

PROCEDURE: POSITIVE BLOOD CULTURES – GRAM STAIN PROCEDURE

I. PRINCIPLE

Blood cultures are one of the most important cultures performed by the laboratory. Diagnosis and interpretation of bacteremia and fungemia depends on appropriate volume per blood culture and number of venipunctures drawn per episode. Automated, continuously monitoring blood culture methods will vary depending on instrumentation. However, all systems are highly sensitive and detect organism growth for almost all organisms within 5 days. Both culture procedures and interpretation of significance of an organism must be carefully controlled to avoid misinterpretation of skin or contaminating flora vs. an agent of infection.

II. SPECIMENS

- A. Blood
 - 1. Blood may be peripheral, arterial, or drawn through a CVL.
 - a) Adults: 10-20 ml of blood per blood culture set (Aerobic/Anaerobic)
 - (1) Blood volume should be evenly divided between 2 bottles per set; 1 aerobic and 1 anaerobic formulation
 - b) Pediatric: 1-5 ml of blood per blood culture set (Pediatric)
 - 2. Autopsy bloods are usually heart blood.
- B. Body fluids in blood culture bottles
- C. Dialysate cultures
- D. Blood Transfusion Bag – Refer to [Procedure: Transfusion Reaction Cultures](#)
- E. AFB & Fungal Blood Isolators – Refer to [Procedure: Isolator™ 10, Isolator™ 1.5 – Blood](#)


III. PROCEDURE

- A. When a Blood culture is flagged as positive on the automated blood culture instrument, document bottle information on “Positive Blood Culture Log”
- B. In LIS, enter order number and check to see if bottle already has a positive counterpart (mate). A counterpart is defined as the aerobic or anaerobic bottle of same order.
 - 1. If new positive, add ^BAP-S, ^BRU-S, and ^\$GS media.
 - 2. If mate (previously positive counterpart), add ^BAP-S, ^BRU-S, and second pos bottle ^GS.
 - 3. Perform methanol-fixed gram stain and read smear.
 - 4. In LIS, add appropriate media for subculture based on gram stain.
- C. If body fluid in blood culture bottle, first compare gram stain of blood culture bottles to gram stain report of the original specimen.

IV. REPORTING RESULTS

- A. “No Growth” Cultures—Preliminaries and Finals will be updated daily by auto resulting worklists.
- B. **Positive Blood Culture:**
 - 1. Organisms Present – First bottle positive of a set
 - a) Positive blood culture gram stains will be reported as a Test Comment
 - b) Using available keypad options report the following:

Gram stain of blood culture medium shows:
[Enter gram stain result]

Gram stain result called to and readback by:
[Document call according to Procedure: Critical Results Notification]
 - c) Set the Significant flag , then set Status to INTERIM. This sets the report in red on LifeChart

2. Organisms Present – Mate

- a) If culture is in current Interim status and gram stain is same as previous bottle, only additional media orders will need to be added.
- b) If culture is in current Interim status and gram stains differ, addition of new gram stain result and notification of result must be documented.
- c) Cultures with a previous Final status may remain as final if gram stain matches previously reported.
- d) If Gram stains differ, culture must be taken out of Final status and put back in an Interim status. Documentation and notification of different gram stain result may then occur.

1ST Positive Blood Culture Set:1ST BOTTLE:

1. GRAM STAIN
2. CALL R.N./PHYSICIAN
3. SUBCULTURE
4. DIRECT BIOCHEMICALS/ADDITIONAL TESTING

2nd BOTTLE:

1. GRAM STAIN
2. SUBCULTURE

2ND, 3RD, 4th, etc. Positive Blood Culture Set:1ST BOTTLE:

1. GRAM STAIN
2. CALL R.N./PHYSICIAN (If needed)
3. SUBCULTURE

2ND BOTTLE:

1. GRAM STAIN
2. SUBCULTURE

3. The verbal notification must indicate # of positive sets, the total sets drawn, and the gram morphology. Refer to [Procedure: Critical Results Notification](#) for additional guidance.

Example:

"1 out of 2 sets drawn on Wednesday (date) positive with gram stain showing GNRs"

C. **Positive Body fluids in blood culture bottles/ Dialysate cultures:**

1. Compare gram stain of body fluid in blood culture bottles to gram stain of the original specimen (if available):
 - a) Original gram stain **positive**:
 - (1) Gram stain of blood culture bottles shows same morphology
 - (a) No additional work up needed, give bottles to appropriate bench
 - (2) Gram stain of blood culture bottles shows *different* morphology
 - (a) Subculture according to subculture protocol
 - (b) Document morphology in the test comment of the culture using }GSBR comment: "Gram stain of broth culture shows" and }RSIP comment: "Restreak of blood culture in progress"
 - (c) Give bottles to appropriate bench
 - b) Original gram stain **negative**:
 - (1) Subculture according to subculture protocol
 - (2) Document morphology in the test comment of the culture using }GSBR comment: "Gram stain of broth culture shows" and }RSIP comment: "Restreak of broth culture in progress"
 - (3) Give bottles to appropriate bench
2. Refer to [Procedure: Critical Results Notification](#) for reporting new or different gram stain morphologies

D. IF GRAM STAIN IS NEGATIVE/ NO ORGANISMS SEEN:

1. Repeat using an air-dried Methanol-fixed method or heat fixed method (no methanol).
2. Sub according to protocol and put back on instrument within 3 hours.
3. Return bottle to instrument and in Soft unset the Significant button by clicking on it twice.
4. The red ✓ in the “ + “ column should appear and then disappear along with the “+” in the Status field. It will now qualify again for Negative Auto resulting Worklist.
5. If Blood bottle flags as positive a 2nd time and gram stain is still ‘No organisms seen’, perform an acridine orange stain.
 - a) If acridine orange stain is negative, do not load bottle back on instrument. Subculture appropriately and place bottle in incubator on blood shelf for remainder of incubation.
 - b) If acridine orange is positive, review/repeat gram stain of bottle. If unable to locate organisms consult with a Sr. tech.

V. INTERPRETATION

- A. A positive blood culture generally means that a person is bacteremic. All positive blood cultures should be plated to appropriate media for work-up.

VI. LIMITATIONS

- A. Low level of organisms may not be detected at all times.
- B. There are fastidious microorganisms that infect the blood that cannot be grown in routine culture of blood.
- C. Some bacteria do not produce enough CO₂ gas for detection in automated systems.

VII. REFERENCES

- A. Leber, Amy L., Clinical Microbiology Procedures Handbook, 2016. 4th edition. Volume 1, Aerobic Bacteriology.
- B. Blood Culture IV, Cumitech 1C.EJ Baron coordinating editor, 2003. ASM Press.

VIII. REVISIONS

- A. 1/17/2020 – Updated subculture protocol for GNR and added referral to procedure for notification guidelines.
- B. 1/20/2022 – Updated subculture protocol to include pediatric bottles and added work-up protocol for body fluids collected in blood culture bottles.
- C. 7/27/2022 – Updated subculture protocol and reflex testing requirements for yeast

POSITIVE BLOOD CULTURE - SUBCULTURE PROTOCOL

**EACH MEDIA PLATE MUST INCLUDE DATE OF SUBCULTURE AND BOTTLE TYPE
BOTTLE TYPE MUST ALSO BE NOTED ON GRAM STAIN SLIDE
A=AEROBIC N=ANAEROBIC P=PEDIATRIC**

Gram Stain:	Subculture to:	Set up direct from bottle:
GNR	BAP W/SS BRUC MAC CNA CHOC (if GNCB) -TAPE PLATES CLOSED, POSSIBLE BT	*Pheno ID/AST BC
Curved GNR	Add extra BRUC and CHOC in Campy jar at 37°C in addition to above mentioned media	
STREP	BAP W/SS BRUC	PTAB
STAPH	BAP BRUC	ATAB **MRSA/SA BC PCR
GPR	BAP BRUC	
YEAST	BAP SAB IMA (If GS mixed w/ bacteria)	***FP Panel
MIXED	BAP W/SS BRUC MAC CNA CHOC (if GNCB) -TAPE PLATES CLOSED, POSSIBLE BT	
NO ORGANISMS SEEN	BAP W/SS CHOC BRUC	Acridine Orange if 2 nd time NOS
FUNGAL ELEMENTS	BAP BRUC SAB IMA (If GS mixed w/ bacteria)	
POS CRYPTO AG	Put tape on bottles indicating they should be given to Mycology on Day 5	Mycology tech sub to SAB and IMA

- * Refer to [Procedure: Rapid identification and sensitivities directly from blood culture using Accelerate Pheno System](#) for acceptable testing requirements.
- ** Refer to [Procedure: Xpert MRSA/SA Blood Culture](#) for acceptable testing requirements.
- *** Refer to [Procedure: Genmark ePlex FPP Procedure](#)