PROCEDURE: POSITIVE BLOOD CULTURES – GRAM STAIN PROCEDURE

1. **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.

1. **SPECIMENS**
   1. Blood
      1. Blood may be peripheral, arterial, or drawn through a CVL.
      2. Autopsy bloods are usually heart blood.
      3. Blood Transfusion Bag – Refer to *Procedure: Transfusion Reaction Cultures*
      4. Recommended volumes:
         1. Adults: 10-20 ml of blood per blood culture set
         2. Children: 1-5 ml of blood per blood culture set
      5. Blood volume should be evenly divided between 2 bottles per set; 1 aerobic and 1 anaerobic formulation.
         1. If the volume of blood obtained is less than 2 ml, as in the case of an infant, inject the total volume into a single aerobic bottle.
         2. Note that only one aerobic bottle was received.
      6. The recommended inoculation volume for each bottle is 0.5 ml to 10 ml of blood. A minimum of 0.5 ml of blood is required for the recovery of *Haemophilus influenzae.* The bottle labels display 5 ml sample fill increments. When using a closed collection system, such as a butterfly setup for direct draw into the bottle, observe the blood flow carefully and remove the fill needle from the bottle when the 10-ml mark is reached**.**
      7. **Do not overfill the bottles.**
      8. Vials are inoculated by the person performing the venipuncture at the site of the draw and sent to the lab.
      9. The initials of the person performing the blood culture draw should be on each bottle.
      10. Each set is logged in and assigned one order number.
      11. Notify Director/Manager/tech-in-charge regarding:
          1. Specimens requesting *Brucella* or *Leptospira*
      12. Improperly collected specimens that are not canceled must include the following disclaimer in the report:
          1. Improperly collected-Recovery of organisms will be compromised, recollection of specimen suggested.
2. Body Fluids – Refer to *Appendix AP20 – Body Fluid or Dialysate Requests*
3. AFB & Fungal Blood Isolators – Refer to *Procedure: IsolatorTM 10, IsolatorTM 1.5 – Blood*
4. PROCEDURE
   1. When a Blood culture is positive on the Versatrek, unload the bottle from the instrument
   2. Check off the bottle on the Verstrek PC and hit [*SEND TO LIS*] button. The significant flag in Soft will be set and can be seen in the Status field as “+”. This will prevent the order from getting any new Negative auto results.
   3. Document bottle information on “Positive Blood Culture Log”
   4. 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.
5. **REPORTING RESULTS**
   1. “No Growth” Cultures—Preliminaries and Finals will be updated daily by auto resulting.
   2. Positive Blood Culture:
      1. Organisms Present – First bottle positive of a set
         1. Positive blood culture gram stains will be reported as a Test Comment
         2. 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*]

* + - 1. Set the Significant flag √ , then set Status to INTERIM. This sets the report in red on LifeChart
    1. Organisms Present – Mate
       1. If culture is in current Interim status and gram stain is same as previous bottle, only additional media orders will need to be added.
       2. If culture is in current Interim status and gram stains differ, addition of new gram stain result and notification of result must be documented.
       3. Cultures with a previous Final status may remain as final if gram stain matches previously reported.
       4. 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
3. SUBCULTURE

2ND BOTTLE:

1. GRAM STAIN
2. SUBCULTURE
   * 1. 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"

* 1. 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.
        1. 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.
        2. If acridine orange is positive, review/repeat gram stain of bottle. If unable to locate organisms consult with a Sr. tech.

1. **INTERPRETATION**
   1. A positive blood culture generally means that a person is bacteremic. All positive blood cultures should be plated to appropriate media for work-up.
2. **LIMITATIONS**
   1. Low level of organisms may not be detected at all times.
   2. There are fastidious microorganisms that infect the blood that cannot be grown in routine culture of blood.
   3. Some bacteria do not produce enough CO2 gas for detection in automated systems.
3. **REFERENCES**
   1. Leber, Amy L., Clinical Microbiology Procedures Handbook, 2016. 4th edition.

Volume 1, Aerobic Bacteriology.

* 1. Blood Culture IV, Cumitech 1C.EJ Baron coordinating editor, 2003. ASM Press.

1. **REVISIONS**
   1. January 17, 2020
      1. Updated subculture protocol for GNR
      2. Added referral to procedure for notification guidelines

**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**

|  |  |  |
| --- | --- | --- |
| Gram Stain: | Subculture to: | Set up direct from bottle: |
| GNR | BAP W/SS | \*Pheno ID/AST BC |
|  | BRUC |  |
|  | MAC |  |
|  | CNA |  |
|  | CHOC (if GNCB) |  |
|  | -TAPE PLATES CLOSED, POSSIBLE BT |  |
| Curved GNR | Add extra BRUC and CHOC in Campy jar at 37°C in addition to above mentioned media |  |
| STREP | BAP W/SS | PTAB |
|  | BRUC |  |
| STAPH | BAP | ATAB |
|  | BRUC | \*\*MRSA/SA BC PCR |
| GPR | BAP |  |
|  | BRUC |  |
| YEAST | BAP | \*\*\*PNA Slides |
|  | SAB |  |
|  | IMA (If GS mixed w/ bacteria) |  |
| MIXED | BAP W/SS |  |
|  | BRUC |  |
|  | MAC |  |
|  | CNA |  |
|  | CHOC (if GNCB) |  |
|  | -TAPE PLATES CLOSED, POSSIBLE BT |  |
| NO ORGANISMS SEEN | BAP W/SS | Acridine Orange if |
|  | CHOC | 2nd time NOS |
|  | BRUC |  |
| 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: Detection of MRSA/SA Directly from blood culture using GeneXpert® Dx System for acceptable testing requirements. |
| \*\*\* | Refer to Procedure: PNA FISH Procedure for acceptable testing requirements. |