Microbiology

Blood Culture Specimen Collection-BACTEC™ 9120 Fluorescent Series

Specimen: This system is designed for blood specimens only.

1. Site Selection
   A. Select a different body site for each culture drawn.
   B. Avoid drawing blood through indwelling intravascular catheters unless blood cannot be obtained by venipuncture or unless diagnosis of catheter sepsis is suspected.
2. Site Preparation
   A. Cleanse selected site with 70% isopropyl alcohol pad by scrubbing vigorously for 60 seconds and allow to dry.
   B. Apply 2% iodine tincture to site starting at center and moving outward in concentric circles. Allow solution to dry prior to venipuncture. After venipuncture, remove iodine solution using a sterile alcohol pad.
3. Disinfecting Blood Culture Bottles
   A. Scrub tops of blood culture bottles with a 70% isopropyl alcohol pad and leave pad on top of bottles until blood is ready to be injected.
   B. Do not use iodine to disinfect tops of bottles.
4. Venipuncture
   A. Avoid touching site of venipuncture.
   B. When using the blood collection set, phlebotomist must carefully monitor volume collected by means of the 5-mL graduation marks on bottle label. If volume is not monitored, the amount collected can exceed the stated maximum amount. This condition may adversely create a false-positive result due to high blood background from increased cell count. It is helpful to use a Sharpie® marker to mark off the level of fluid and level to achieve. Bottle should be standing in order to monitor fill level. Collect aerobic bottle first in case you are unable to collect both bottles.
   C. When using a 20-mL syringe, draw 16 mL to 20 mL of blood for 1 blood culture set (aerobic and anaerobic). Aerobic bottle should be inoculated with 8 mL to 10 mL of blood and the anaerobic bottle should be inoculated with 8 mL to 10 mL of blood.
5. Volume - Volume of blood is critical because the concentration of organisms in most cases of bacteremia is low. Use of higher or lower volumes may adversely affect recovery and/or detection times.
   A. Children (<12 years): 1 mL to 3 mL of blood per venipuncture (0.5-5 mL is acceptable); transfer entire amount to BACTEC™ Peds Plus™ bottle.
   B. Adult (>12 years): 16 mL to 20 mL of blood per venipuncture. If it is impossible to draw required amount, draw aerobic bottle first and aliquot as follows:

Amount per Venipuncture	BACTEC™ Aerobic Bottle with Blue Cap (Standard)	BACTEC™ Anaerobic Bottle with Purple Cap (Lytic)
16 mL to 20 mL	Split equally between aerobic and anaerobic bottles	
13 mL to 16 mL	8 mL	Remaining blood
10 mL to 12 mL	6 mL	Remaining blood
5 mL to 9 mL	Entire blood amount	None
3 mL	Use a BACTEC™ Peds Plus™ bottle **Note:** A blue-capped (standard) bottle is acceptable if not anticipated.	

6. Number and Timing - Most cases of bacteremia are detected using 2 or 3 sets of separately collected blood cultures. More than 3 sets generally 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. Optimally, cultures should be separated by intervals of 1 hour.
7. Specimen Labeling
   A. Each bottle must have a label with the following information:
      1)Patient's first and last name and middle initial
      2)Date and time of collection
      3)Medical record number
      4)Collector's initials
      5)Site of venipuncture (if other than peripheral)
   B. Do not cover BD barcode.

Collection and Transport of All Other Microbiology Specimens

The following are some general guidelines for collection, labeling, and transport of common routine microbiological specimens. See each individual test for additional instructions.

Specimens **must** be labeled with patient's full name (first, last, and middle initial), medical record number (if available), date and time of collection, test(s), location of patient, and type of specimen. Transport to the laboratory within 24 hours of collection.

1. *AFB* - Collect most specimens as for routine culture and refrigerate until processing.
2. *Anaerobes* - Collect specimen in a syringe with needle removed and capped, sterile container, or special anaerobic transport swab. Transport to the laboratory at ambient temperature.
3. *Blood* - To be collected by laboratory staff, emergency department, and ICU nurses.
4. *Body Fluid (not spinal fluid)* - Collect in a screw-capped, sterile container and transport at ambient temperature. A syringe can be sent (with needle removed).
5. *Clostridium difficile* - Collect stool specimen as for routine culture and refrigerate until processing. Freeze if specimen needs to be held overnight.
6. *Ear* - Cleanse external ear. Obtain a regular culture swab collecting any exudate and transport at ambient temperature. Collect aspirate, if possible.
7. *Eye* - Collect a regular culture swab or a mini-tip BBL™ CultureSwab™ of the conjunctiva or exudate and transport at ambient temperature. Do not touch external skin.
8. *Fungus* - Collect scrapings in a sterile container or use a regular culture swab, if necessary. Transport at ambient temperature.
9. *GC-Outpatient* - Collect 1 regular culture swab. Transport to the laboratory at ambient temperature as soon as possible.
10. *Genital* - Collect a regular culture swab and transport at ambient temperature.
11. *India Ink* - Collect in usual spinal fluid tube. Refrigerated until processing.
12. *KOH* - Collect specimen in screw-capped, sterile container; swab without preservative; or place on glass slide, if necessary. Transport at ambient temperature and process as soon as possible if on slide.
13. *Nose* - Collect a nasopharyngeal swab or a regular culture swab and transport at ambient temperature. Specify if specimen is drainage or nasopharyngeal.
14. *Sputum* - Collect sputum (not saliva) in a screw-capped, sterile container. If >1 hour is anticipated before pickup, send specimen refrigerated.
15. *Stool* - Collect in a clean, **tightly-sealed** container. If there is a delay in transport of >1 hour, send specimen refrigerated. **Multiple specimens should be spaced at least 24 hours apart**. **Do not** send more than 1 specimen per day.
16. *Throat* - Collect a regular culture swab and transport at ambient temperature.
17. *Urine* - Collect in a sterile container, cup, or vial with preservative. Use preservative (yellow-top) vial only for cultures, not for urinalysis (UA). Refrigerate specimen up to 24 hours. **First-morning** specimens are preferred for both UA and culture.
18. *Viral* - Specimen for culture is placed in special transport media (M5), standard transport swab with Stuarts media, or a sterile container and refrigerated during transport.
19. *Wet Prep-Genital* - Collect swab placed in approximately 2 mL to 3 mL sterile saline. Transport at ambient temperature as soon as possible.
20. *Wound* - Collect 2 swabs (1 for Gram stain) or use dual collection transport swab. Store at ambient temperature.

Sensitivity Testing for Microbiology

Breakpoint testing (S-susceptible, I-intermediate, R-resistant) is performed on appropriate pathogens. Minimum inhibitory concentrations (MICs) are performed by request. *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* are screened for ESBL (extended spectrum beta-lactamase). Gatifloxacin (Tequin®) is routinely reported on respiratory specimens. In most cases, no more than 3 susceptibility tests will be set up per culture.

Note: When ordering a culture, indicate any antibiotics patient is taking so that drug will be included in testing. Other antimicrobials not routinely reported are also tested. Contact Microbiology Laboratory if in need of these results.

List of Antibiotics Routinely Included in Sensitivity Testing

Antimicrobials Routinely Reported Gram Positive	
Gram Positive (predominately *Staphylococcus* species)	***Streptococcus* species (not *Enterococcus* or *Streptococcus pneumoniae*)**
Amoxicillin/K Clavulanate (Augmentin™)	Azithromycin
Amoxicillin/Sulbactam (Unasyn™)	Erythromycin
Azithromycin	Penicillin (if beta-strep only)
Cefazolin	Clindamycin
Clindamycin	Vancomycin
Erythromycin	Gatifloxacin (if respiratory)
Gentamicin Oxacillin/Nafcillin	Alpha strep is sent to the State laboratory for a Penicillin MIC if from a sterile site
Penicillin Tetracycline/Doxycycline	Beta-strep is sent to the State laboratory for a Penicillin MIC if from a sterile site
Trimethoprim/Sulfamethoxazole (Bactrim™)	
Vancomycin	**Spinal Fluid (Gram Positive)**
Plus:	Ceftriazone (Rocephin™)
Chloramphenicol (if eye or cornea)	Chloramphenicol
Gatifloxacin (if respiratory)	Penicillin
Nitrofurantoin (if urine)	Vancomycin
Enterococci	
Ampicillin	
Gentamicin Synergy (non-urine)	
Streptomycin Synergy (non-urine)	
Tetracycline/Doxycycline	
Vancomycin	
Ciprofloxacin (if urine and amp resistant)	
Nitrofurantoin (if urine)	
Streptococcus pneumoniae	
Azituromycin	
Chloramphenicol	
Erythromycin (non-spinal fluid)	
Penicillin	
Tetracycline (non-spinal fluid)	
Trimethoprim/Sulfamethoxazole (non-spinal fluid)	
Vancomycin	
Gatifloxacin (if respiratory)	

Antimicrobials Routinely Reported Gram Negative	
Gram-Negative Organisms (Enterobacteriaceae)	**Spinal Fluid (Gram Negative)**
Ampicillin	Ampicillin
Amoxicillin/K Clavulante (Augmentin™)	Ceftriazone (Rocephin™)
Amoxicillin/Sulbactam (Unasyn™)	Chloramphenicol
Cefazolin	Trimethoprim/Sulfamethoxazole (Bactrim™)
Cefotetan	
Ceftriaxone (Rocephin™)	***Salmonella*/*Shigella***
Ciprofloxacin/Gentamicin	
Tetracycline/Doxycycline Tobramycin (if gentamicin resistant)	Ampicillin Ciprofloxacin
Trimethoprim/Sulfamethoxazole (Bactrim™)	Trimethoprim/Sulfamethoxazole (Bactrim™)
Plus:	Plus:
Chloramphenicol (if eye)	Cefotaxime (if extra-intestinal)
Gatifloxacin (if respiratory)	Chloramphenicol (if extra-intestinal)
Nitrofurantoin (if urine)	
***Pseudomonas aeruginosa* and non-Enterobacteriaceae**	
Ceftazidime (Fortaz)	
Ciprofloxacin	
Gentamicin	
Piperacillin/Tazobactam (not reported for *Acinetobactor* species or *Stenotrophomonas maltophilia*)	
Tobramycin (if gentamicin resistant)	
***Haemophilus influenza* (Beta-Lactamase Positive or Sterile Site)**	
Ampicillin	
Cefaclor	
Ceftriaxone (Rocephin™)	
Cefuroxime	
Trimethoprim/Sulfamethoxazole (Bactrim™)	
Plus:	
Ceftriaxone (if blood or spinal fluid)	
Chloramphenicol (if blood or spinal fluid)	
Gatifloxacin (if respiratory)	