

Weight	Volume in aerobic bottle	Volume in anaerobic bottle
<1.5 Kg / <3.3 lbs.	1ml	3 ml
1.5 – 3.9 Kg / 3.4 – 8.6 lbs.	1 ml	3 ml
4.0 – 13.9 Kg / 8.7 -31 lbs.	2 ml	5 ml
>14 Kg / >31 lbs.	3 ml	8 ml

B. Sampling Time
- Draw 2 to 3 sets of blood cultures per febrile episode at least 60 minutes apart. Do not draw more than 3 sets in a 24-hr period. This provides maximum recovery of microorganisms in patients with intermittent bacteremia, and documentation of persistent bacteremia in patients with intravascular infections (e.g. endocarditis, intravenous catheter site infections).

C. Special instructions
- Inoculated vials should be transported as quickly as possible to the laboratory.
- If only the minimum volume of blood can be drawn, inoculate the Bactec™Peds Plus/F only.
- If the purple anaerobic bottle is received in the lab after the pink aerobic bottle has already been received in Sunquest, or vice versa, follow these steps:
  - Do not discard the purple anaerobic bottle

- If the purple anaerobic bottle is received within 1 hour of the pink aerobic bottle, place the purple bottle into the Bactec FX using the accession number.
- If the purple anaerobic bottle is received greater than 1 hour from the pink aerobic bottle, **re-order the BC as a new collection**.
- **St Paul**: if the purple anaerobic bottle is received within 1 hour of the pink aerobic bottle, cannot be received in Sunquest and cannot be tracked in SMART, hand write the data on the tracking form. Purple anaerobic bottles received greater than 1 hour from the pink aerobic bottle, re-order as new collection.

- Bottles should not be refrigerated or frozen.
- **DO NOT USE** culture bottles past their expiration date.
- **DO NOT USE** culture bottles that exhibit any cracks or defects; discard the vial in the appropriate manner.
- **DO NOT USE** culture bottles that have had their caps removed prematurely.

Reference document in the Lab Test Directory: Blood Culture

Special Safety Precautions

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

- Wear gloves while handling inoculated vials.
- Perform all blood culture processing in a biological safety cabinet.
- Properly dispose of all contaminated materials. Place syringes, needles and other sharp contaminated materials in a puncture proof container.
- NEVER ATTEMPT TO RECAP A NEEDLE.

Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**

- *Biohazard Containment*
- *Biohazardous Spills*
- *Safety in the Microbiology Laboratory*

Storage

- Bottles are stored at 2° to 25° C.

Remote Access to BD

Use these instructions to connect to the internet for remote access to BD.

1. Click on Window key and R at same time.
2. To open the Control program, hit Okay.
3. Click on Networking and Sharing Center
4. Click on Change Adaptor Setting
5. Highlight Facility HAN and right click
6. Select Enable
7. Click on RSS Carefusion Link on desktop to allow BD to remotely access.
8. Disable when remote access is complete.

Quality Control

Media Quality Control
Commercially prepared blood culture media do not require additional in-laboratory QC per CLIA and CLSI M22-A3.
Each case of media has a Quality Control certificate from BD indicating the organisms tested and the acceptability of those tests. An example from each media type is kept on file.

Bactec FX Instrument Maintenance
The following procedures should be performed daily:

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. Next tap the “yellow” button to illuminate the yellow station indicators. Make a note of any station that does not illuminate yellow.
6. Repeat Steps 3 - 5 for each of the drawers in the system.
7. Close the drawer.
8. Tap the “alarm” button to verify that the audible alarm is functioning.
9. 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.
10. Check the temperature on the temperature vial(s) in each drawer.
11. Information should be recorded on the Maintenance QC Log, which is located in the Daily Bactec Maintenance binder. Daily Bactec Maintenance QC reports that print automatically should be checked and filed in the Bactec Maintenance binder according to each instrument.

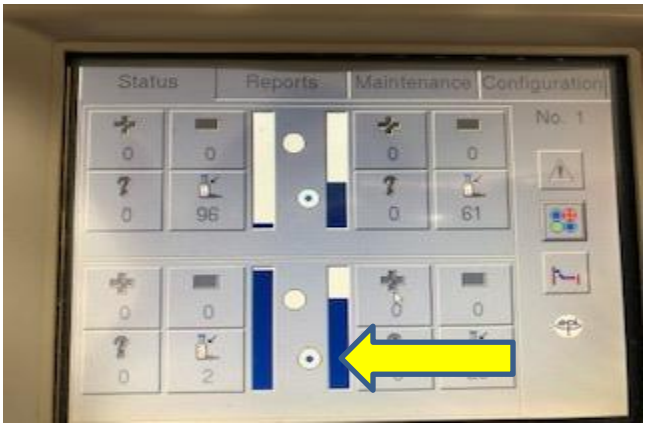
Daily Backup:
The automatic Epicenter Backup is programmed to happen overnight.

Monthly Maintenance:
Change both sets of filters on each Bactec instrument. Rinse filters thoroughly with water and allow to dry completely.

Procedure

Entering Data and Loading Instrument

A. To enter vials in the instrument, select a drawer using the indicator where there are available stations.
B. Do not select a drawer with the blue dot in the white circle. Select a different drawer.



The blue dot indicates the instrument is reading. Pick a drawer that does not have a blue dot in the white circle.

C. Then follow one of the two methods described below.

Method 1 (Vial Activated)

1. Select a drawer that has available stations. Use the indicator on the front of the drawer that indicates which drawer is currently in use.
2. Check if the drawer is currently reading bottles by looking for the blue dot in the white circle (see yellow arrow). Select a different drawer without the blue dot and open that drawer.
3. The barcode scanner turns on.
4. Scan a vial sequence barcode label and the Accession barcode.
5. The Vial Entry display appears and 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)

Method 2 (Icon Activated)

1. Select a drawer that has available stations. Use the indicator on the front of the drawer that indicates which drawer is currently in use.
2. Check if the drawer is currently reading bottles by looking for the blue dot in the white circle (see yellow arrow). Select a different drawer without the blue dot and open that drawer.
1. Tap the “vial entry” button on the Status display
2. The Vial Entry display appears and the barcode scanner turns on
3. Scan the vial sequence barcode label
4. 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)
8. When a vial is placed into the last available station in a drawer, the Activity Complete tone sounds (3 beeps).
9. 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 hemolytic, 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. Continuous loud alarm will sound if drawer is open more than 10 minutes.

Make sure all vials are fully inserted in the stations before closing the drawer.

Vials that are not read for 40 minutes (because of an open drawer or being unseated) need to be subbed and an AO performed. If AO stain is positive, perform Gram stain.

WARNING

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

Vials Delayed in Transport—

Add code **DELA** to the **SDES** if received after 36 hours from collection.

Bottles can be held up to 36 hours at room temperature and still be placed into the Bactec for reading.

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. The instrument recognizes anonymous vials 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.

These anonymous vials need to be identified in the system using the ID(entify). Do not perform Negative Vial Removal until all Anonymous vials have been resolved. You could lose data if you accidentally remove an Anonymous vial.

To identify anonymous vials:

1. Open drawer and remove vial from flashing yellow station or open drawer and tap ? to activate ID Anonymous workflow.
2. Scan the sequence and accession for the anonymous vial. The patient information is filled on the workflow display and the station the vial was pulled from will be flashing green.
3. Return the vial to the flashing green station.

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.

Positive / Negative/ Ongoing 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 Epicenter 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
   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
   3. In Protocol Negatives (ongoing) are indicated by LED with no light lit up.

B. **Removing positive vials**

   1. Print “**Current positive** report”. At the FX screen, touch the **Reports** tab. Touch the drop-down menu and select **Current positives.** Touch the **Print** button at the bottom of the screen.
   2. 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
   2. The Positive Removal display appears. Scan vial sequence. (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.

C. **Negative Vial Removal**
   1. Negative bottles with be removed at the beginning of each shift.
   2. Open drawer.
   3. Remove negative vial from Flashing Green station (Vial activated workflow).

OR:

4. Tap the “remove negatives” button to activate Negative Removal Workflow (Icon Activated workflow).
5. “Remove Negative” Workflow display is activated.
6. Only negative vial station LEDs are illuminated flashing green and the barcode reader is not turned on.
7. Continue removing negative vials until all vials with flashing green LEDs are removed.
8. Dispose of bottles in biohazard waste containers.
9. Retain bottles labeled with white tape “flag” labeled TSUB. Follow False Positive Bottle instructions.
10. If a completed Out-of-Protocol vial is accidentally left in the instrument, it will remain negative and can be removed at a later time.
11. Triple beep (workflow complete) will sound.

D. **Processing an Instrument – Positive Vial**

1. Remove the vial from the instrument and place in a biological safety cabinet.
2. Reprint the specimen label to use on the subculture plates.
3. If it is necessary to release pressure in the vial, place a 70% isopropyl alcohol wipe over the septum and insert a venting needle through the alcohol wipe and septum. Remove the needle after the pressure is released. Place the venting needle into a sharps container.
4. Invert the vial to mix the contents.
5. Disinfect the septum of the vial with a 70% isopropyl alcohol wipe. Allow to dry.
6. Attach a 1 ml syringe to a blood transfer device.
7. Push the blood transfer device into the septum of the vial, invert and withdraw 1ml.
8. Remove the blood transfer device with the syringe from the vial.
9. Remove the syringe from the blood transfer device and discard the blood transfer device into a sharps container.
10. Using the contents from the syringe, inoculate a CHOC & SB from the aerobic bottle. Inoculate a CHOC, SB and ASB2 from the anaerobic bottle. Label the plates with the current date, the current time, mark them “A” for the aerobic bottle and “N” for the anaerobic bottle (use the barcode labels).
11. Streak plates semi-quantitatively for primary isolation.
12. Positive vials suspicious of Brucella should be sealed with tape to prevent exposure upon opening. Label with red or orange sticker to work under the hood.
13. Make a Gram stain slide.
14. Expel the remaining sample into a sterile and labeled snap cap tube.
15. Incubate CHOC, SB, and CNA in 4-10% CO_2 at 35ºC.
16. Incubate MAC, CCAN and SAB in ambient air incubator at 35ºC.
17. Incubate the ASB2 in the ambient air incubator at 35ºC in anaerobic conditions.
18. After the slide is dry and heat fixed, perform the Gram stain procedure as soon as possible.
19. Read and report the Gram stain results. (See Reporting section).
16. Determine if BioFire FilmArray BCID panel should be performed.
    1. Perform BioFire FilmArray BCID if it is the first positive blood culture, using the labeled snap cap tube.
    2. Perform BioFire FilmArray BCID if it is the first positive blood culture greater than or equal to 5 days since previous result, using the labeled snap cap tube.
    3. Perform BioFire FilmArray BCID if the Gram Stain morphology/reaction is different from previous positive Blood Cultures, using the labeled snap cap tube.
    4. Enter code **BFTP** (BioFire testing in process) after gram stain result when you have determined that BCID is needed and are waiting for BioFire FilmArray BCID panel results.
    5. **Do not** perform if patient has had previous positive blood cultures identified on BioFire FilmArray with **same** Gram stain morphology/reaction, within the previous 5 days.
    6. **Do not** perform on related bottle (other bottle with same accession number) with the **same** Gram stain morphology/reaction.
    7. **Do not** perform if no organisms are seen on the Gram Stain.
    8. **Do not** perform on deceased patients or collected during an autopsy (MCAL or SCAL).
    9. Refer to MC 1.03f1 Positive Blood Culture Workflow for further instructions.

17. If no organisms are seen, refer to False Positive Bottle section.
    - **Day shift:** Perform an AO if Gram stain is negative.
18. Results of Gram stain may require additional plates to be inoculated.
    - Gram negative rod: CNA and MAC from the positive bottle.
    - Yeast: CCAN and SAB from the positive bottle.

19. **If No Organisms Detected on the BCID panel:** culture plates should be taped closed and labeled to 'work up in the hood' or with a red or orange sticker.
    These cultures may be a slow growing highly infectious organism and should be worked up in the BSC for our own safety. Please refer to the MCVI 3.60 Bioterrorism Protocols procedure for more specific information.
20. **Culture plates that are no growth or hazy growth after 1 day** should be taped closed and labeled as 'NG1-work up in hood' with a red or orange sticker.
    These cultures may be a slow growing highly infectious organism and should be worked up in the BSC for our own safety. Please refer to the MCVI 3.60 Bioterrorism Protocols procedure for more specific information.
21. Perform identification and susceptibility (AST) of organism(s) grown on solid media according to laboratory protocol.
22. Always consult caregiver regarding AST, if not performed, except for cultures with multiple isolates drawn from IV start (IVS) drawn in E.D.
23. Positive bottles are saved for one month in case of additional testing.
24. Returning 'False' (smear negative) Positive Vials
    - Place tape on the neck of the vial to mark it for a terminal subculture and AO stain.
    - Go to Vial Entry, scan sequence, and place vial in flashing green station.
    - False positive vials must be returned to the instrument within 5 hours.
25. For positive related vials, follow steps 1-20 in this section. A related vial is the second bottle in the blood culture set (aerobic and anaerobic bottle) to become positive. The related vial will have the same Accession number, therefore the same collection date, time and source, as the first positive bottle.

MANUAL BLOOD CULTURES

If blood bottles are received into the laboratory that does not meet the criteria for the Bactec™ system, they will be monitored off-line for growth.

1. Place the bottle(s) into the 35° C incubator.
2. Macroscopically examine the bottle(s) twice a day for first 2 days. Record in workups.
3. Macroscopically examine the bottle(s) for five days. Record in workups.
4. Perform blind subcultures to CHOC from aerobic bottles and to CHOC and ASB2 from anaerobic bottles at 24 hours, 48 hours and 5 days.
5. Perform Acridine Orange (AO) stains at 24 hours, 48 hours and 5 days.
6. Examine plates at 24 hours and 48 hours before discarding as negative.
7. Perform identification and susceptibility of organism(s) grown on solid media according to laboratory protocol.

Workups:		
	Wkup # 1 Med : BPNK Desc : DAY 1 Id : UNKN	Workup components: SC: CHOC AO: NEG COM: VISUAL EXAM NEG
	Wkup # 1.1 Med : BPNK Desc : DAY 1 Id : UNKN	Workup components: COM: VISUAL EXAM NEG
	Wkup # 2 Med : BPNK Desc : DAY 2 Id : UNKN	Workup components: SC: CHOC AO: NEG COM: VISUAL EXAM NEG

Wkup # 2.1
Med : BPNK
Desc : DAY 2
Id : UNKN

Workup components:
COM: VISUAL EXAM NEG

ANAEROBIC BOTTLE ONLY

If only an anaerobic bottle is received, contact the floor and see if the patient is still available to be drawn for an aerobic bottle. Incubate the anaerobic bottle in Bactec™ FX. Leave the label for day shift stating anaerobic bottle only received. Day shift micro tech will manually update.
Observations:

- Line 1: Enter RCUL-NG1
- Line 2: Enter **ANABC** that states: **Inadequate draw. Only anaerobic bottle received for culture. Please draw an aerobic bottle given the low prevalence of anaerobic bacteremia.**
- Final culture on day 5.
- Example:

Observations: 1. Routine Culture: No Growth 1 day
2. Inadequate draw. Only anaerobic bottle received for culture. Please draw an aerobic bottle given the low prevalence of anaerobic bacteremia.

FALSE POSITIVE BOTTLE

1. If no organisms are seen on Gram Stain, mark the **False Positive** (Gram stain negative) bottles with a tape “flag” labeled **TSUB**. This will alert day shift techs for AO stain and terminal subculture.
2. Return the flagged bottle to the Bactec. Save sample in snap cap for day staff.
3. Do not call results to provider.
4. Day shift: When the False Positive bottle becomes a Bactec out of protocol negative at 5 days:
    a. Perform terminal subculture (TSUB): Inoculate CHOC from aerobic bottle and CHOC and ASB2 plates from anaerobic bottles.
    b. Label the plates with the current date, the current time, mark them “A” for the aerobic bottle or “N” for the anaerobic bottle (use the barcode labels) and incubate.
    c. Perform AO stain from these TSUB bottles.
5. Examine plates at 24 hours and 48 hours before discarding as negative.

Method Performance Specifications

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

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.

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 0.5 mL or less for Bactec™ Peds Plus/F or 3.0 mL or less for Bactec™ Lytic 10 Anaerobic/F, an appropriate supplement may be required for recovery of these organisms. Bactec™ BRAND FOS™ Fastidious Organism Supplement may be used as nutritional supplements.

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.

Antimicrobial Activity

Neutralization of the antimicrobial activity by resins varies depending on dosage level and timing of specimen collection. Studies have demonstrated that the resins present in this medium do not adequately neutralize meropenem preparations. Studies have demonstrated that the resins present in this medium adequately neutralize the antifungal agent fluconazole with *Candida albicans*.

Susceptibility Testing of *Salmonella* isolates

To set up susceptibilities on *Salmonella* isolates, use the Vitek AST-N806 card or the EBAC HAE NMEN KB disks on MH agar. Report ampicillin, ciprofloxacin, trimethoprim-sulfa, and ceftriaxone.

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.

Subacute Bacterial endocarditis-SBE

The causative agents of bacterial endocarditis grow on the valves of the heart, and often are shed intermittently, and at a low level. Therefore, in order to allow them time to grow for detection by the Bactec system, the protocol should be changed to 14 days.

Recovery of *Brucella* spp.

Special handling is required for the recovery of *Brucella spp*. from blood cultures.

- Incubate *Brucella* (BRCL) for **10** days.
- At day **5,** do a blind subculture, Gram and Acridine orange stain.
- At day **10**, do terminal subculture with Gram and Acridine Orange stain.
- Refer to the LRN Level Bioterrorism Laboratory Protocols Procedure, in the Safety folder for more specific information.

Affected Vial Report

If the instrument loses power or there is an alert on the instrument with corrective action recommending to print an **Affected Vial Report** (see instrument quick reference guide for code and corrective action):

- Select Report tab
- Select Affect Vials (it is the first report on the list)
- Click Print
- Vials that appear on the Affected Vials list should be pulled and processed as a Manual Blood Culture. Refer to Manual Blood Culture section above with these additional instructions:
  - Start process on the same day of incubation as it was in the BACTEC FX instrument. Examples below:

- Day 2 in instrument = day 2 of manual processing. Perform macroscopic exams, subculture and AO.
- Day 3 in instrument = day 3 of manual processing. Perform macroscopic exam.

➢ Hold bottle for 5 days. Perform terminal subculture and AO on day 5.

Optimum recovery

- 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 CO_2 to be detected by the system or if significant growth has occurred before placing the vial into the system. False positives 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.

Critical Results

All positive Blood Culture results are critical and are reported within 60 minutes to the provider. Additional identification results also require notification to the provider. Refer to MCVI 4.0 Critical Results procedure for full list. Organisms to watch for include:

- MRSA
- ESBL or Carbapenemase producers
- Agents of Bioterrorism--*Bacillus anthracis, Brucella, Burkholderia mallei/pseudomallei, Francisella tularensis, or Yersinia pestis*
- Fungal or Yeast isolates:
  - Mucorales-all genera included in this Order, including those listed below
    - *Mucor spp.*
    - *Rhizomucor spp.*
    - *Rhizopus sp.*
    - *Syncephalastrum spp.*
    - *Lichtheimia spp.*
    - *Cunninghamella spp.*
  - *Cryptococcus neoformans*
  - *Coccidioides immitis*
  - *Histoplasma capsulatum*
  - *Blastomyces dermatitidis*
  - *Sporothrix schenkii*
- VRE
- *Corynebacterium diphtheriae*
- *Neisseria meningitis*

Result Reporting

REPORTING POSITIVE BLOOD CULTURES ON EVENING AND NIGHT SHIFTS

1. Record ALL results on the Bactec "Current Positive" print-out.
   Record whether 'A" or 'N"
   Record/write the Gram results.
   Record the "Called to", with date and time.
   Record tech initials

2. **Critical Value:** All positive blood cultures Gram stains are reported immediately by phone to the provider, excluding those pending BioFire FilmArray BCID result.
   - If BioFire FilmArray is **not** performed, Gram stain will be reported immediately by phone to the provider.

- Call Infection Prevention with Gram stain results that appear to be Gram-negative diplococci/Gram negative cocci.

3. Critical Value: If BioFire FilmArray BCID panel is performed, calling the Gram stain result can wait until the BCID results are complete and then the BioFire results are relayed to the physician and pharmacy. Refer to MC 10.4 BioFire FilmArray BCID 2 panel for further instructions on reporting with BCID results.

4. A related vial Gram stain does not need to be called if the results are identical. New organisms in a related vial will qualify for immediate reporting.

5. Document in the computer, the person called, their credentials (MD, RN, CNP, etc.) and the date and time of the call.

6. If No Growth is already recorded, replace No Growth result with the Gram stain result.

7. Report the Gram stain results using codes or the F8 function keys. Gram stain results are not quantified for blood cultures.

RESULTS:	Codes:	Function keys:
GRAM POSITIVE COCCI	GPC	key 2
IN CLUSTERS	CLS	key 3
IN PAIRS	PA	no key for PA
IN PAIRS AND CHAINS	PCHS	no key for PCHS
IN CHAINS	CHS	key 4
GRAM NEGATIVE RODS	GNR	key A
GRAM POSITIVE RODS	GPR	no key for GPR
GRAM NEGATIVE COCCI	GNC	no key for GNC
GRAM NEG COCCOBACILLI	GNEG-CC	no keys
YEAST	YEAS	key O
BEING ISOLATED AND IDENTIFIED	BIID	key >
**Called to and read back by	CAL	key C
GRAM STAIN	GMS	no key for GMS

EXAMPLES:

Observations: 1. GRAM POSITIVE COCCI IN CLUSTERS BEING ISOLATED AND IDENTIFIED
2. **Called to ER (Dr. Smith) 2230 05/19/2008 GRAM STAIN

Using codes: 1. **GPC** (tab) **CLS** (tab) **BIID** (down arrow)
2. **CAL** (tab) (2 semicolons) **ER (Dr. Smith) 2230 05/19/08** (tab) **GMS**

Using the Function keys:
1. **key 2** (tab) **key 3** (tab) **key >** (down arrow)
2. **key C** (tab) (2 semicolons) **ER (Dr. Smith) 2230 05/19/08** (tab) **GMS** (no key, have to use code)

Observations: 1. GRAM NEGATIVE RODS BEING ISOLATED AND IDENTIFIED
2. **Called to L8 (Mary, RN) 1715 05/20/2008 GRAM STAIN

Using codes: 1. **GNR** (tab) **BIID** (down arrow)
2. **CAL** (tab) (2 semicolons) **L8 (Mary P.,RN) 1715 05/20/08** (tab) **GMS**

Using the Function keys:
1. **key A** (tab) **key >** (down arrow)
2. **key C** (tab) (2 semicolons) **L8 (Mary P.,RN) 1715 05/20/08** (tab) **GMS** (have to use code, no key)

Observations: 1. YEAST BEING ISOLATED AND IDENTIFIED
2. **Called to NICU (Dan, RN) 0320 05/21/2008 GRAM STAIN

Using codes: 1. **YST** (tab) **BIID** (down arrow)
2. **CAL** (tab) (2 semicolons) **NICU (Dan B., RN) 0320 05/21/08** (tab) **GMS**

Using the Function keys:
1. **key O** (tab) **key >** (down arrow)
2. **key C** (tab) (2 semicolons) **NICU (Dan B., RN) 0320 05/21/08** (tab) **GMS** (have to use code, no key)

REPORTING POSITIVE BLOOD CULTURES ON DAY SHIFT

8. Critical Value: All positive blood cultures Gram stains are reported immediately by phone to the provider, excluding those pending BioFire FilmArray BCID result.
    - If BioFire FilmArray is **not** performed, Gram stain will be reported immediately by phone to the provider.
    - Call Infection Prevention with Gram stain results that appear to be Gram-negative diplococci/Gram negative cocci
    - Call Infection Prevention with identification of *Neisseria meningitidis* isolates.

9. Critical Value: If BioFire FilmArray BCID panel is performed, calling the Gram stain result can wait until the BCID results are complete and then the BioFire results are relayed to the physician and pharmacy. Refer to MC 10.4 BioFire FilmArray BCID 2 panel for further instructions on reporting with BCID results.

10. Critical Value: Call provider with results of additional organisms. Examples:
    - Call results from related bottle with different Gram stain
    - Call results of additional organisms that grow after first organism was resulted.

11. A related vial Gram stain does not need to be called if the results are identical. New organisms in a related vial will qualify for immediate reporting.

12. Document in the computer, the person called, their credentials (MD, NP, CNP, etc) and the date and time of the call.

13. If No Growth is already recorded, replace No Growth result with the Gram stain result.

14. Report and record all results and workups in Sunquest Microbiology Result Entry, in the Culture Entry tab using customized keyboards or by entering a code in the result box.

Observations:	1. GRAM POSITIVE COCCI IN CLUSTERS BEING ISOLATED AND IDENTIFIED 2. **Called to Dr. Plouff at 0830 09/23/2006 GRAM STAIN 3. Susceptibilities to follow	
Workups:	Wkup # 1 Med : BPNK Desc : POS Id : UNKN	Workup components: SC : CHOC SB GMS : STPH
	Wkup # 2 Med : BPRL Desc : POS Id : UNKN	Workup components: SC : CHOC SB ASB2 MAC CNA GMS : GNR
Observations:	1. GRAM NEGATIVE RODS BEING ISOLATED AND IDENTIFIED 2. **Called to HOC (Mary P.,RN) at 0830 09/23/2006 GRAM STAIN 3. Susceptibilities to follow	
Workups:	Wkup # 1 Med : BPNK Desc : POS Id : UNKN	Workup components: SC : CHOC SB CNA MAC GMS : GMNR

15. Negative blood cultures are updated each day in Sunquest Microbiology Automatic No-Growth Result Entry.
    - Enter **BC** in the Worksheet box and click **Add**.
    - Click the **Add** button a little lower on the screen.
    - Click the **Start Update** button at the bottom of the screen.
16. Sunquest will complete the update and the window will close.

17. Blood cultures are automatically finalized as: No Growth 5 Days

18. **Call Infection Prevention with Gram stain results that appear to be Gram-negative diplococci morphologically resembling *Neisseria* sp. Also inform Infection Prevention when *Neisseria meningitidis* has been isolated and confirmed. Document date and time called in the computer.**

19. If growth should occur or additional testing should be requested after the culture has been finalized, remove the final status and send out a supplementary report using the code SRPT in SREQ or CULTURE RESULTS. Re-final the culture when identifications and/or testing is complete. If a culture requires a correction, the code **CORR** (corrected report) must be used in CULTURE RESULTS. Refer to the procedure MCVI 5.1 Mislabeled / Unlabeled Specimens and Correcting Patient Data.

References

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5. Versalovic, James, editor in chief, Manual of Clinical Microbiology. 10th ed. American Society for Microbiology, Washington, DC, 2011
6. Recommendations for preventing transmission of Human Immunodeficiency Virus and Hepatitis B Virus to patients during exposure-prone invasive procedures. MMWR 1991, Vol. 40, No RR-8.
7. Blood borne Pathogens. Code of Federal Regulations, Title 29, Part 1910.1030 Federal Register 1991, 56:64175-64182.
8. Baron E. J, M.P Weinstein, W.M. Dunne, Jr., P. Yagupsky, D.F. Welch, and D.M. Wilson. 2005. Cumitech 1C, Blood Culture IV. Coordinating ed., E.J.Baron, American Society for Microbiology, Washington, DC.
9. Principles and Procedures for Blood Cultures, CLSI Guidelines, 2007, M47-A, Vol. 27.
10. Quality Control for Commercially Prepared Microbiological Culture Media, CLSI, 2004, M22-A3, Vol. 24.

Appendices

WORKLABEL MEDIA-FORM DEFINITION
BATTERY: BC

SPEC	MEDIA
0	BPNK, BPRL

Training Plan/ Competency Assessment

Training Plan	Initial Competency Assessment
1. Employee must read the procedure. 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.	1. Direct observation.

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.0	Eileen Brinkman	6/28/2010	Initial Version
1.1	Eileen Brinkman	10/14/2010	Deleted related vial information and added delayed vial entry.
1.2	Becky Carlson	1/05/2014	Added positive culture AST statement
1.3	Becky Carlson	11/01/2014	Added gram review by 2nd tech
2	Becky Carlson	4/14/205	Re-numbered from MC 403
3	Susan DeMeyere	5/23/2017	Changed procedure for Salmonella testing.

	4	Susan DeMeyere	8/30/2017	Added removal of negative bottles on all shifts. Added retaining negative bottles with TSUB flag for false positive workup.
	6	Susan DeMeyere	10/31/2018	Removed back up DVD from Maintenance. Added use of Clinical Collect sufficient for bar code labeling. Change orange anaerobic bottle to purple bottle. Removed anaerobic culturing from aerobic bottles. Removed venting anaerobic only bottles.
	7	Susan DeMeyere	5/13/2019	Added instructions for false positive bottles. Added BCID and gram stain calling instructions.
	8	Susan DeMeyere	6/24/2019	Added instructions for macroscopic exam of manual blood culture.
	9	Susan DeMeyere	2/8/2021	Added instructions to select different drawer when instrument is reading, indicated by blue dot in white circle.
	10	Susan DeMeyere	9/15/2021	Added instructions with No Organisms Detected on BCID to work under the hood, label with sticker.
	11	Susan DeMeyere	9/19/2022	Updated to repeat BCID after 5 days.
	12	Susan DeMeyere	5/18/2023	Added Bactec Maintenance QC Report will be reviewed and saved in the Daily Maintenance Binder for each instrument.
	13	Susan DeMeyere	2/20/2024	Add Affected Vial Report instructions and changed delayed vial instructions.
	14	Susan DeMeyere	1/30/2025	Added instructions for when the purple bottle is received after the pink bottle.