WOUND CULTURES – GENERAL PROCEDURE

I. Principle
Wound infections may be caused by one or more organisms. Postoperative wounds are often infected with a mixture of aerobes and anaerobes, while superficial skin wounds are often caused by aerobes only. Deep wound infections such as internal body or organ infections can be caused by one or several aerobes and/or anaerobes.

Wounds, especially postoperative wounds can become colonized with potential pathogens. A gram stain is a useful diagnostic tool in determining colonization versus infection. A gram stain showing few or no polymorphonuclear cells with relatively large amounts of normal skin flora are consistent with colonization. However, wound gram stains showing moderate to many polymorphonuclear cells usually are indicative of infection.

II. Specimen Collection:
A. Specimen types and collection
1. Eye cultures
   a. The specimen of choice is a corneal scraping. Other specimens include: eye swab, conjunctiva swab, sclera, cornea, vitreous fluid, eye fluid, and biopsy.
   b. Corneal scrapings are typically inoculated to the media at the bedside.
   c. Other specimens are received already plated to the media, or are sent as swabs or other transport containers.

2. Ear cultures
   a. The specimen most often received by the lab is an aerobic swab of the outer ear canal to diagnosis skin infection.
   b. To culture fluid from the middle ear, a needle is inserted through the eardrum and fluid is withdrawn by syringe (performed by a physician and called tympanocentesis). The air is then expelled from the syringe, the needle removed and the stopper put in place to prevent oxygen exposure. If transportation time will be prolonged, the specimen should be injected into an anaerobic transport tube, as oxygen will slowly diffuse through a plastic syringe.

3. Wound Cultures: The ideal specimen for wound culture is sterile body fluid, tissue biopsy or aspirate from undrained abscesses. Sterile body fluid, tissue biopsy and aspirated abscess are suitable specimens for both aerobic and anaerobic culture. More commonly the lab receives wound cultures on aerobic and anaerobic swabs because they are more easily collected than an aspirate or a tissue. Swabs are less desirable because of the smaller amount of specimen that is sampled and the fact that they are often contaminated with normal skin flora, making interpretation of results difficult.
   a. Sterile body fluids such as abdominal, amniotic, ascites, bile (liver aspirate), bone marrow, culdocentesis fluid, joint, paracentesis, pericardial, peritoneal, pleural, synovial and thoracentesis should be collected aseptically with a needle and syringe.
      i. If anaerobes are suspected excess air should be expelled from the syringe and a needle cap used to close the syringe. If transportation time will be prolonged, the specimen should be injected into an anaerobic transport system, as oxygen will slowly diffuse through a plastic syringe.
      ii. See Body Fluid and Tissue procedure for specimen processing and work up.
   b. Tissue specimens
      i. Tissue specimens are obtained by surgical or needle biopsy procedures after careful preparation of the skin site.
      ii. A tissue biopsy should be placed in a sterile cup and sent to the lab immediately for processing to prevent drying and oxygen exposure.
      iii. If there will be a delay in transporting the tissue, it should be placed in an anaerobic transport system.
      iv. See Body Fluid and Tissue procedure for specimen processing and work up.
c. Aspirated Abscess
   i. Ideally an aspirate of an undrained abscess should be collected in a sterile syringe – any air bubbles should be expelled and the needle tightly capped to prevent exposure to oxygen.
   ii. If anaerobes are suspected excess air should be expelled from the syringe and a needle cap used to close the syringe. If transportation time will be prolonged, the specimen should be injected into an anaerobic transport tube, as oxygen will slowly diffuse through a plastic syringe.

d. Aerobic Swabs:
   i. Specimens that are not suitable for anaerobes or anaerobes are not suspected are received on aerobic swab only.
   ii. Superficial and skin wounds are often sent on aerobic only swabs.

e. Aerobic and Anaerobic Swabs:
   i. If anaerobes are suspected, both an aerobic and anaerobic swab are collected.
   ii. Closed or open deep wounds or abscess, deep drainages or exudates are often sent on both aerobic and anaerobic swabs.

III. Specimen transport and handling:
   A. Specimen transport should be immediate.
   B. If there will be delay in specimen transport the specimen should be place in transport system to minimize drying and minimize exposure to oxygen if anaerobes are suspected.

IV. Specimen processing:
   A. Sterile Body Fluids and other clear fluids including CSF
      1. Sterile Body Fluids and other clear fluids including CSF greater than 1 ml should be centrifuged 2,500 rpm for 10 minutes to concentrate any organisms present.
      2. Cloudy specimens may not be centrifuged.
      3. The sediment is plated to media and gram stained.
      4. If less < 1 ml of body fluids is received, the specimen should be inoculated directly to the media.

   B. Tissue specimens
      1. Prepare a “touch prep” to preserved characteristic cellular elements and bacterial arrangements.
         a. Mince tissue with sterile scissors or scalpel.
         b. Use sterile forceps to hold pieces of tissue, touch the sides of one or more of the minced fragments to a sterile glass slide.
      2. Tissue specimens should be homogenized with a tissue grinder and small amount of saline. This process should release microorganisms deep within the tissue. The homogenized material should then be inoculated to the media with a sterile swab.
         a. If fungus is suspected it is recommended to mince the large tissue into smaller pieces with a scalpel or sterile scissors. Grinding the tissue can often destroy fungal elements.

   C. Aspirates:
      1. If specimen received in syringe, transfer contents to sterile vial. Vortex specimen if necessary.
      2. A representative area of the specimen (purulent or blood-tinged portions) should be selected using a sterile swab.
      3. Roll swab gently across the gram stain slide to avoid destruction of cellular elements and disruption of bacterial arrangements.
      4. Roll the swab gently across the media to inoculate 1/3 to 1/4 of the plate to make the primary streak.
      5. Alternatively a drop of the specimen can be place on the media using a sterile pipette.

D. Aerobic and anaerobic swabs:
1. Roll swab gently across the gram stain slide to avoid destruction of cellular elements and disruption of bacterial arrangements.
2. Roll the swab gently across the media to inoculate 1/3 to 1/4 of the plate to make the primary streak to the appropriate media (aerobic swab = aerobic media, anaerobic swab = anaerobic media)
3. If only one swab received, roll swab on sterile slide for gram stain, inoculate plates, and place swab in broth last.

V. Direct Examination
A. Gram stain
1. Report gram stain results as part of culture

VI. Culture Setup
A. Examples of media to inoculate: non inhibitory media should always be inoculated first
1. Wound specimens collected on aerobic and anaerobic swabs should be plated to the following media:
   a. Sheep blood agar (use aerobic swab)
   b. MacConkey agar (use aerobic swab)
   c. Chocolate agar (use aerobic swab)
   d. Kanamycin, Vancomycin (KV) agar (use anaerobic swab)
   e. Supplemented anaerobic blood agar (use anaerobic swab)
   f. Ana PEA (use anaerobic swab)
   g. Thioglycollate broth (use anaerobic swab)
2. Specimens collected on only an aerobic swab should be plated to the following media:
   a. Sheep blood agar
   b. MacConkey agar
   c. Chocolate
   d. PEA
   e. Thioglycollate broth
3. Tissue specimens (after it has been ground) should be plated to the following media:
   a. Sheep blood agar
   b. Supplemented anaerobic blood agar – for anaerobes
   c. MacConkey
   d. Chocolate
   e. Thioglycollate

B. Incubate media
1. Temperature: 35ºC
2. Atmosphere:
   a. BAP, CHOC, PEA - CO₂, MAC, THIO - ambient air,
   b. Supplemented anaerobic blood agar, KV agar, and Ana PEA - anaerobically
3. Time:
   a. Aerobically incubated media: 18-24 hours before 1st read, total 48 hours incubation or until identification is complete
   b. Anaerobically incubated media: 48 hours before 1st read unless anaerobic chamber is used, total 72 hours or until identification is complete
   c. Thioglycollate broth: typically 5 to 7 days or longer if serious infection suspected (2 weeks)
C. Pathogens

1. Eye
   a. \textit{Staphylococcus aureus}
   b. \textit{Haemophilus} species
   c. \textit{Streptococcus pneumoniae}
   d. Beta hemolytic \textit{Streptococcus} species
   e. \textit{Neisseria gonorrhoeae}
   f. Enteric gram-negative rods
   g. \textit{Pseudomonas} species
   h. \textit{Moraxella lacunata}
   i. \textit{Moraxella (Branhamella) catarrhalis}
   j. Fungi

2. Ear
   a. \textit{Staphylococcus aureus}
   b. \textit{Streptococcus pneumoniae}
   c. Beta hemolytic \textit{Streptococcus} species
   d. \textit{Haemophilus} species
   e. \textit{Pseudomonas} species
   f. \textit{Proteus} species and other enteric gram-negative rods
   g. \textit{Moraxella (Branhamella) catarrhalis}
   h. Anaerobes

3. Wound
   a. \textit{Staphylococcus aureus}
   b. Group A \textit{Streptococcus}
   c. Enteric gram-negative rods
   d. Group D \textit{Streptococcus}
   e. \textit{Pseudomonas} species
   f. Anaerobes
   g. \textit{Enterococcus} species

D. Normal Flora

1. Normal Eye Flora
   a. Coagulase negative \textit{Staphylococcus} species
   b. Non-hemolytic and alpha hemolytic streptococci
   c. Diphtheroids
   d. \textit{Propionibacterium} species

2. Normal Ear Flora
   a. Same as the eye

3. Normal Skin Flora
   a. \textit{Staphylococcus} species and \textit{Micrococcus} species
   b. Diphtheroids
   c. \textit{Propionibacterium} species
   d. \textit{Peptostreptococcus} species
   e. \textit{Bacillus} species
   f. \textit{Streptococcus} species viridans group
   g. Yeast

4. Contaminants: Normal skin flora organisms can contaminate cultures during the specimen processing step if sterile technique is not followed.
VII. Culture Interpretation
A. See Sterile Body Fluid and Tissue procedure for culture interpretation of Sterile Body Fluid and Tissue specimens.

B. Quantitation of growth:
1. Guidelines for estimating amount of growth of an individual organism:
   a. rare - growth present in first quadrant only (<15 colonies)
   b. few - growth present in quadrants 1 and 2 (>15 colonies in quadrant 1 and <15 colonies in 2nd quadrant)
   c. moderate - growth in quadrants 1, 2, and 3 (>15 colonies in 2nd quadrant and <15 colonies in quadrant 3)
   d. many - confluent growth, extending to all 4 quadrants.

C. For non-sterile wound (body sites that may contain normal flora) cultures, aerobic plates are held for a minimum of 2 days. Observe all aerobic plates at 1 day and 2 days post inoculation for potential pathogens.
   1. The following is list of general guidelines to follow when interpreting wound cultures: For all isolates, identify to the appropriate level per source.
      a. Moderate to many WBC seen on specimen gram stain
         i. Potential aerobic pathogens are work up in any amounts
            1. Perform and report identification and appropriate susceptibilities
            2. For example, if many Group A streptococcus is isolated from a foot wound susceptibilities are not performed because beta-hemolytic streptococcus is still know to be sensitive to penicillin.
         b. No to few amounts of WBC seen on specimen gram stain
            i. Potential aerobic pathogens in predominant amounts are worked up
               1. Typically three pathogens are worked up and the rest are characterized in the report. For example, Few lactose fermenting gram negative rods.
               2. Perform and report identification and appropriate susceptibilities
            ii. Potential aerobic pathogens not in predominant amounts are characterized in the final report. Susceptibilities and full identification are not performed.
      c. Normal skin flora:
         i. Normal skin flora is identified to the level to rule out potential pathogens. Sensitivities are not performed.
         ii. Normal skin flora is then characterized or reported as Normal skin flora in the final report.
            1. Examples:
               Many *Escherichia coli*
               Few Normal Skin Flora
               Or
               Many *Escherichia coli*
               Rare diptheroids
               Few coagulase negative staphylococcus
      d. Specimen gram stains with squamous epithelial cells presents, suggest skin contamination during the collection process.

D. For non-sterile wound (body sites that may contain normal flora) cultures, anaerobic plates are held for a minimum of 3 days. Observe plates on 2 day and 3 day post inoculation for potential pathogens. If an anaerobic chamber is utilized, plates can be examined at 1 day post inoculation.
   a. If cultures are no growth, reincubate for 1 to 2 days, minimum of 3 days.
   b. At 2 days, anaerobic culture plates should be inspected for colony types that do not appear on the aerobic plates. Indications that anaerobes are present include the following:
      i. Colony morphotypes present anaerobically, but not present on aerobic culture plates
      ii. Selective anaerobic media supporting growth
      iii. Foul odor upon opening anaerobic jar or chamber
      iv. Characteristic anaerobic morphologies (molar tooth)
c. For colonies suspected of being anaerobes:
   i. As part of the preliminary identification of the anaerobes, describe the colony morphology.
   ii. Check aerotolerance (Mahon, page 615, Table 23-18)
      1. Subculture a colony to CHOC (incubate in CO2), and AnaBAP (incubate in anaerobic conditions).
      2. If colony growth on AnaBAP and not on CHOC it is determined to be an anaerobe.
   iii. Once it is proven that an anaerobe is isolated, identifications can be performed.
      1. Non-sterile wound cultures
         a. Mixed aerobic cultures
            i. When 1 or 2 anaerobes are isolated with mixed aerobic cultures the anaerobes can be characterized.
               1. Example: Few anaerobic gram negative rods
            ii. If it is determined that 3 or more anaerobic organisms are isolated form anaerobic cultures the organisms can be reported as mixed anaerobic flora.
               1. Example: Few mixed anaerobic flora
         b. If it is determined that the 3 or more anaerobic organisms are isolated from anaerobic cultures with no aerobes isolated the organisms can be reported as mixed anaerobic flora.
            i. Example: Few mixed anaerobic flora
      c. Pure culture
         i. Full identifications should be performed
         ii. Beta lactamase testing on Gram negative rods.
      d. Anaerobes isolated that are normal skin flora can be reported as such in mixed cultures. Example: Peptostreptococcus

E. Hold no growth Thioglycollate broths for 7 days for possible growth.
   1. No further work up is needed if the broth is no growth in 7 days.
   2. If the broth grows it should be gram stained and subcultured for possible workup.

F. Report results
   1. Correlate all information – Does it make sense?
      a. Preliminary report – should include:
         i. As much information about the organism identification as possible
            Example: Many Lactose Fermenting Gram negative rods (Identification and susceptibility to follow)
                     Few Normal Skin Flora
      b. Final report – should include:
         i. Organisms identification (if pathogen)
         ii. Organisms susceptibility results (if pathogen and appropriate)
         iii. Quantitation of Normal Skin Flora or other organisms present
         iv. Example:
             Many Escherichia coli, MIC results
             Few Normal Skin Flora

VII. References
A. Textbook of Diagnostic Microbiology, Mahon & Manuselis, 3rd edition, Chapter 33, pages 935-956; Chapter 39, pages 1047-1054; Chapter 41, pages 1072-1104.
D. Manual of Clinical Microbiology, Murrary PK, et. al., 9th ed