Nebraska Medicine

The Nebraska Medical Center Procedure Adopted: 12/02

Clinical Laboratory Procedure Supersedes: 6/01

Microbiology/Virology Last Edited: 1/2/2025

**Blood Culture Collection**

**(For Bacteria, Fungus/Yeast or Mycobacteria/AFB)**

**(BLDCU, BLDA, FUNBL, AFBBL)**

**Principle:**

The detection of living microorganisms in a patient’s blood has great diagnostic importance. When bacteria multiply at a rate that exceeds the capacity of the reticuloendothelial system to remove microorganisms, bacteremia and/or fungemia results. Blood is drawn and cultured to identify the bacteria and/or fungus and determine susceptibility patterns before antibiotic therapy is started.

The volume of blood drawn is critical because the concentration of organisms in the majority of bacteremia is low, especially if the patient is on antimicrobial therapy. For optimal detection of organisms, the appropriate volume of blood is critical because the sensitivity of blood cultures in detecting pathogens is proportional to the volume of blood collected. A significant number of bacteremias will be missed in adults with samples less that 10 ml, with 20 ml per culture bottle being an optimal volume for an aerobic and anaerobic blood culture. The routine draw will be 20 ml of blood, divided equally between an Aerobic bottle and an Anaerobic bottle. During a febrile episode, two sets of blood cultures should be obtained. If possible, each set should be drawn from a separate site. In infants and children, the concentration of organisms during bacteremia is higher than in adults, so less blood is required for culture. Pediatric bottles should only be used for pediatric patients whose age and condition prevent drawing greater than 3 ml of blood. Drawing from an indwelling catheter is not acceptable unless diagnosis of catheter sepsis is suspected.

Blood cultures can be drawn to detect bacteria (BLDA, or BLDCU), fungus/yeast (FUNBL), or Acid-Fast bacteria known as mycobacteria (AFBBL).

**Reagents, Supplies and Equipment:**

1. Bactec bottles. All bottles are stored at room temperature. Bottles must be stored upright.

Plus Aerobic/F Bottle (grey label, blue top)

Lytic Anaerobic/F Bottle (purple label and top)

Peds Plus/F Bottle (pink label and silver top) for pediatric patients only

Mycolytic/F Lytic Bottle (red label and top)

2. 70% isopropyl alcohol pads

3. Chloraprep

4. Syringe, Safety Needles, and tourniquet

5. Gloves, and lab coat

6. BD Transfer Device

Nursing line collection supplies:

1. Two normal saline syringes
2. Two empty 10 mL syringes
3. Passive disinfection caps

**Procedure:**

|  |
| --- |
| **Gloves must be worn when performing this procedure. Add additional PPE as necessary based on patient isolation precautions.** |

The appropriate volume of blood is critical because the sensitivity of blood cultures in detecting pathogens is proportional to the volume of blood collected. A significant number of bacteremias will be missed in adults with samples less than 10 ml, with 20 ml per culture being an optimal volume for an aerobic and anaerobic blood culture. When less than the recommended volume of blood is drawn for culture, the blood should be inoculated into the aerobic vial first with any remaining blood then inoculated into the anaerobic vial. Inoculating **more** than recommended for the particular blood bottle will interfere with the automated instrument’s ability to detect a positive culture.

a. In infants and children, the concentration of organisms during bacteremia is higher than in adults, so less blood is required for culture. Pediatric bottles should only be used for pediatric patients whose age and condition prevent drawing greater than 3 ml of blood.

a. Listed below is a summary of blood culture orders with appropriate Bactec bottle types and blood volumes required:

**Blood per Bottle\* Total Standard**

**Order BACTEC Bottle/Label Color Maximum Minimum Set Draw**

**\*\***Culture, Aer & Ana Blood Plus Aerobic/F (grey) 10 ml 3 ml 6 to 20 ml split equally

(BLDCU) Lytic/Anaerobic/F (purple) 10 ml 3 ml between bottles.

**If <6 ml total drawn:**

**add to Aerobic**

**bottle only;**

**reorder as Culture, Aerobic Blood, BLDA**

**Table 1: Pediatric patients only – Volume of blood collection based on weight.**

**FOR MULTIPLE DRAW SITES: (assuming 2 sets of cultures) volume to be put into each bottle:**

|  |  |  |  |
| --- | --- | --- | --- |
| Patient Dosing Weight | Total Volume  (ml) | Pediatric Vial (Pink)  Line site/ Peripheral site  (ml) | Regular Vial (Blue)  Line site/ Peripheral site  (ml) |
| 3.9 kg or less | 2 | 1/1 |  |
| 4-7.9 kg | 3 | 1.5/1.5 |  |
| 8-13.9 kg | 6 | 3/3 |  |
| 14-18.9 kg | 12 |  | 6/6 |
| 19-25.9 kg | 16 |  | 8/8 |
| 26-39.9 kg | 20 |  | 10/10 |
| 40kg or greater | 40 |  | 2sets per adult protocol |

**FOR A SINGLE DRAW SITE with entire volume <10 mL, place entire volume into 1 bottle with one order:**

|  |  |  |  |
| --- | --- | --- | --- |
| Patient Dosing Weight | Total Volume  (ml) | Pediatric Vial (Pink)  All from same site  (ml) | Regular Vial (Blue)  Line site/ Peripheral site  (ml) |
| 3.9 kg or less | 1 | 1 |  |
| 4-7.9 kg | 3 | 3 |  |
| 8-13.9 kg | 6 |  | 6 (adult bottle) |

**Patients >14 kg can follow the multiple draw site table, collecting 2 aerobic bottles from same site with volumes specified.**

\*\*\*Culture AFB, Blood Myco/F Lytic (red) 5 ml 3 ml 1 bottle/order/day

(AFBBL)

**Pediatric patients only** 3 ml 1 ml

\*\*\*Culture Fungus, Blood Myco/F Lytic (red) 5 ml 3 ml 1 bottle/order/day

(FUNBL) Add order comment if

specific yeast or mold

suspected

**Pediatric patients only** 3 ml 1ml

\*For optimal yield, it is best to collect maximum volume of blood for each bottle.

**\*\***For BLDCU add maximum volume of blood to Aerobic bottle first and remainder to Anaerobic bottle.

\*\*\*Simultaneous orders for both FUNBL and AFBBL must have SEPARATE Myco/F Lytic bottles collected.

**Peripheral Collection**

* 1. Collect blood for cultures by venipuncture of peripheral veins. Draw blood from two separate peripheral sites, if possible. Follow clinician’s orders for specimen site if applicable. If possible, draw blood below an existing intravenous line to prevent dilution of the blood with infusion fluid. No more than 3 peripheral cultures may be drawn in a 24-hour period (multiple line draws are acceptable) without prior review by Microbiology Medical Director.
  2. **Patient identification:**
     + - 1. Perform hand hygiene upon entering the room. Do not wear gloves into the room. Put clean gloves on after hand hygiene is performed.
         2. Assess patient status. If isolation precautions, use appropriate PPE. Follow precautions listed on isolation signage. Bring in only what is needed for the collection(s). Carts should only enter rooms that are not in isolation precautions or ensure not to pass the parameter square on the floor.
         3. Per Nebraska Medicine policy positive identification of the patient is mandatory.

**Inpatient/ED**: scan the patient arm band. Verify that the information in Collect matches the information on the patient arm band while verbally confirming patient full name and date of birth.

**Outpatient**: Call the patient to exam room by first name or preferred name listed in One Chart. Once in the room, ask the patient to state their first name, last name, and date of birth. Confirm all labels printed belong to the identified patient.

* 1. **Patient preparation:**
     + - 1. Place tourniquet.
         2. Select appropriate site(s) for collection during initial assessment to prepare for 2nd set or if a miss on the first attempt.
         3. Once the site has been selected by palpating with the tourniquet in place, remove the tourniquet and begin setting up the supplies.
         4. Disinfect cart or countertop with bleach wipe. Once dry, follow up with sani-cloth and allow to dry.
         5. Gather supplies taking care not to remove sterile equipment from package until ready for collection. Set out on clean cart or disinfected countertop.
         6. Cleanse the site first by using alcohol prep, scrubbing for 30-60 seconds and then allow to air dry.
         7. Secondarily cleanse using Chloraprep. Begin in the center of the site and scrub with friction in back-and-forth motion. Cleanse for 30-60 seconds. Allow 30-60 second dry time.
         8. **All steps are necessary to ensure aseptic site.** The site is not aseptic until the disinfectant has fully absorbed and the site is dry. **DO NOT touch the site once cleansed.** Advise the patient not to move or touch the cleansed site.
         9. Alternative aseptic cleansing solutions: Iodine, warm water and soap, or Betadine. Must follow aseptic guidelines listed on each option.
  2. **Supply preparation:**
     + - 1. Mark the fill line on all bottles before collection.
         2. While the site is drying, remove the blood culture bottle caps and scrub the bottle tops with individual alcohol prep pads. Once the bottle tops have been scrubbed, discard used pads and allow bottle tops to dry completely.
  3. **Collection:**
     + - 1. Replace tourniquet and perform venipuncture.
         2. **Re-palpating is not acceptable without fully re-cleansing the site.** If re-palpating must be performed, the aseptic cleansing must be performed again.
         3. Once total volume needed for collection is reached for one set, remove the tourniquet and then remove the needle and activate the safety device.
         4. Apply appropriate and adequate pressure to the site. Check for bleeding and bandage accordingly.
         5. Follow the order of draw for the method chosen (see options in 7. Collection Methods below).
         6. Label all specimens at the bedside. Do not cover the barcode or fill line window. Place Collect label or the label space on the bottle.
         7. Dispose of all used supplies and trash in the appropriate receptacles. If in isolation, follow appropriate steps upon exiting the room, including wiping down the handheld and printer.
  4. **Collection Methods:**
     + - 1. **Direct inoculation – best practice**

A person injecting a needle into a person's arm

Description automatically generated

1. When collecting directly into the blood culture bottles via evacuated tube and winged needle method, adequate volume is necessary.
2. Connect the Aerobic bottle to the needle inside the hub and fill the bottle to the appropriate fill line.
3. Be aware of the fill lines on the bottle and ensure that the appropriate amount is collected before removing the bottle from the hub. 8-10 mL volume for each bottle is recommended for adult draws. Refer to the pediatric draw chart for pediatric volumes.
4. Remove the bottle from the hub, mix, and repeat with the Anaerobic bottle (if indicated). Do not lay the bottles on the patient’s bed. Place bottles upright on a clean counter or cart. Collect any additional orders, following the correct order of draw. Blood cultures are always first.
5. Enter an order comment stating the site each set was collected from. Example “L.A.C” for left antecubital.
   * + - 1. **Needle and syringe:**

A person injecting a needle into a mannequin

Description automatically generated

1. Connect needle to either collection hub or syringe. Do not touch the connectors with fingers. Use the sterile packaging as a holder for the supplies. **Do not** pop the sterile syringe out of the sterile container. Open the package as directed to keep the package intact and sterile.

A person wearing purple gloves holding a plastic bag

Description automatically generated

1. First draw and discard a waste syringe of 3-5 mL.
2. It is recommended that the full volume is collected at once. If unable to collect total volume for 1 set, smaller syringes can be used. If switching syringes is needed, take care not to touch the connector of the butterfly or syringe. To protect the integrity of the patient sample, place the full syringe back into the sterile packaging. **DO NOT** lay on the patient’s bed, tray table, or cart.
3. When using the syringe method, the order of draw is reversed. The Anaerobic bottle should be inoculated first, followed by the Aerobic bottle.

A person in gloves holding a syringe with a liquid in a tube

Description automatically generated

* 1. Follow same steps for 2nd set. If the same site is needed for the second collection, allow no less than 30 minutes between collections. The time on the label must be accurate for both collections.
  2. Bottles are received into the LIS before being delivered to the Microbiology department.

**Nursing Line Collection:**

* + - 1. Blood cultures should only be drawn from a Central Venous Catheter (CVC) if the line is suspected to be the source of infection. If the line is the suspected source, paired cultures are desired with one sample from the CVC and the other from the periphery. It is important to clearly indicate the time the sample is obtained and the source of the blood (e.g. CVC vs peripheral).
      2. If the CVC is **NOT** suspected as the source of infection, then draw both blood cultures peripherally. Whenever possible, draw one from the right arm and one from the left arm.

NOTE: Patients with dialysis fistulas or arms that are marked to have a fistula implanted can NOT have blood drawn from that arm. Both paired cultures should be drawn peripherally from the same arm as long as one is obtained distally, and the other one is drawn proximally.

* + - 1. It is not necessary to wait 20 minutes between blood culture draws.
      2. DO NOT obtain blood cultures from the line if an antibiotic is currently infusing. Instead, obtain two sets of peripheral blood cultures if able.
      3. Collection of blood culture from CVC:
         1. Perform hand hygiene at the beginning and appropriately throughout the procedure.
         2. Put on mask and eyewear. Place mask on patient.
         3. Prepare the field for collection of blood cultures including wiping down the tray table or area for supplies with disinfection wipe for appropriate wet time and opening the blood culture collection kit.
         4. Turn off solutions infusing into any lumens of the catheter. Wait 60 seconds prior to drawing blood culture specimens.

NOTE: consideration must be given to the medications infusing. If vasoactive medications or other critical infusions are running, they should not be stopped.

NOTE: Do not draw cultures from lumens where antibiotics are currently running. If an antibiotic is being infused through the line that is being cultured, pause the IV for 1-5 minutes and draw from a lumen that is not infusing antibiotics. If needing to draw from the single lumen infusing antibiotics, pause the IV for 1-5 minutes, flush with 10 mL of saline, pull back 10 mL of waste and discard, then collect blood cultures.

* + - * 1. Aseptically connect needleless connectors to syringes.
        2. Perform hand hygiene and don gloves.
        3. Clamp the central line for lumen(s) utilized for culture.
        4. Remove the existing needleless connector of the lumen.
        5. Scrub the hub of the central line with 70% alcohol for a minimum of 15 seconds using friction.
        6. Attach a new needleless connector with syringe to the lumen.
        7. Unclamp the central line.
        8. Withdraw into the syringe(s) that blood for one or both cultures. If not able to achieve blood return, flush the lumen, pull back 10 mL of waste and discard, then withdraw the specimen. Draw no less than 10 mL of blood per blood culture bottle. (For pediatric collections, refer to Table 1.)

NOTE: There is not waste for blood cultures unless a flush is needed.

NOTE: The appropriate volume of blood is critical because the sensitivity of blood cultures in detecting pathogens is proportional to the volume of blood collected. Bacteremia will not be reliably detected in adults with samples less than 10 mL per bottle. See Table 1 for volume guidelines for pediatric patients. If not enough blood is available for both the aerobic and anaerobic bottles, place all the blood into the aerobic bottle (up to 10 mL).

m. Flush each lumen of the CVC with saline, using push pause technique.

n. Repeat step m. for the second lumen if using two lumens

o. Remove gloves, perform hand hygiene, and don new gloves.

p. Ensure that no blood remains in the needleless connector or connect new needleless connector using aseptic technique.

q. Restart any IV infusions.

r. Remove gloves, perform hand hygiene, and don new gloves.

s. Prepare blood culture vials by removing the caps. Scrub the top of the vials with 70% alcohol for a minimum of 5 seconds using friction.

t. Transfer the blood using a blood collection assembly into the appropriate blood culture bottles.

u. Gently mix the contents of the bottles by inverting 4-5 times.

v. Carefully document on the bottle the site from where the blood was obtained and the time of draw.

NOTE: When peripheral and central line sourced blood cultures are ordered, it is imperative that blood cultures taken from the peripheral and central line be labeled as such. Document in the electronic medical record and on the bottle label, indicating the specific line or extremity that the blood was collected from.

w. DO NOT cover the BACTEC bottle barcode.

x. Send specimens immediately to the laboratory making sure to double bag the bottles (may be together).

**Procedural Notes:**

1. If there is a delay in transporting bottles to the Microbiology Laboratory, store at room temperature away from direct sunlight and/or ventilation sources.

**NOTE: DO NOT PUT IN THE INCUBATOR OR THE REFRIGERATOR.**

1. When multiple blood cultures are ordered for collection at the same time, draw the samples from separate venipuncture sites if possible.
2. The sensitivity of blood cultures in detecting pathogens is proportional to the volume of blood collected. To optimize the yield from blood cultures, a 16-20 ml volume (8-10 ml into each bottle) is recommended. The manufacturers of blood culture instrumentation have a requirement for the optimal amount of blood which can be placed into the blood culture bottle/tube.
3. Blood culture bottles **cannot** be drawn directly using a disposable vacutainer hub.
4. Blood for culture should not be withdrawn through an indwelling intravenous or intra-arterial catheter unless it cannot be obtained by venipuncture due to contamination of the catheter by skin flora. If an intravascular catheter site is used, a second culture should be drawn by a venipuncture for comparison.
5. Annually a study of blood volume is performed. From the BACTEC FX Epicenter software, under Blood Volume Monitoring Reports, the Blood Volume Summary ran. Acceptable level of performance is >80% of bottles inoculated with 3-10 ml of blood and less than 5% inoculated with < 3ml of blood.

Unacceptable results, with corresponding accession numbers, are shared with phlebotomy management to address with staff. If an unacceptable level of performance is obtained, the study is repeated 6 months later, and every six months until an appropriate level of performance is met.

1. Contamination rates are monitored via LIS generated crystal reports each month. Data included in these reports are single positive blood cultures growing the following isolates:

* *Bacillus* species
* *Corynebacterium* species
* *Cutibacterium acnes*
* *Micrococcus* species
* Viridans streptococci
* Coagulase negative staphylococci

Other normal flora/environmental isolates will be evaluated on a case-by-case basis to determine if a probable contaminant.

Acceptable level of performance is <=1% of all blood cultures drawn classified as contaminants.

**References:**

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