STOOL CULTURES – GENERAL PROCEDURE

I. Principle
Stool cultures are performed to detect enteric pathogens or potential pathogens. Routine stool cultures in the United States should be examined for the presence for *Salmonella*, *Shigella*, and *Campylobacter* spp at minimum. It should be noted that media routinely set to detect these pathogens will also detect *Aeromonas hydrophila* and *Plesiomonas shigelloides*. *Vibrio*, *Yersinia* and *E. coli* O157:H7 may not be part of the routine stool culture at your clinical site. Often cultures for *Vibrio*, *Yersinia* and *E. coli* O157:H7 are performed upon doctor request. However, the age of the patient and incidence of the particular pathogen in a geographical area, may dictate routine screening for these pathogens. Bloody stool specimens are routinely screened for *E. coli* O157:H7 or shiga-like producing strains of *E. coli*.

Recently, the CDC has recommended routine screening of stool culture for the presence of *E. coli* strains that produce a Shiga-like cytotoxin. Enterohemorrhagic *E. coli* (EHEC) has been isolated from patients who have hemorrhagic colitis and hemolytic-uremic syndrome (HUS). One virulence trait of all EHEC strains is the ability to produce cytotoxin(s) called Shiga-like toxin (SLT) or verotoxin (VT). SLT-I and SLT-II are the two most common toxins and individual EHEC strains have the ability to produce both or either, in varying quantities. Therefore, SLT production and not individual (O157:H7) serotype identification is a better diagnostic strategy for the determination of EHEC associated disease.

II. Specimen Collection, Transport and Handling
A. Specimen types and collection
1. Feces – collected in sterile/nonsterile clean container and sent to the laboratory for immediate processing. The pH of the specimen will begin to drop after collection and this could reduce the quantity of pathogens present.
2. The number of stool specimens submitted to the lab is controversial. It is recommended if more than one specimen is submitted that it should be on different days.

B. Specimen transport and handling
1. Stool held at room temperature should be cultured within 1 hour of collection.
2. Refrigeration of stool specimens is not recommended as some pathogens, especially *Shigella* species, are very susceptible to lower temperatures and will die rapidly.
3. Specimens that cannot be sent immediately to the lab or processed shortly after collection should be placed in an appropriate enteric transport media.
4. Specimens containing barium, mineral oil or urine should be rejected.

III. Direct Examination – upon doctor's request
A. Leukocyte evaluation
1. Perform a Wright's or Gram stain on the stool specimen to evaluate for the presence of leukocytes. A Direct Gram stain is not useful beyond its determination of the presence of leukocytes, as it will not differentiate suspected pathogens from normal microbial flora.
   a. Place a drop of feces on a slide, spread out, and allow to air dry
   b. Stain slide using Wright’s or Gram’s stain procedure
   c. Evaluate slide under oil immersion for PMN’s
   d. Report results
2. Latex agglutination for detection of lactoferrin released by fecal leukocytes in diarrheal stool specimens.
   a. See site specific procedures for instructions
   b. Not appropriate for detection of leukocytes in breastfeeding babies. Lactoferrin present in breast milk.
IV. Culture Setup

A. General comments

1. The purpose of a stool culture is to use selective and routine media to screen for the presence of stool pathogens. In many cases, full identification of a suspicious colony is not performed. Instead, screening tests are performed to rule out potential stool pathogens. If suspicious colonies are noted then full identification performed.

2. Routine stool cultures should always include testing for the presence of *Salmonella* species, *Shigella* species, and *Campylobacter jejuni*. It is recommended that routine stool cultures include testing for the presence of *Aeromonas* species, and *Plesiomonas* species. The CDC recommends routine screening of stool for *E. coli* strains that produce a Shiga-like cytotoxin.

3. When required by the physician, media may also be included to detect *Yersinia* species or *Vibrio* species. However, in areas where these pathogens are common including media for these organisms is part of the routine culture.

4. The CDC recommends testing to detect shiga-like toxin producing strains of *E. coli* in addition to culture for *Escherichia coli* O157:H7. It is no longer recommended to do culture alone. Clinical sites may perform culture and toxin testing or toxin testing alone.

5. A macroscopic exam is reported with every culture.

6. Pathogens commonly isolated in stool
   a. *Salmonella* species
   b. *Shigella* species
   c. *Campylobacter jejuni*
   d. *Vibrio cholera*
   e. *Vibrio parahaemolyticus*
   f. *Yersinia enterocolitica*
   g. *Clostridium difficile*
   h. *Staphylococcus aureus*
   i. *Aeromonas* species
   j. *Plesiomonas* species
   k. Hemorrhagic *Escherichia coli* O157:H7
   l. Shiga-like toxin producing strains of *Escherichia coli*

7. Normal flora commonly isolated in stool
   a. Enteric organisms
   b. *Staphylococcus* species
   c. *Streptococcus/Enterococcus* species
   d. Anaerobic organisms

B. Inoculate media

1. Use a swab to inoculate plates using representative areas of the specimen. Inoculate the media making the first streak then use a sterile loop to streak for isolation. Non inhibitory media should always be inoculated first.

2. Routine stool culture
   a. Blood agar
   b. MacConkey agar
   c. Hektoen enteric (HE) agar (or XLD agar or SS agar)
   d. Selective agar for Campylobacter (i.e., CVA, Campy BAP, Skirrow)
   e. GN broth (or Selenite F) – selectively enhances the growth of *Salmonella* and *Shigella* while suppressing the growth of normal bowel flora. Also, used for the detection of shiga-like toxin producing strains of *E. coli*. This will increase the chances of isolating these pathogens when they are present in small numbers.
3. Yersinia culture
   a. Cefsulodin-Irgasan-Novobiocin (CIN) agar or Yersinia Selective agar
   b. Phosphate buffered saline (PBS) broth – suppresses the growth of normal bowel flora allowing easier detection of Yersinia species, but it also slows the growth of the Yersinia.

4. Vibrio culture
   a. TCBS agar
   b. Alkaline peptone broth

C. Incubate media
   1. Temperature:
      a. Campy BAP: 42ºC
      b. PBS broth: 4ºC
      c. CIN plate: room temperature
      d. All other plates: 35ºC
   2. Atmosphere:
      a. Campy BAP: microaerophilic (increased CO₂)
      b. BAP: either ambient air or CO₂
      c. All other plates: ambient air
   3. Time: 18-24 hours
      48 to 72 hours total

V. Culture Interpretation
A. Evaluation for Salmonella and Shigella
   1. After 24 hours incubation, examine the HE and MAC plates for non-lactose fermenting colonies and H₂S producing colonies. These colony types are suspicious for Salmonella species and Shigella species, but may also be normal enteric flora – so biochemical screens must be performed to rule in or out the presence of these pathogens.
      a. For colonies that are suspicious for Salmonella or Shigella perform KIA and LIA slants.
      b. See Table 1 for interpretation of KIA and LIA results.
      c. If screen is positive perform biochemical ID panels such as API 20 E.
      d. Colonies that appear to be Salmonella species or Shigella species are confirmed by performing serological agglutination tests (see Section VI.)
   2. If enrichment broth (i.e., GN broth or Selenite F) is used, they are subcultured to HE at 24 hours. Suspicious colonies from the enrichment broth subculture plates are screened biochemically.
   3. A susceptibility test is performed only on any confirmed colonies of Salmonella and Shigella species when requested. Treatment with antibiotics is not recommended for Salmonella species because it may induce a carrier state in the patient. In most case treatment of the clinical symptoms such as dehydration is sufficient.
   4. If no suspicious colonies are found or if all biochemical screens are negative, the report is sent out as:
      a. No Salmonella or Shigella isolated

B. Campylobacter jejuni
   1. Campy plates are examined at 48 hours and 72 hours.

   2. Colonies growing on the original Campy BAP are tested for oxidase. Any oxidase positive colonies are Gram stained to look for the typical curved rods of Campylobacter species. Pseudomonas species will occasionally grow on Campy BAP and the gram stain will quickly exclude them from further testing.
3. Oxidase positive curved gram-negative rods should be further tested to confirm the presence of *Campylobacter* species. A positive Sodium Hippurate test will confirm the species *Campylobacter jejuni*.

4. Susceptibility tests are not run on *Campylobacter* species because their resistance patterns are well established.

C. *Aeromonas* and *Plesiomonas*
   1. After 24 hours incubation, examine the BAP for large gray colonies that are gram-negative rods in predominance.
   2. Do an oxidase on each different colony type of gram-negative rods. If a colony type is oxidase positive and has a smooth morphology, perform a spot indole test. An oxidase positive indole positive gram-negative rod is suspicious for *Aeromonas* species or *Plesiomonas* species.
   3. Confirm identification of suspicious organisms with various biochemical ID panels.
   4. *Aeromonas* species are slightly beta hemolytic on the BAP but *Plesiomonas* species are nonhemolytic.
   5. Do not use the MAC to screen for *Aeromonas* or *Plesiomonas* as these organisms can be lactose variable.

D. *Yersinia*
   1. After 24 hours incubation, examine the CIN plate for dark red colonies with a “bull’s eye” center surrounded by transparent border colonies that indicate mannitol fermentation and is suspicious for *Yersinia*. Confirm identification of suspicious organisms with various biochemical ID panels.
   2. CIN plates are held for 72 hours at room temperature.
   3. After 1 week, 2 weeks, and 3 weeks incubation, subculture the PBS broth to a CIN plate. Screen any suspicious colonies that grow. Although overwhelming infections are usually detected on primary culture, studies show that the 3-week incubation period is needed to detect some cases of *Yersinia*.

E. *Vibrio*
   1. After 24 hours incubation, examine the TCBS agar for yellow colonies that indicate sucrose fermentation. Screen suspicious colonies biochemically.
   2. After 8-24 hours of incubation, subculture alkaline peptone broth to a TCBS agar. Screen any suspicious colonies that grow.

F. *Staphylococcus aureus* or Yeast
   1. After 24 hours incubation, examine the BAP for *Staphylococcus aureus* or yeast. If organism is in moderate to many amounts or as the predominant organism, work up the organism.

G. *Escherichia coli* O157:H7
   1. Setup stool culture with a MacConkey agar with 1% D-sorbitol (instead of lactose).
   2. After 24 hours of incubation, examine Mac-Sorbitol plate for colorless colonies (*E. coli* O157:H7 is sorbitol negative and most other normal flora strains of *E. coli* are sorbitol positive).

H. Shiga-like toxin producing strains of *E. coli*
   1. EIA or molecular testing is performed to detect these strains.

VI. Serological Testing for *Salmonella* and *Shigella*
   A. All isolates that biochemically resemble either *Salmonella* species or *Shigella* species must be confirmed by serological methods following site specific procedures.
   
   B. For suspected *Shigella* species, test the following somatic ("O") antigens:
      1. Antigen A = *Shigella dysenteriae*
      2. Antigen B = *Shigella flexneri*
      3. Antigen C = *Shigella boydii*
      4. Antigen D = *Shigella sonnei*
   
   C. For suspected *Salmonella* species, test the following somatic ("O") antigens:
      1. Polyvalent A-E, Vi serum
      2. Polyvalent F-I serum

VII. *Clostridium difficile*
   A. *Clostridium difficile* is an anaerobic gram-positive spore-forming rod that causes antibiotic-associated pseudomembranous enterocolitis. Since *C. difficile* is found as part of the normal fecal flora in many individuals, isolation of the organism does not prove the presence of disease. In cases of *C. difficile* diarrhea, the patient’s normal fecal flora is suppressed by prolonged antibiotic therapy allowing the *C. difficile* to multiply and produce a toxin.
   
   B. Detection of the presence of the toxin is used to diagnose the disease. Toxin may be detected by tissue culture assay, a latex agglutination test, or an ELISA test.
      1. Stool specimens collected for toxin studies should be refrigerated until testing to preserve the toxin that is rapidly labile at higher temperatures.

VIII. References
   A. Textbook of Diagnostic Microbiology, Mahon & Manuselis, 3rd edition, Chapter 34, pages 957-978.
   
   