

Notes

The rate of isolation of micro-organisms from blood is directly related to the volume of blood collected; **therefore, it is best to fill each blood culture bottle to the maximum (optimal) blood volume for each bottle (see page 2 Materials Required).**

However for pediatric patients there must be a balance between the total volume of blood collected, the patient body weight, and the clinical condition of the patient. **For pediatric patients weighing 5.1 to 30 kg whether or not a specimen is obtained from a second site is at the discretion of the clinician.** Collection from two sites identifies blood culture contaminants and/or line infections.

Table 1 –Blood Culture Collection Volumes According to Body Weight

Body Weight (kg)	Site 1	Site 2	Number of bottles to be collected
Less than or equal to 5 kg	Pediatric Bottle minimum 1 mL*	NA	1
5.1 - 12.7 kg♦	Pediatric Bottle 4 mL	Pediatric Bottle 2-4 mL	1-2
12.8 kg - 30 kg	Aerobic + Anaerobic 10 mL + 10 mL	Aerobic 10 mL	2-3
Greater than 30 kg	Aerobic + Anaerobic 10 mL + 10 mL	Aerobic + Anaerobic 10 mL + 10 mL	4

♦For pediatric patients weighing 5.1 to 12.7 kg a second pediatric bottle can be collected from the same site if the decision is to collect from one site only instead of the recommended 2 sites.

***More blood (up to 4 mL per bottle) can be collected if clinically appropriate.**

Refer to zone specific documents for pediatric weight-based maximum blood volume drawn in 24 hours. Blood culture volumes can be in addition to the maximum volume allowed for a single blood collection.

Calgary Zone: [Monitoring Blood Volume](#)

Edmonton Zone: [Maximum Blood Draw Protocol for Pediatric Patients](#)

North, Central, South Zone: follow current practices

- For patients weighing greater than 30 kg with suspected bacteremia or candidemia, draw two (2) sets of blood cultures from **two different** venipuncture sites. Each set consists of 1 aerobic **and** 1 anaerobic bottle. In cases of suspected infectious endocarditis, a third set may be collected 1 hour after the collection of the first two sets. Each set is considered a separate test request therefore each set requires its own separate paper requisition or order in the hospital information system.
- Peripheral venipuncture (not indwelling line) is the technique of choice for obtaining blood for culture unless an intravascular catheter-associated infection is suspected.
 - For patients with suspected intravascular catheter-associated infection 1 set (1 aerobic and 1 anaerobic bottle) should be obtained from the catheter along with a second set (1 aerobic and 1 anaerobic bottle) from a peripheral site. Withdrawal of a discard tube or flushing of the indwelling line is not required when line sepsis is suspected. Ensure the indwelling line collection site is indicated on the bottle(s) and the test request.
- In hemodialysis units, blood cultures may be collected in the dialysis blood lines during a dialysis session
- In NICU Advanced Starters may collect blood culture during PIV insertion if sample contamination is avoided at all times. Healthcare providers (non-lab) follow site specific nursing procedures for *Blood Sample Collection During Intravenous Cannula Insertion*. Blood samples may be collected from a newly inserted Umbilical Vascular catheter line prior to initiation of IV fluids.

Risks or Cautions

- It is critical that the skin be meticulously prepared prior to venipuncture to prevent contamination of the specimen, which could result in inappropriate antimicrobial therapy. **Please follow the site preparation guidelines carefully and perform collection while practicing sterile technique.**
- Blood cultures should, whenever possible, be collected before administration of antimicrobial agents.
- **Overfilling:** The vacuum in the bottle will usually allow for the optimal blood volume to be exceeded. Therefore it is important to observe the volume of the bottles as they are filled using the visual volume markers on the sides of the bottles, to ensure they are not overfilled.
- **Underfilling:** If unable to collect optimal amount, place as much blood as possible into the aerobic bottle or pediatric bottle then place remaining blood into anaerobic bottle. For pediatric patients weighing 5.1 to 12.7 kg, place remaining blood into a pediatric bottle.
- **False negative results** may occur if the optimal volume of blood is not placed into the blood culture bottle.
 - Overfilling of blood culture bottles dilutes the media and antibiotic absorbing resin, reducing frequency of detection or/ and increasing time to detection of organisms
 - Underfilling reduces sample size of specimen and increases the relative concentration of the culture bottle media components, reducing frequency of detection, or increasing time to detection of organisms
- **False positive results** can occur during the collection process (contamination). Refer to the [test directory page](#) for test interpretation.

Materials Required

- Routine venipuncture supplies
- 70% Isopropyl alcohol swabs or wipes
- Antiseptic agent (2% chlorhexidine gluconate (CHG) with 70% isopropyl alcohol [preferred] or 10% povidone iodine (PVP-I; iodopovidone). For NICU collections healthcare providers (non-lab) should follow site specific nursing guidelines for selecting appropriate chlorhexidine solution strength
Note: Use the type of antiseptic agent as available or prescribed by institutional protocols.
- Winged collection set units with adapter for blood culture bottles or syringes for pediatric or difficult collections
BD BACTEC™ bottles use a vacutainer holder, whereas Biomerieux BacT/ALERT® bottles must be used with the BacT/ALERT® Blood Collection Adapter Cap
- Sterile needleless transfer device (syringe collections or vascular access collections only)
- Appropriate blood culture bottles (Aerobic/Anaerobic/Pediatric):

BD BACTEC™



BACTEC Plus Aerobic/F REF442023 Blue ring/grey lid
BACTEC Lytic/10 Anaerobic/F REF442021 Purple ring/purple lid

Maximum optimal volume is 10 mL



BACTEC PEDs Plus/F REF442020 Silver ring/pink lid

Maximum volume is 5 mL

Biomerieux BacT/ALERT® (Calgary Zone only)



Aerobic FA Plus REF410851 Green lid
Anaerobic FN Plus REF410852 Orange lid

Maximum optimal volume is 10 mL











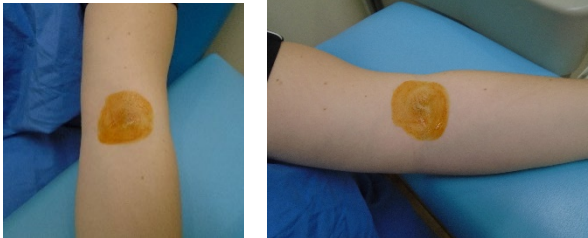
Pediatric PF Plus REF410853 Yellow lid







Maximum volume is 4 mL

Collecting the Sample

The collection instructions provided below represent best practice recommendations. However it should be noted that there may be situations that fall outside normal processes; in these situations sites should follow local institutional protocols.

STEP	ACTION	NOTES				
1.	Confirm patient identity using two approved identifiers					
2.	<p>Check bottles for signs of contamination. Signs of contamination include:</p> <ul style="list-style-type: none"> • Bottle media appears cloudy • Bulging or indented septa or visible gas • Bottom of the Biomerieux BacT/ALERT® bottle is bright yellow <p>Check bottles for signs of damage:</p> <ul style="list-style-type: none"> • Check for cracked or distended bottles or bottles that have lost their caps. A star-like crack pattern on the bottom of the bottle is consistent with a drop or impact that has compromised the container • Check that the fluorescent material at the base of the BD BACTEC™ bottle has not separated from the glass 	<p>DO NOT USE contaminated or damaged bottles:</p> <ul style="list-style-type: none"> • Bottles that have been dropped should be discarded and not used for collection • Discard cracked or distended bottles or bottles without caps • If the bottom of the Biomerieux BacT/ALERT® is a bright yellow then do not use; this is a sign the Biomerieux BacT/ALERT® is compromised 				
3.	Check the expiry date printed on each blood culture bottle label.	If expired, DO NOT use. Discard expired bottles.				
4.	<p>Optimal Volume:</p> <table border="1"> <thead> <tr> <th>BD BACTEC™</th> <th>Biomerieux BacT/ALERT®</th> </tr> </thead> <tbody> <tr> <td> <p>Mark optimal volume on the blood culture bottle</p>  </td> <td> <p>Do not draw lines on Biomerieux BacT/ALERT® Aerobic FA Plus or Anaerobic FN Plus blood culture bottles; these bottles include a manufacturer indicated FILL line. Refer to Filling and Labelling Biomerieux BacT/ALERT® Blood Culture Bottles MI6644.</p>  <p>BACT/Alert® Pediatric (PF Plus) do not have a manufacturer indicated fill line.</p> </td> </tr> </tbody> </table> <p>For BACTEC PEDs Plus/F and BACT/Alert® Pediatric (PF Plus) draw two lines to mark the appropriate volume for the weight of the pediatric patient.</p>	BD BACTEC™	Biomerieux BacT/ALERT®	<p>Mark optimal volume on the blood culture bottle</p> 	<p>Do not draw lines on Biomerieux BacT/ALERT® Aerobic FA Plus or Anaerobic FN Plus blood culture bottles; these bottles include a manufacturer indicated FILL line. Refer to Filling and Labelling Biomerieux BacT/ALERT® Blood Culture Bottles MI6644.</p>  <p>BACT/Alert® Pediatric (PF Plus) do not have a manufacturer indicated fill line.</p>	
BD BACTEC™	Biomerieux BacT/ALERT®					
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STEP	ACTION	NOTES		
5.	Select a blood collection site.	<ul style="list-style-type: none"> Healthcare providers (non-lab) should follow site specific nursing procedures when collecting from an indwelling device. Laboratory staff are permitted to perform venipuncture collections only and must follow APL venipuncture procedures. 		
6.	<p>Cleanse the site:</p> <ul style="list-style-type: none"> Cleanse the site with 70% isopropyl alcohol for a minimum of 30 seconds using a circular motion, starting at the center of the site and moving outward to the periphery (5 cm diameter). Do not return the 70% isopropyl alcohol swab or wipe to the center of the site. Allow to dry. Do not blow on, waft air over or wipe off the alcohol. Allow to completely air dry. 	<ul style="list-style-type: none"> If patient is allergic to 70% isopropyl alcohol, use double application of 10% povidone iodine. Consult with site medical or microbiologist if patient is allergic to both cleansing agents. 		
7.	<p>Disinfect the site: Select skin antiseptic in use by your institution:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; text-align: center;"> <p>3M SoluPrep* (2% chlorhexidine gluconate with 70% isopropyl alcohol)</p> <ul style="list-style-type: none"> Using a continuous back and forth friction motion move first horizontally across the center of the treatment area to the periphery and repeating the motion over the desired treatment area for 15 seconds. Continuous back and forth motion is then repeated over the same area moving in a vertical direction for an additional 15 seconds </td> <td style="width: 50%; text-align: center;"> <p>10% povidone iodine (PVP or PVP-I)</p> <ul style="list-style-type: none"> Disinfect the site with 10% povidone iodine using a circular motion for a minimum of 30 seconds Start at the center of the cleansed site moving to the periphery (5 cm diameter) Do not return the swab or wipe to the center of the site </td> </tr> </table> <p>*For those institutions where 3M Soluprep is not available follow the manufacturer's instructions for the brand of 2% chlorhexidine gluconate with 70% isopropyl alcohol utilized.</p> <ul style="list-style-type: none"> Allow to dry, preferably for 2 minutes, minimum dry time is 1 minute Do not touch the venipuncture site after preparation and prior to phlebotomy. 	<p>3M SoluPrep* (2% chlorhexidine gluconate with 70% isopropyl alcohol)</p> <ul style="list-style-type: none"> Using a continuous back and forth friction motion move first horizontally across the center of the treatment area to the periphery and repeating the motion over the desired treatment area for 15 seconds. Continuous back and forth motion is then repeated over the same area moving in a vertical direction for an additional 15 seconds 	<p>10% povidone iodine (PVP or PVP-I)</p> <ul style="list-style-type: none"> Disinfect the site with 10% povidone iodine using a circular motion for a minimum of 30 seconds Start at the center of the cleansed site moving to the periphery (5 cm diameter) Do not return the swab or wipe to the center of the site 	<ul style="list-style-type: none"> 2% chlorhexidine gluconate with 70% isopropyl alcohol is the preferred antiseptic for skin disinfection. Refer to local practices/guidelines for antiseptic use in neonates If patient is allergic to iodine, use a double application of 70% isopropyl alcohol. Allow to dry.  <ul style="list-style-type: none"> Do not re-palpate the site again to avoid transferring microorganisms to the venipuncture site. If palpation is required either repeat the disinfection process or don a sterile glove if trained in aseptic technique.
<p>3M SoluPrep* (2% chlorhexidine gluconate with 70% isopropyl alcohol)</p> <ul style="list-style-type: none"> Using a continuous back and forth friction motion move first horizontally across the center of the treatment area to the periphery and repeating the motion over the desired treatment area for 15 seconds. Continuous back and forth motion is then repeated over the same area moving in a vertical direction for an additional 15 seconds 	<p>10% povidone iodine (PVP or PVP-I)</p> <ul style="list-style-type: none"> Disinfect the site with 10% povidone iodine using a circular motion for a minimum of 30 seconds Start at the center of the cleansed site moving to the periphery (5 cm diameter) Do not return the swab or wipe to the center of the site 			
8.	Remove the cap and disinfect the septum (rubber stopper) with a 70% isopropyl alcohol swab/ wipe and allow to dry for 1 minute.	<ul style="list-style-type: none"> DO NOT use iodine as it can damage the septum 		
9.	<p>Review Order of Draw:</p> <ul style="list-style-type: none"> Collect blood cultures first starting with the aerobic bottle or pediatric followed by the anaerobic bottle from the first site. 	<ul style="list-style-type: none"> <i>Blood specimens should be drawn according to approved Order of Draw and Order of Transfer (PA03-005).</i> Other blood work (e.g. Chemistry, Hematology, etc.) can be drawn from the first site after the blood culture bottles have been collected. 		

STEP	ACTION	NOTES				
10.	<p>Winged collection set (e.g. Butterfly needle) peripheral venipuncture procedure is the preferred method except for neonatal patients. Healthcare providers (non-lab) should follow site specific nursing procedures when collecting from neonatal patients.</p> <ul style="list-style-type: none"> Remove winged collection set and tubing from the package. Do not touch the rubber cover to prevent contamination. Attach winged collection set to vacutainer holder or blood collection adaptor as shown: <table border="1" data-bbox="207 451 1511 978"> <thead> <tr> <th data-bbox="207 451 857 485">BD BACTEC™</th> <th data-bbox="870 451 1511 485">Biomerieux BacT/ALERT®</th> </tr> </thead> <tbody> <tr> <td data-bbox="207 485 857 978"> <p>Vacutainer holder</p>  </td> <td data-bbox="870 485 1511 978"> <p>BacT/ALERT® Blood Collection Adapter Cap</p>  <p>Source image courtesy of Biomerieux, Inc., Recommendations for Blood Culture Collection PRN 16-0390-00/MK</p> </td> </tr> </tbody> </table> <ul style="list-style-type: none"> Place the vacutainer holder (BD BACTEC™) or adaptor (BacT/ALERT®) over the blood culture bottle. Holding the bottle upright, press straight down to pierce the septum. The needle and vacutainer holder must be held down to keep the needle from popping out of the bottle. Keep the bottle upright during collection in order to monitor the fill line; and collect desired volume of blood. DO NOT allow the bottle contents to touch the stopper or the end of the needle during collection. DO NOT collect all 4 bottles from the same venipuncture. Remove the bottle when the blood flow has reached the FILL line as indicated in step 7. Ensure bottles are not underfilled or overfilled. This is important to ensure appropriate ratio of blood to culture media for optimal recovery and detection of pathogenic bacteria. <p>Collection using a syringe:</p> <ul style="list-style-type: none"> Collections using syringes are not recommended due to risk of needle stick injury. If a syringe collection is required: <ul style="list-style-type: none"> Following institution specific procedures draw appropriate volume of blood as indicated in Table 1. Immediately discard needle into approved biohazard sharps container. Transfer specimen to the culture bottle using needleless transfer device (connector). Transfer the blood into the appropriate blood culture bottles following the <i>approved Order of Draw and Order of Transfer (PA03-005)</i> Be sure to transfer the correct volume into each bottle. <p>Collection from Intravascular Catheters (Non-Lab Healthcare Providers only):</p> <ul style="list-style-type: none"> The patient care unit must collect one set from the line and may request the lab to draw the other set by venipuncture. Never directly attach the blood culture bottle to a vascular access device due to risk of reflux of the broth media back flowing into the patient's due to the short distance and potential for incorrect volume collected. Follow site specific nursing procedures for indwelling device collections. 	BD BACTEC™	Biomerieux BacT/ALERT®	<p>Vacutainer holder</p> 	<p>BacT/ALERT® Blood Collection Adapter Cap</p>  <p>Source image courtesy of Biomerieux, Inc., Recommendations for Blood Culture Collection PRN 16-0390-00/MK</p>	
BD BACTEC™	Biomerieux BacT/ALERT®					
<p>Vacutainer holder</p> 	<p>BacT/ALERT® Blood Collection Adapter Cap</p>  <p>Source image courtesy of Biomerieux, Inc., Recommendations for Blood Culture Collection PRN 16-0390-00/MK</p>					

STEP	ACTION	NOTES
11.	Collect blood culture from a second site, selecting a separate venipuncture site from the other arm. Start with the aerobic bottle or pediatric followed by the anaerobic bottle.	<ul style="list-style-type: none"> If only one arm is available, a different location on the same arm may be used for the second site draw. If a different location on the same arm is not possible then use of same vein is acceptable.
12.	Gently mix bottles by inversion 8 to 10 times to prevent clotting.	
13.	Remove residual iodine/CHG from skin with 70% isopropanol after venipuncture	If the patient is allergic to 70% isopropyl alcohol, use soap and water to remove iodine residue.

Labelling the Blood Culture Bottles (at patient bedside)

- Label the blood culture bottle(s) with the patient **full first and last name, date and time of collection, Provincial Health Number (PHN/ULI)**, or Government issued identification such as Federal, Military, RCMP, or Immigration.
 - If patient label(s) has been provided then apply the label **vertically** to the outside of the container. **DO NOT** place a label on the bottom of the bottle.
 - DO NOT OBSCURE THE BARCODES.** Do not obscure the adjacent sequence number on the original BD BACTEC™ bottle label or the the optical volume read window on the BacT/ALERT® bottle.
- Notes:** For additional information refer to [Filling and Labelling Biorerieux BacT/ALERT® Blood Culture Bottles MI6644](#).

Lab Test Request

- For each blood culture set collected, the following must be indicated on the paper requisition or in the electronic ordering system:
 - site(s) used
 - date and time of collection
- Indicate on the primary patient label in the empty area after the patient name the site location (left arm, right hand, line type, etc.)
- Place each blood culture set collected into a separate plastic resealable specimen bag. If submitting test requests on paper requisitions fold and place the requisition in the **outside** pocket of the plastic bag.

Delivery to Lab

- Blood culture specimens must be sent to the laboratory immediately after collection and should be received within 12 hours.
- Transport at room temperature. **DO NOT** refrigerate or incubate.
- Bottles transported through the pneumatic tube system should be packaged with extra padding to ensure bottles are not damaged from impacts inside the carrier.
- If you have any questions regarding collection consult your local laboratory or page the Microbiologist or Pathologist on-call for your site.

References

1. Leber, et al. Clinical Microbiology Procedures Handbook, 4th ed. May 2016.
2. Miller, et al. A Guide to Utilization of the Microbiology Laboratory for the Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology. Clin Infect Dis 2018; 67(6):e1-94.
3. Strand, et al. JAMA 1993. 269(8):1004-6.
4. Mimoz, et al. Ann Intern Med. 1999. 131(11):834-7 5. Little, et al. Am J Med.1999. 107(2):119-25.
5. Clinical Laboratory Standards Institute (CLSI). Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, 7th ed. Approved Standard GP41. Wayne, Pennsylvania: Clinical Laboratory Standard Institute, April 2017.
6. BioMerieux, Inc. BacT/ALERT FA Package Insert. Publication 043784-03 2020-02.
7. BioMerieux, Inc. BacT/ALERT FN Package Insert. Publication 043785-01 2020-08.
8. Clinical Laboratory Standards Institute (CLSI) Principles and Procedures for Blood Cultures, 2nd ed. Guideline M47. Wayne, Pennsylvania: Clinical Laboratory Standard Institute, May 2022.
9. Howie, SRC. Blood sample volumes in child health research: review of safe limits. *Bull World Health Organ* 2011; 89:46–53. doi:10.2471/BLT.10.080010.