

Sanford Policy ENTERPRISE Laboratory Phlebotomy/Processing:	Blood Culture Collection - Enterprise
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DATE REVIEWED/REVISED: 04/01/2026	FORMULATED BY: SENIOR LABORATORY QUALITY SPECIALIST

SCOPE:

Enterprise – All employees, all entities

PURPOSE:

To define a procedure for the proper collection of blood culture specimens.

PRINCIPLE:

Blood cultures are often collected from patients who have fevers of unknown origin (FUO), a sudden, relative increase in the pulse rate, chills, prostration, and hypotension. Blood cultures are preferably drawn prior to antibiotic therapy. Proper collection technique during phlebotomy is vital to successfully detecting septicemias. Deviating from proper technique may result in contaminated specimens and cause a delay in proper diagnosis as well as increase cost to the medical facility.

MATERIALS:

1. Tourniquet (latex-free)
2. 1.75mL or 3mL Chloraprep Single Swab Applicator - 2% chlorhexidine-based preparation
NOTE: Chloraprep cannot be used on infants under 2 months of age as it may cause irritation or chemical burns.
3. Betadine preps or 10% providone-iodine swabs, if applicable
4. 70% alcohol wipes
5. Blood culture bottles – Store at 15-30°C in an upright position, protected from light
 - A. BacT/Alert FA Plus aerobic (green, REF #410851) and BacT/Alert FN Plus anaerobic (orange, REF#410852)
 - OR**
 - B. BacT/Alert PF Plus pediatric (yellow, REF#410853)
6. Fungal blood culture tubes
7. Butterfly needle and hub (male or female) or 5, 10 or 20cc syringes
8. Gauze pads
9. Gloves
10. Biohazard transport bags
11. Computer label or requisition
12. Adhesive bandage, Coban, or paper tape

PROCEDURE:

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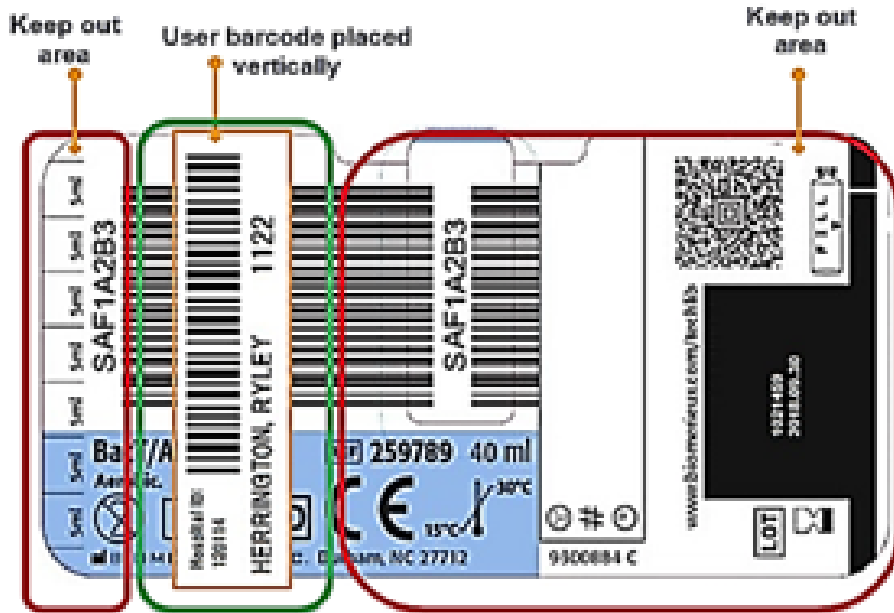
1. Introduce yourself to the patient.
2. Perform hand hygiene per policy [Hand Hygiene- Enterprise](#) and [Standard and Transmission Based Precautions \(Isolation\)- Enterprise](#) for direct patient care and exposure to body fluids.
3. Identify the patient using at least 2 unique identifiers per policy [Patient Identification and Wrist Band Identification- Enterprise](#).
4. Explain the procedure to the patient.
 - A. **Inpatients:** check for the green latex allergy band, red allergy band and any limb alert bands, as applicable.
 - B. **Outpatients:** review allergies and any "FYI Flags" in patient's medical record. These flags are patient-specific and may provide valuable collection information.
5. Apply gloves.
6. Aseptically select and assemble necessary blood culture supplies.
7. Apply tourniquet and select vein. Release tourniquet once vein is selected. Utilize different collection sites for each culture, as applicable. Do not leave tourniquet on for longer than 1 minute.
8. Site preparation:
 - A. **Chloraprep (>2 months old)**
 - 1) Scrub the venipuncture site (approximately three to four square inches) with a Chloraprep single swab applicator.
 - a. Pinch wings once to activate the ampule and release the antiseptic.
 - b. Wet the sponge by pressing and releasing the sponge against the treatment area until liquid is visible on the skin.
 - c. Completely wet the treatment area with antiseptic.
 - d. Use gentle repeated back-and-forth strokes of the swab.
 - e. If the site was dry prior to scrubbing, you must scrub for 30 seconds; if the area was moist prior to scrubbing, it must be scrubbed for 2 minutes.
 - 2) Allow to air dry for 30 seconds if the site was dry before scrubbing and 1 minute if the site was moist before scrubbing.
 - B. **Betadine (≤2 months old)**
 - 1) Cleanse the site for 30 seconds with a 70% alcohol wipe working outward from the venipuncture site in a circular motion and allow to air dry for 30 seconds.
 - 2) Cleanse the site with a 10% providone-iodine swab or betadine prep working outward from the venipuncture site in a circular motion.
 - 3) Cleanse the site with a second, fresh 10% providone-iodine swab or betadine prep working outward from the venipuncture site in a circular motion and allow to air dry for 60 seconds.
 - C. **70% alcohol (Chloraprep/Betadine sensitivity and/or ≤2 months old)**
 - 1) Cleanse the site for 30 seconds with a 70% alcohol wipe and allow to air dry for 30 seconds.
 - 2) Cleanse the site with a second 70% alcohol wipe for 30 seconds and allow to air dry for 30 seconds.
 - 3) Patients under 2 months of age: cleanse the site a third time with a 70% alcohol wipe as described above.
 - D. **DO NOT BLOT OR FAN. DO NOT REPALPATE** the vein after cleansing and disinfection has occurred.
 - E. Perform venipuncture.
9. Collection and Inoculation

- A. The preferred method for collection is a butterfly and male or female hub. A syringe method may also be used. If the first attempt is unsuccessful and a second venipuncture is attempted, the area must be re-cleaned and a new needle must be used. Every effort should be made to obtain optimal blood volumes.
- B. Obtaining blood cultures from peripheral catheters at insertion or during the dwell (IV starts, PICC lines, VAD lines, etc.) is **NOT** recommended. Venipuncture is the preferred and best practice method to collect blood cultures. A doctor's order is typically required to collect blood cultures from these sites. Refer to [Intravenous Therapy \(IV\)- Enterprise](#) for details.
- C. Blood culture bottles should be first in the order of draw. Refer to the [Laboratory Order of Draw - Enterprise](#) policy.
- D. Prepare bottles for inoculation
 - 1) Inspect bottles to make sure there are no cracks and the sensor at the bottom is grey. Do not use bottles if there are cracks or yellow sensors.
 - 2) Verify the expiration date. If expired, discard and obtain new unexpired bottles and inspect.
 - 3) Remove the plastic flip cap and clean the exposed rubber septum with 70% isopropyl alcohol. Fungus tubes do not have a plastic cap but the top of the tube must be cleaned prior to inoculation with 70% isopropyl alcohol. Scrub tops of bottles and/or fungus tube vigorously. Allow to air dry for 30 seconds.
- E. Butterfly and hub/transfer device method
 - 1) Reapply tourniquet.
 - 2) Open butterfly package and connect the hub/transfer device to the connector of the butterfly collection set.
 - 3) Perform venipuncture.
 - 4) Using a butterfly and hub or transfer device, inoculate the bottles with the appropriate volume of blood (see [Bottle Filling Requirements](#)). The aerobic bottle should be filled first due to air present in the tubing.
 - 5) Release tourniquet.
- F. Syringe (with butterfly and transfer device) method
 - 1) Reapply tourniquet
 - a. Use a single syringe large enough to draw an appropriate amount of blood (see [Bottle Filling Requirements](#))
 - 2) Open the syringe, butterfly package and transfer device packages and connect the syringe to the connector of the butterfly set.
 - 3) Perform venipuncture
 - 4) Withdraw the required amount of blood (see [Bottle Filling Requirements](#)).
 - 5) After deploying the safety sheath for the butterfly needle, remove butterfly from the syringe; attach the transfer device and inoculate the bottles using the syringe markings as a guide.
 - a. Fill the anaerobic bottle first. The aerobic bottle should be filled second due to air at the seal of the syringe.
 - 6) Release tourniquet.
- G. Fungus tubes should be inoculated after the anaerobe and aerobe bottles.
- H. Once all bottles/tubes are inoculated, mix by gentle inversion 3-5 times.
- I. The provider will order the designated number of cultures to be drawn and the time interval in which to draw them. If no time is specified, it is acceptable to collect the

second set immediately after the first, providing a separate venipuncture is performed for each set.

10. Labeling

- A. Bottles should be properly labeled with the collection site and type, collector's initials, and time (and/or documented in the LIS).
- B. When the label is attached to the bottle, the bar code label on the bottle should NOT be covered.



11. Collection Time

- A. Scan to record the collection time for the first set of cultures on the Rover after completing the draw, if applicable. If the laboratory does not utilize Rover, Receive specimen into the laboratory and enter collection date, time, and collector.
- B. If there are two sets ordered, skip the collection activity for one set to properly document the collection time.
- C. If collecting multiple sets of cultures, collection times should be different for each set. If a second set of cultures is ordered, select a second site and repeat the steps, starting in Procedure, Section 6.

12. Transport

- A. Culture bottles and tubes are transported to the lab immediately. If sent by a pneumatic tube, bottles must be placed in a sealed biohazard bag and stabilized with foam inserts. A maximum of two sets with two bottles each are transported at one time.
- B. Transport from off-site locations occurs at room temperature ASAP.

13. Bottle Filling Requirements:

Recommended (IDEAL) Volumes**For Pediatric and NICU patients:**

Health Care Providers should consider the **maximum safe volume** that can be drawn from each patient on a case-by-case basis. For babies and children, the **blood volume drawn for culture should not exceed 1% of the patient's blood volume**³. Keeping in mind that studies have indicated that the volume of blood collected is related to the pathogen yield. So, the more blood that is tested the higher the rate of identifying the infection.

The blood volumes of children change with age. They initially range from 76 to 86 mL/kg at birth and decrease to the adult value of 70 mL/kg by 15 years of age. Therefore, until additional clinical data are available, a recommendation of obtaining no more than 1% of a child or adolescent's total blood volume per collection makes physiological sense.³

Weight of Patient (kg)	Culture Set #1	Culture Set #2	Total Volume for Culture	Bottle(s)	Allocation
≤ 1	0.7mL	NA	1% of patient's total blood volume	Peds bottles; yellow cap (BacT/Alert PF or PF Plus)	Entire amount into 1 peds bottle
1.1 – 2	0.7mL	0.7mL	1% of patient's total blood volume	Peds bottles; yellow cap (BacT/Alert PF or PF Plus)	Entire amount into 1 peds bottle for each culture
2.1 - 6.0	2mL	2mL	4mL	Peds bottles; yellow cap (BacT/Alert PF or PF Plus)	Entire amount into 1 peds bottle for each culture
6.1 – 12.7	3mL	3mL	6mL	Peds bottles; yellow cap (BacT/Alert PF or PF Plus)	Entire amount into 1 peds bottle for each culture
12.8 – 36.3	10mL	10mL	20mL	Aerobic bottle; green cap (BacT/Alert FA or FA Plus)	Entire amount into 1 aerobic bottle for each culture. DO NOT exceed 10mL per bottle.
> 36.3 & (adults)	16-20mL	16-20mL	32-40mL	Aerobic; green cap (BacT/Alert FA or FA Plus) and anaerobic; orange cap (BacT/Alert SN or FN Plus)	Equal Split; 8-10mL into each bottle type, for each culture. DO NOT exceed 10mL per bottle.

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Less Than Ideal Volume Collected (Irrespective of Patient Weight)

Total Volume Collected	Bottle(s)	Allocation
0.5 – 2mL (≤ 18 years)	Peds bottles; yellow cap (BacT/Alert PF or PF Plus)	Entire amount into 1 peds bottle (yellow cap)
0.5 – 10mL (≥19 years)	Aerobic bottle; green cap (BacT/Alert FA or FA Plus)	Entire amount into 1 aerobic bottle (green cap)
11 – 15mL	Aerobic; green cap (BacT/Alert FA or FA PLUS) AND anaerobic; orange cap (BacT/Alert SN or FN PLUS)	10mL into 1 aerobic bottle (green cap), remainder into anaerobic bottle (orange cap). DO NOT exceed 10mL per bottle.

Fungal Blood Culture Recommended (IDEAL) Volumes

Patient	Collection Tube	Recommended Volume	Minimum Volume
Pediatric	Lithium or Sodium Heparin (no gel)	4.0mL	0.5mL Note: NICU acceptable volume 0.4mL
Adult	SPS (Sodium Polyanethol Sulfate)	8.3mL	4.0mL

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A “set” of blood cultures consists of an anaerobic and an aerobic bottle.

It is best practice to fill each bottle with 8-10mL (16-20mL total volume per set) to get the best microorganism growth and prevent contamination. Only when circumstances do not allow for optimal blood volume collection is it acceptable to place less than optimal volumes in blood culture bottles/tubes.

The vacuum in the bottle will usually exceed 10mL; monitor the volume collected by means of the 5mL incremental markings on the bottle label.

LIMITATIONS:

1. Chloraprep cannot be used for:
 - A. Patients under 2 months of age.
 - B. Patients with an allergy to isopropyl alcohol.
 - C. Patients also being drawn for a blood alcohol level.

REFERENCES:

1. bioMérieux BACT/ALERT FA Plus, IFU, 2-2022.
2. Chloraprep One-Step 2% w/v chlorhexidine gluconate (CHG) and 70%v/v isopropyl alcohol (IPA), IFU.
3. CLSI. *Principles and Procedures for Blood Cultures*. 2nd ed. CLSI guideline M47. Clinical and Laboratory Standards Institute; 2022.
4. Sanford Laboratories Test Catalog

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