LABORATORY: Blood Culture
Skills= 22.5 points

Objectives:
At the completion of this activity, the student should be able to:
1. Describe the Gram stain reaction and morphology for each organism observed on the patient slide.
2. Explain the significance of using specific types of media in set-up of positive blood cultures.
3. Identify an unknown organism in a positive blood culture.
4. Call critical results providing pertinent details.

Materials:
Microscope slide(s)
Venting needles
Alcohol pads
Blood agar media (BAP)
Chocolate agar media (CHOC)
MacConkey Media (MAC)
Gram stain materials
Microscope
Immersion Oil
1 Unknown Patient Positive Blood Culture

References:
1. Henry's Clinical Diagnosis and Management by Laboratory Methods, 22nd Edition, 2011. Multiple editors; page 1164

Discussion:
Establishing the presence of bacteria in the blood stream is the definitive confirmation of the diagnosis of sepsis. Since blood is normally sterile and bacterial invasion occurs only during febrile conditions, the isolation and identification of an organism has great diagnostic significance.

Proper collection of blood for culture is essential for proper treatment of the patient.

1. Number and Timing
   Most cases of bacteremia are detected by using three sets of separately collected blood cultures. More than three sets of blood cultures yield little additional information. Conversely, a single blood culture may miss intermittently occurring bacteremia and make it difficult to interpret the clinical significance of certain isolated organisms. Organisms may be held for extended incubation at physician request.

   A. Acute sepsis-
      a. Collect two or three cultures from separately prepared sites prior to starting therapy.
   B. Endocarditis
      a. Acute
         i. Obtain three blood cultures with three separate venipunctures over 1 to 2 hours of evaluation, and begin therapy.
      b. Subacute
         i. Obtain three blood cultures on day 1 (15 min or more apart). If all are negative 24 hours later, obtain three more.
   C. Fever of unknown origin
      Obtain two separate blood cultures initially. If these are negative, obtain two more 24 to 36 hours.

2. Volume of blood
   The volume of blood is critical because the concentration of organisms in most cases of bacteremia is low, especially if the patient is on antimicrobial therapy. In infants and children, the concentration of organisms during bacteremia is higher than in adults, so less blood is required for culture. The recommended volume for adults is 10-30 mL of blood per venipuncture. For children, the recommended volume is 1-5 mL of blood per venipuncture.

3. Additional information can be found in the bacteremia lecture for culture medium consideration and collection guidelines.

Procedure:

1. Procedure for Processing Positive Blood Cultures

1. Each student will be given a blood culture bottle that has been flagged as "positive" by the blood culture instrument. The student will notate the patient's name and source in the report form.
2. The instructor will demonstrate how to "tap" the specimen onto slides and

media using a venting needle directly inserted into the positive blood culture bottle.

3. The student will first prepare a smear and allow it to dry, approximately 10-15 minutes. After the slide has thoroughly dried, the smear should be gram stained.
4. Although the bottle is a patient clinical specimen- only the gram reaction and morphology will be reported. We do not quantitate numbers of bacteria due to the bottles being a broth medium. In addition, no PMNs or RBCs are reported. Indicate the results of your gram stain in the report sheet.
5. Call the critical value to the nurse taking care of the patient. Information given to the nurse should include: patient name and ID, source (RAC/LAC etc.), and blood culture set (1 of 2 etc.). Once the nurse has read back the information, document the call on the report sheet including the nurse's name, the date and time of the call.
6. Use the chart below to select which media to inoculate.
   ***Inoculate only if indicated by the gram stain.**

Bottle Detected Positive	**Initial Media for Bottle Sub**	**Additional Info/Media**
Aerobic Bottle Only or Pedi Bottle	BAP/MAC*/Chocolate	Plates to CO_2 incubator/bag
Anaerobic Bottle Only	BAP sub both Aerobic and Anaerobic /MAC*/Chocolate	Aerobic plates to CO_2 incubator/bag Anaerobic plate to anaerobic bag
Aerobic- and Anaerobic Bottle	Aerobic bottle: BAP/MAC*/Chocolate Anaerobic bottle: BAP	Note when both bottles are positive at the same time, no anaerobic plate sub is needed.

7. After a 24-hour incubation, examine the plates for growth and record results in the report form.
   a. If there is no growth at 24 hours, an initial report of no growth is issued. The plates are held for up to five (5) days before reporting as "No growth 5 days".
   b. If growth is observed, continue identification work-up as necessary.
8. Perform a gram stain on any suspected isolates and continue with organism work-up as indicated.
   a. For gram-positive cocci, follow previous identification methods.
   b. For yeast/fungal elements
      i. Germ tube
         1. Label a 12 x 75 mm test tube
         2. Using a pipette, dispense 3 drops of serum into the tube.
         3. With a wood applicator stick or loop, lightly touch a yeast colony, and then put the stick in the serum. Gently mix.
         4. Incubate the test at 35°C for 2.5- 3 hours.
         5. Place a drop of the suspension on a clean microscope slide

and cover with a cover glass.

6. Examine the suspension under the low-power objective (40x), then switch to high-power (100x) to confirm the presence or absence of germ tubes.
    a. A positive reaction is the presence of a germ tube, which is a short filamentous extension arising laterally from a yeast cell, with no constriction at the point of origin. The germ tube is half the width and 3 to 4 times the length of the yeast cell and there is no presence of nucleus. Report as "positive" in the result form.
    b. A negative reaction is observing no filamentous extension arising from a yeast cell or a short hyphal extension constricted at the point of origin. Reported as "negative" in the result form.

Procedural Notes

Most cultures, if positive, yield a single organism. Polymicrobial cultures are rare and are associated with higher mortality rates.

The growth of microorganisms in a blood culture may be delayed or prevented if an anticoagulant is not used in the culture medium since the organisms may become trapped in the fibrin clot. Antibiotics in the blood may greatly reduce, if not completely eliminate, the chances of obtaining a positive culture.

These obstacles may be overcome by the use of sodium polyethanol sulfonate (SPS), a nontoxic anticoagulant which enhances bacterial growth by obstructing the natural bacterial inhibitors of blood. Since SPS inhibits the activity of streptomycin, polymyxin B, kanamycin and gentamycin, therapy with these antibiotics should not interfere with microbial growth in blood cultures containing this anticoagulant. SPS inhibits the growth of certain mycoplasmas and should not be used for their isolation.

A gram-stained smear from a culture medium may contain small numbers of non-viable organisms derived from medium constituents, staining reagents, immersion oil, glass slides, and specimens used for inoculation. In addition, the patient specimen may contain organisms that will not grow in the culture medium or in media used for subculture.

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Points= 22.5

STUDENT NAME: _____DATE: _____

Unknown #/Patient Name: _____ Source:_____

Bottle Information: Circle One from bottle type and one from bottle number:
Aerobic Anaerobic Pedi Set 1 of 2 Set 2 of 2

Patient Specimen Gram Stain:_____

Called Report:_____

Growth on Culture Media

Date	Media	Amt of Growth	Colony Morphology	Gram Stain- if performed

PRELIMINARY Identification:
_____

Work-Up/Identification Tests

Date	Tests	Results

Final Report:_____