BLOOD CULTURES – GENERAL PROCEDURE

I. Principle
Microorganisms present in the circulating blood are a threat to every organ in the body. This is true even if the organisms are present continuously, intermittently, or transiently in the blood. For this reason, blood cultures are one of the most important specimens submitted to the Microbiology Laboratory for examination. Blood cultures are important in the diagnosis and treatment of many conditions including bacteremia, fungemia, sepsis, infections of native and prosthetic heart valves, suppurrative thrombophlebitis, and infections of vascular grafts. The presence of microorganisms in the patient’s blood can be indicative of septicemia, a serious life threatening disease. Therefore, rapid detection and identification of bloodborne pathogens is very important, as well as notification of the clinician.

Common pathogenic organisms in blood culture sites include coagulase-negative Staphylococcus, Staphylococcus aureus, Streptococcus pneumoniae, Alpha hemolytic Streptococcus viridans group, Enterococcus species, Enterobacteriaceae, Pseudomonas species, Haemophilus influenzae, Bacteriodes fragilis, and Candida albicans. Other bacteria, yeast, molds and mycobacteria have also been associated with blood infections. All organisms are potential pathogens in an immunosuppressed host. Several sources of bacterial sepsis include infective endocarditis, infected arteriovenous fistulas, mycotic aneurysms, suppurrative thrombophlebitis, infected intravenous catheters, infected indwelling arterial catheters, genitourinary tract, respiratory tract, abscesses, surgical wounds, and biliary tract. Understanding the circumstances in which bacteremia and sepsis can occur is helpful in interpreting results of blood cultures.

From adult patients it is recommended that two sets of blood cultures should be collected per febrile episode to help distinguish probable pathogens from possible contaminants, some sources indicate three sets be drawn. A set includes both an aerobic bottle and an anaerobic bottle. Collection of multiple sets of blood cultures is especially useful when determining the significance of coagulase-negative staphylococcus, Diptheroids (Corynebacterium species), Streptococcus viridans group, and Bacillus species. These organisms can potentially be skin contaminants associated with improper collection techniques.

Automated blood cultures machines incubate routine blood cultures for 5 days, this is sufficient time to recover most bacteria while decreasing the isolation of contaminants. Blood cultures that are incubated manually should be held a minimum of 7 days with a series of blind subcultures. Blind subcultures are when you subbed the bottle to appropriate media and perform a gram stain even though there is no visual signs of growth. When an unusual organism is suspected such as Mycobacterium special media is used and extended incubation times are utilized.

II. Specimen Collection, Transport and Handling
A. Indication for drawing blood cultures:
   1. Increased temperature
   2. Chills
   3. Leukocytosis - especially with a left shift
   4. Suspected meningitis
   5. Suspected pneumonia
   6. Fever associated with a heart murmur (suspected endocarditis)
   7. Typhoid fever
   8. Brucellosis

B. Specimen types and collection
   1. Timing of Blood Culture Drawing
      a. Generally blood cultures are drawn as a set (1 aerobic, 1 anaerobic) times 2 or 3 (obtaining more than three blood cultures within 24 hours does not result in a significant increase in positive results)
b. The ideal time to draw a set of blood cultures is one-half hour before an expected chill or temperature spike as the patient’s blood will contain the highest numbers of bacteria at this time. Once the temperature spikes the numbers of bacteria present will rapidly decrease.

c. Ideally blood cultures should be drawn prior to administering antibiotic therapy. If this is not possible, draw blood in bottles containing resin beads that nonspecifically absorb antibiotics allowing for better growth of organisms that may be present.

2. Volume of Blood Culture Drawing
   a. Volume is system specific.
      i. In general, for adults 10-20 ml per draw is recommended.
      ii. For children, since they have a smaller blood volume, 1-5 ml is recommended.
      iii. The optimal ratio of blood to culture medium is 1:5 or 1:10. The dilution aids in negating the bactericidal effect of serum.

   b. Too Little Blood
      i. It will alter the appropriate anticoagulant to blood ratio. Too little blood will not allow for optimal recovery of the organism due to small volume.

   c. Too Much Blood
      i. It will alter the appropriate anticoagulant to blood ratio and there will not be enough nutrients in the system to produce positive results in patients with bacteremias.
      ii. It will result in an increase in the amount of inhibitors present from the blood.

3. Procedure for Drawing Blood Cultures
   a. After locating a vein, cleanse the site with isopropyl alcohol followed by an iodine solution.
   b. Repeat cleansing procedure two more times.
   c. Allow the site to dry completely before drawing the specimen.
   d. Do not palpate the site further after cleansing unless your finger has been cleaned in the same manner. It is important not to introduce skin contaminants into a blood culture as they can make interpretation of results difficult.
   e. When sufficient blood has been collected for culture, introduce blood into appropriate bottles.
   f. Mix the bottles well to prevent clotting of the specimen and transport to the lab immediately.
   g. Do not refrigerate after drawing, hold at room temperature while transporting.
   h. Considerations must be made for those patients allergic to iodine. In those cases cleanse the site with isopropyl alcohol only.

III. Direct Examination
   A. Not routinely performed – gram stain not sensitive enough.

IV. Blood Culture Bottle Systems Setup
   A. Manual (Conventional) Blood Culture Systems
      1. Blood cultures should be incubated at 35ºC and examined visually for evidence of growth (hemolysis, turbidity, gas production, colony formation) for a minimum of 7 days.
      2. Blind subcultures and microscopic examination of the bottles should be performed at 12-24 hours, 48 hours, and some time before the bottles are discarded.
      3. Some systems have paddles of agar incorporated in the lid of the blood culture bottles. Sub-culturing can be accomplished by tipping the broth over the paddles of agar.

   B. Manual Lysis-Centrifugation Blood Culture System (Isolator System)
      1. The Isolator is a special tube that contains saponin, a chemical that lyses cells.
      2. Approximately 7.5 to 10 ml of blood is placed in the tube.
      3. The tube is then centrifuged for 15 minutes at 3000 rpm to concentrate any microorganisms that may be present.
4. After centrifugation, the sediment is sub-cultured to appropriate media.
5. Literature indicates this system increases the yield of fastidious bacteria, yeasts, dimorphic fungi and mycobacteria that are causing systemic infections.

C. Continuous Monitoring Blood Culture Systems
1. BacT/Alert
   a. Detects CO₂ production via a pH sensitive membrane in the bottom of the bottles.
   b. The sensor visibly turns from gray to yellow when CO₂ is present because of the pH change.
   c. A light-sensitive detector in the instrument continually monitors each bottle’s sensor.
2. BACTEC 9000 Series
   a. Detects CO₂ production via a gas-permeable sensor in the bottom of the bottles.
   b. Similar to the BacT/Alert except fluorescent, rather than spectral, light is used to detect increased CO₂ levels.
   c. As CO₂ is produced in each bottle, its sensor emits a fluorescent light that passes an emission filter on the way to a light sensitive diode.
3. VersaTREK
   a. Both gas production and consumption are monitored.
      i. Production of CO₂ and H₂
      ii. Consumption of O₂
   b. Changes in the concentration of CO₂, H₂, and O₂ are monitored via detecting any change in pressure in the headspace of each bottle.

D. Blood Culture Bottle Media
1. The media used in most blood culture systems is multi-purpose and nutritionally enriched.
2. Most commercially available media use the anticoagulant SPS (sodium polyetholysulfonate) in concentrations varying from 0.025% to 0.05%.
   a. SPS also inactivates neutrophils, certain antibiotics (aminoglycosides & polymyxin), and inactivates complement.
   b. SPS may inhibit growth of Peptostreptococcus anaerobius, Neisseria gonorrhoeae, Neisseria meningitidis, and Gardnerella vaginalis.
3. Synthetic antibiotic-removing resin beads can be added to bind antibiotics. The literature is inconclusive as to whether or not these beads are effective.

E. Pathogens commonly isolated in blood.
1. Staphylococcus aureus
2. Coagulase negative Staphylococcus species – the most common cause of SBE associated with prosthetics devices
3. Beta hemolytic strep including Group B Streptococcus - in infants
4. Streptococcus pneumoniae
5. Alpha hemolytic Streptococcus viridans group - the most common cause of SBE
6. Haemophilus influenza
7. Enteric gram-negative rods
8. Pseudomonas species
9. Enterococcus species
10. Yeasts and molds
11. Anaerobes – Bacteroides and Clostridium species
12. Neisseria species

F. Common contaminants for both blood due to improper collection techniques (Normal skin flora)
1. Coagulase negative Staphylococcus species
2. Propionibacterium acnes
3. Alpha hemolytic Streptococcus viridans group
4. Bacillus species
V. Culture Interpretation

A. Reporting Results
1. An initial report is sent out at 24 or 48 hours and the final report is sent out at 5 days for all no growth specimens that are incubated with a continuous monitoring system. It is recommended that cultures not monitored continuously be held for 7 days before a final report is sent out.
2. Cultures requested Brucella species are held for 3 weeks due to the growth rate of these organisms. Brucella can be isolated in as little as 3 days but due to its slow growth rate it is recommended that cultures be held for 21 days.
3. Cultures requested for yeast should be held for 7 days; those for fungus are held for 21-28 days (recommended).

B. Positive Cultures
1. Gram stain the flagged positive bottle to determine the morphology of the organisms present.
   a. Acridine Orange stain (AO) – may be routinely performed in some laboratories on flagged positive cultures when no organisms are seen on gram stain.

2. Call the results of the gram stain to the physician or primary care provider so that appropriate therapy can be initiated if necessary.

3. Subculture to appropriate media based on gram stain results. Incubate subculture plates in appropriate atmosphere.
   a. Always include an anaerobic subculture when indicated.
   b. Include selective media when appropriate (i.e. MacConkey for gram negative rods)
   c. Include direct Coagulase when appropriate (i.e. GPC w/clusters)
      i. Inoculate 100 ul of well mixed blood with coagulase plasma
   d. Include differential discs on media when appropriate for quick identification of organisms. (i.e. GPC in chains)
      i. Optochin disk, Bacitracin disk and/or Vancomycin disk
   e. Perform identification of the organism from the isolation plates the next day.
      i. Perform susceptibility on appropriate isolates from the isolation plates.

4. Isolation of an organism in multiple sets suggests bacteremia or fungemia rather than contamination when isolating common skin contaminants.

5. Report organism identification and sensitivities if appropriate. Clinical significance is dependent on identification of the recovered isolate and the number of positive cultures.

C. Report Results
1. Correlate all information – Probable Contaminant vs. Pathogen
   a. Preliminary report – should include
      i. As much information as possible
      ii. Number of sets positive

   b. Final report – should include:
      i. Organisms identification (if pathogen)
      ii. Organisms susceptibility results (if pathogen and appropriate)
      iii. Number of sets positive
      iv. Information to other blood culture set(s) positive if correspond
2. Example Report:
   a. Probable Contaminant:
      i. Gram Stain:
         GPC in clusters, probable Staphylococcus species (aerobic bottle)
         Results called to Superwoman, RN at 0800 on 5/7/16. Batman, MLS.

      ii. Preliminary:
         Coagulase-negative Staphylococcus species (1 of 3 sets)

      iii. Final:
         Coagulase-negative Staphylococcus species (1 of 3 sets), Probable
         Contaminant: no susceptibility performed. Contact the clinical microbiology
         laboratory if you would like susceptibility testing performed.

   b. Probable Pathogen
      i. Gram Stain:
         GPC in clusters, probable Staphylococcus species (aerobic and anaerobic
         bottles)
         Results called to Spiderman, RN at 0956 on 5/4/12. Batgirl, MLS.

      ii. Preliminary:
         Coagulase-negative Staphylococcus (2 of 2 sets)

      iii. Final:
         *Staphylococcus epidermidis* (2 of 2 sets); MIC results
         See blood culture #1234 for corresponding blood culture set results

V. References
   C. Bailey & Scott’s Diagnostic Microbiology, Forbes, 11th edition, Chapter 55, pages 865-883