WOUND CULTURES – GENERAL PROCEDURE

I. Principle
Wound infections may be caused by one to many organisms. Postoperative wound 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 may 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, Transport and Handling
A. Specimen types and collection
1. Eye cultures
   a. The specimen of choice is a corneal scraping.
   b. The specimen should be directly inoculated to media at the bedside due to the small amount of specimen available.
   c. In practice, however, it is more common to receive swabs that can be plated in the laboratory.

2. Ear cultures
   a. The specimen most often received by the lab is an aerobic swab of the outer ear canal.
   b. To culture fluid from the middle ear, a needle is inserted through the eardrum and fluid is withdrawn by syringe (performed by a physician and called tympanocentesis).

3. Wound Cultures
   a. The ideal specimen is an aspirate from a previously undrained abscess, or a tissue biopsy.
      i. The aspirate should be collected in a sterile syringe – any air bubbles should be expelled and the needle tightly capped to prevent exposure to oxygen.
   b. A tissue specimen should be placed in a sterile cup and sent to the lab for immediate processing if anaerobes are suspected.
      i. If there will be a delay in transporting, the tissue should be placed in an anaerobic transport system.
   c. More commonly the lab receives wound cultures on aerobic and anaerobic swabs because they are more easily collected than an aspirate or a tissue.
      i. 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.

B. Specimen transport and handling
1. Specimen transport should be immediate.
   i. 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.
C. Specimen processing
   1. Tissue specimens are prepared for plating by grinding in a mortar and pestle or by using a tissue grinder.
      a. When using a mortar and pestle, always work under a safety hood to prevent exposure to aerosols.
      b. The tissue is ground with a small amount of sterile saline to release organisms that are trapped in the tissue.

III. Direct Examination
   A. Gram stain
      1. A gram stain on the specimen is performed if adequate specimen
         a. If swabs (one anaerobic and two aerobic) are submitted for culture, use second swab for making gram stain
         b. The gram stain is omitted if a second aerobic swab is NOT submitted.
         c. If tissue is submitted, make Gram stain from ground tissue.
      2. Method – aerobic swab
         a. Place a drop of saline on a slide and roll swab in saline. Allow to air dry
         b. Stain slide using Gram stain procedure
         c. Evaluate slide under oil immersion for bacteria, PMN's, and other cells
      3. Method – tissue
         a. Grind tissue using mortar and pestle.
         b. Place a drop of ground tissue on a slide. Allow to air dry
         c. Stain slide using Gram stain procedure
         d. Evaluate slide under oil immersion for bacteria, PMN's, and other cells
      4. Report gram stain results as part of culture

IV. Culture Setup
   A. Examples of media to inoculate
      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 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 (use specimen after it has been ground) should be plated to the following media:
         a. Sheep blood agar
         b. Supplemental blood agar – for anaerobes
         c. MacConkey
         d. Chocolate
         e. Thioglycollate

   B. Incubate media
      1. Temperature: 35°C
2. Atmosphere: BAP, CHOC, PEA - CO₂, MAC, THIO - ambient air, Supplemented blood agar, KV agar, and Ana PEA - anaerobically

3. Time:
   a. Aerobically incubated media: 18-24 hours
   b. Anaerobically incubated media: 48 hours

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

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

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

D. Normal Flora
1. Eye
   a. Coagulase negative *Staphylococcus* species
   b. Non-hemolytic and alpha hemolytic streptococci
   c. Diphtheroids
   d. *Propionibacterium* species

2. Ear
   a. Same as the eye

3. Wound
   a. No normal flora present; however, there may be skin contaminants present due to the method by which the specimen was obtained.
4. Skin
   a. *Staphylococcus* species and *Micrococcus* species
   b. Diphtheroids
   c. *Propionibacterium* species
   d. *Bacillus* species
   e. *Streptococcus* species viridans group
   f. Yeast

V. Culture Interpretation
   A. Quantitate, identify and perform sensitivities on all potential pathogens (See Sec. IV).

   B. Quantitate and report normal flora organisms (See Sec. IV) without sensitivities.

   C. Hold all plates for 48 hours before sending out the report.

   D. Hold no growth Thioglycollate broths (on sterile body sites) 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.

   E. At 48 hours, anaerobic culture plates should be inspected for colony types that do not appear on
      the aerobic plates.
      1. Perform gram stains on the suspected anaerobes and re-isolate them anaerobically and
         subculture them aerobically. The aerobic subculture is necessary to prove that the organism
         is an anaerobe, as facultative organisms occasionally grow on the anaerobic plates without
         growing on the aerobic plates.
      2. Anaerobic organisms that are considered pathogenic are identified.

V. References
   A. Textbook of Diagnostic Microbiology, Mahon & Manuselis, 2\textsuperscript{nd} edition, Chapter 27, pages 919-944; Chapter 33, pages 1045-1052; Chapter 35, pages 1083-1113.

   B. Color Atlas and Textbook of Diagnostic Microbiology, Koneman, 5\textsuperscript{th} edition, Chapter 3, pages 152-153, 162.

   C. Bailey & Scott's Diagnostic Microbiology, Forbes, 11\textsuperscript{th} edition, Chapter 59, pages 917-926, and
      Chapter 63, pages 972-984.