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| 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
 | * 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, 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
* Inoculating loop
* Microscope
 | * 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
* 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)
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| **Specimen** | 1. 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:

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| --- | --- | --- |
| 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 |

1. 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).
1. 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.
* 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](https://starnet.childrenshc.org/References/labsop/micro/cultpro/mc-1.03-bactec-fx-blood-brucella-culture.pdf) |
| **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***.**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
3. [*Safety in the Microbiology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)
 |
| **Storage** | * Bottles are stored at 2° to 25° C.
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| **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 Chart, which is located in the Bactec Maintenance book.

**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** | * + - 1. **Entering Data And Loading Instrument**
1. To enter vials in the instrument, select a drawer using the indicator where there are available stations.
2. Do not select a drawer with the blue dot in the white circle. Select a different drawer.

C:\Users\CE004159\AppData\Local\Temp\1\XPgrpwise\60128759secmpsmps4po10016132741106631\IMG_1969.JPG The blue dot indicates the instrument is reading. Pick a drawer that does not have a blue dot in the white circle.1. Then follow one of the two methods described below.
	* + 1. Method 1 (Vial Activated)
2. Select a drawer that has available stations. Use the indicator on the front of the drawer that indicates which drawer is currently in use.
3. 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.
4. The barcode scanner turns on.
5. Scan a vial sequence barcode label and the Accession barcode.
6. The Vial Entry display appears and the Sequence, Media, and default Protocol are automatically entered.
7. If you did not scan the Accession, scan or enter it now
8. To change the protocol tap the “modify” button, then tap the up arrow to increase or down arrow to decrease the protocol length.
9. Place the vial into an available station (solid green indicator)
	* + 1. Method 2 (Icon Activated)
10. Select a drawer that has available stations. Use the indicator on the front of the drawer that indicates which drawer is currently in use.
11. 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.
12. Tap the “vial entry” button on the Status display
13. The Vial Entry display appears and the barcode scanner turns on
14. Scan the vial sequence barcode label
15. The Sequence, Media, and default Protocol are automatically entered
16. If you did not scan the Accession, scan or enter it now
17. To change the protocol tap the “modify” button, then tap the up arrow to increase or down arrow to decrease the protocol length
18. Place the vial into an available station (solid green indicator)
19. When a vial is placed into the last available station in a drawer, the Activity Complete tone sounds (3 beeps).
20. To continue entering vials, select another drawer with available stations.
	* + 1. **Inserting Vials in the Instrument**
			2. 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.
			3. After all vials have been inspected and inserted in stations, close the drawer.
			4. 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.
			5. Once vials are placed in their stations, you should avoid moving them to other stations unnecessarily.
			6. 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.
			7. 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.* + - 1. **Vials Delayed in Transport—**(add **DELA** to the **SDES** when receiving these cultures).

Vials that are delayed in transportation to lab 8 hours or more need to be subbed to CHOC, SB, and ASB2 plates, and a Gram stain and AO performed before being placed into Bactec. Subculture the bottle(s) according to the positive bottle BC protocol. Read the preliminary Gram stain. Leave results for day shift Micro with a label indicating the status.If the Gram stain is negative, put the bottle(s) in the Bactec according to the processing new vials protocol.Bottles can be held up to 48 hours at room temperature and up to 24 hours in a 35-degree incubator, and still be placed into the Bactec for reading.* + - 1. **Anonymous Vial Entry**
			2. 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.
			3. 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.
			4. 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.
	* + 1. 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** 1. **Notification of positive and negative vials**
	1. The system notifies you of new positive cultures in several ways
		1. Positive Vial audible alarm sounds
		2. Station Indicators: FLASHING RED or FLASHING AMBER / RED (alternating) -Anonymous Positive
		3. Message box appears on Epicenter screen.
		4. Positive vial system indicator for that drawer illuminates
		5. 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
			1. Out-of-Protocol Negatives are indicated by the following
				1. Negative vial system indicator for that drawer illuminates
				2. On the Status display, the “negatives” icon is active and the number of negative vials in the drawer is shown
				3. Station indicators: FLASHING GREEN

In Protocol Negatives (ongoing) are indicated by LED with no light lit up.**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.

The barcode scanner turns on. All positive, final negative, available, and anonymous (all variations) are indicated by the appropriate lit or flashing station indicators.Tap the “remove positives” button on the Status display, OR Remove a vial from a FLASHING RED (positive) or FLASHING AMBER / FLASHING RED (anonymous-positive) stationThe 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.* + - * 1. **Negative Vial Removal**

Negative bottles with be removed at the beginning of each shift. Open drawer.Remove negative vial from Flashing Green station (Vial activated workflow). OR:Tap the “remove negatives” button to activate Negative Removal Workflow (Icon Activated workflow).“Remove Negative” Workflow display is activated.Only negative vial station LEDs are illuminated flashing green and the barcode reader is not turned on.Continue removing negative vials until all vials with flashing green LEDs are removed.Dispose of bottles in biohazard waste containers. Retain bottles labeled with white tape “flag” labeled TSUB. Follow False Positive Bottle instructions.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.Triple beep (workflow complete) will sound.**Processing an Instrument – Positive Vial**Remove the vial from the instrument and place in a biological safety cabinet.Reprint the specimen label to use on the subculture plates.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.Invert the vial to mix the contents.Disinfect the septum of the vial with a 70% isopropyl alcohol wipe. Allow to dry.Attach a 1 ml syringe to a blood transfer device.Push the blood transfer device into the septum of the vial, invert and withdraw 1ml.Remove the blood transfer device with the syringe from the vial.Remove the syringe from the blood transfer device and discard the blood transfer device into a sharps container.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). 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. Make a Gram stain slide. Expel the remaining sample into a sterile and labeled snap cap tube.After the slide is dry and heat fixed, perform the Gram stain procedure as soon as possible.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 during hospital admission using the labeled snap cap tube.
2. Perform BioFire FilmArray BCID if the Gram Stain morphology/reaction is different from previous positive Blood Cultures during the same hospital admission using the labeled snap cap tube.
3. 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.
4. **Do not** perform if patient has had previous positive blood cultures identified on BioFire FilmArray with **same** Gram stain morphology/reaction during hospital admission.
5. **Do not** perform on related bottle (other bottle with same accession number) with the **same** Gram stain morphology/reaction.
6. **Do not** perform if no organisms are seen on the Gram Stain.
7. **Do not** perform on deceased patients or collected during an autopsy (MCAL or SCAL).
8. If no organisms are seen, refer to False Positive Bottle section.
9. Day shift: Perform an AO if Gram stain is negative.
10. Results of Gram stain may require additional plates to be inoculated.

 A) Gram negative rod: CNA and MAC from the positive bottle. B) Yeast: CCAN and SAB from the positive bottle.1. **If No Organisms Detected on the BCID panel:** culture plates should be taped closed and labeled as NG1-work up in the 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](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.60%20BioTerrorism%20Protocol.docx) procedure for more specific information.1. **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](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.60%20BioTerrorism%20Protocol.docx) procedure for more specific information.1. Perform identification and susceptibility (AST) of organism(s) grown on solid media according to

 laboratory protocol.1. Always consult caregiver regarding AST, if not performed, except for cultures with multiple isolates drawn from IV start (IVS) drawn in E.D.
2. Positive bottles are saved for one month in case of additional testing.
3. 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 hr.
4. 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 CULTURESIf 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 24h, 48h, and 5 days.
5. Perform Acridine Orange (AO) stains at 24h, 48h, and 5 days.
6. Examine plates at 24hand 48h before discarding as negative.
7. Perform identification and susceptibility of organism(s) grown on solid media according to laboratory protocol.

Workups: Wkup # 1 Workup components: Med : BPNK SC: CHOC  Desc : DAY 1 AO: NEG  Id : UNKN COM: VISUAL EXAM NEG  Wkup # 1.1 Workup components: Med : BPNK COM: VISUAL EXAM NEG  Desc : DAY 1  Id : UNKN Wkup # 2 Workup components: Med : BPNK SC: CHOC  Desc : DAY 2 AO: NEG Id : UNKN COM: VISUAL EXAM NEG  Wkup # 2.1 Workup components: Med : BPNK COM: VISUAL EXAM NEG  Desc : DAY 2  Id : UNKNANAEROBIC BOTTLE ONLYIf 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. 1. Return the flagged bottle to the Bactec. Save sample in snap cap for day staff.
2. Do not call results to provider.
3. Day shift: When the False Positive bottle becomes a Bactec out of protocol negative at 5 days:
4. Perform terminal subculture (TSUB): Inoculate CHOC from aerobic bottle and CHOC and ASB2 plates from anaerobic bottles.
5. 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.
6. Perform AO stain from these TSUB bottles.
7. Examine plates at 24h and 48h before discarding as negative.
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| **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 and *Streptobacillus* sp), the presence of optimum volumes of blood, based on media type, benefits the recovery of these organisms. To enhance the growth of SPS sensitive organisms when less than optimum volumes of blood are inoculated, additional whole human blood may be added. It is suggested to inoculate twice the amount of blood or joint fluid recommended by the manufacture into the blood culture bottle. 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 or whole human blood 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 imipenem-cilastatin antimicrobial preparations.**Susceptibility Testing of *Salmonella* isolates**To set up susceptibilities on *Salmonella* isolates use 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. Do a blind subculture, Gram and Acridine orange stain at 5 days and terminal subculture with Gram and AO at 10 days. Refer to the LRN Level Bioterrorism Laboratory Protocols Procedure, in the Safety folder for more specific information.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 CO2to 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. |
| **Result Reporting** | **REPORTING POSITIVE BLOOD CULTURES ON EVENING AND NIGHT SHIFTS**1. Record ALL results on the Bactec “Current Pos” print-out.

Record whether ‘A” or ‘N”Record/write the Gram results.Record initials and initials of second tech.Record the “Called to”, with date and time.1. **Critical Value:** All positive blood cultures are reported immediately by phone to the physician, excluding those pending BioFire FilmArray BCID result. Call Infection Control with Gram stain results that appear to be Gram-negative diplococci/Gram negative cocci and also all *Neisseria meningitidis* isolates.
2. 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. If BioFire FilmArray is not performed, Gram stain will be reported immediately by phone to the physician. Refer to MC 10.1 BioFire FilmArray BCID panel for further instructions on reporting with BCID results.
3. 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.
4. Document in the computer, the person called, their credentials (MD,RN,CNP,etc) and the date and time of the call.
5. 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 3IN PAIRS PA no key for PAIN PAIRS AND CHAINS PCHS no key for PCHSIN CHAINS CHS key 4GRAM NEGATIVE RODS GNR key AGRAM POSITIVE RODS GPR no key for GPRGRAM NEGATIVE COCCI GNC no key for GNCGRAM NEG COCCOBACILLI GNEG-CC no keysYEAST YEAS key OBEING ISOLATED AND IDENTIFIED BIID key >\*\*Called to and read back by CAL key CGRAM 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 STAINUsing 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 STAINUsing 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 STAINUsing 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 SHIFT1. Critical Value: All positive blood cultures are reported immediately by phone to the Physician, excluding those pending BioFire FilmArray BCID result. Call Infection Control with Gram stain results that appear to be Gram-negative diplococci/Gram negative cocci and also all *Neisseria meningitidis* isolates.
2. 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. If BioFire FilmArray is not performed, Gram stain will be reported immediately by phone to the physician. Refer to MC 10.1 BioFire FilmArray BCID panel for further instructions on reporting with BCID results.
3. Document in the computer, the person called, their credentials (MD, NP, CNP, etc) and the date and time of the call.
4. 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 followWorkups: Wkup # 1 Workup components: Med : BPNK SC : CHOC SB  Desc : POS GMS : STPH Id : UNKN Wkup # 2 Workup components: Med : BPRL SC : CHOC SB ASB2 MAC CNA Desc : POS GMS : GNR Id : UNKNObservations: 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 followWorkups: Wkup # 1 Workup components: Med : BPNK SC : CHOC SB CNA MAC Desc : POS GMS : GMNR Id : UNKN1. Negative blood cultures are updated each day in Sunquest Microbiology Automatic No-Growth Result Entry.
2. Enter BC in the Worksheet box and click **Add**. Then enter BC2 and click **Add**.
3. Click the **Add** button a little lower on the screen.
4. Click the **Start Update** button at the bottom of the screen.
5. Sunquest will complete the update and the window will close.
6. Blood cultures are automatically finalized as: No Growth 5 Days

**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.**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 5.0 Micro Computer Training](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%205%20Computer%5CMCVI%205.0%20Micro%20Computer%20Training.docx)1. If organism is detected by BCID and not isolated in culture, submit the sample to MDH for further testing.
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| **References** | 1. Bactec™ Peds Plus/F Culture Vials Insert. Document PP-091-JAA, 2008. Becton Dickinson Microbiology Systems.
2. Bactec™ Plus Anaerobic/F Culture Vials Insert. Document 8085859, 2009. Becton Dickinson Microbiology Systems.
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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.
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| **Appendices** | WORKLABEL MEDIA-FORM DEFINITIONBATTERY: BCSPEC MEDIA0 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.
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| **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.  |  | 5 | Susan DeMeyere | 9/5/2017 | Added instructions to tape plates closed that are suspicious of Brucella. Added to tape closed and label plates with no growth as NG1-work up in hood. |
|  | 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. |