Stool (Fecal) Cultures – Culture Setups

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Transport and handling

• Stool for bacterial culture should be received in the laboratory promptly for immediate processing
  – Samples that are delayed in transport should be inoculated to an appropriate enteric transport media
  • Enteric transport media is different than parasitic transport
  – Refrigeration of samples is not recommended
  • Pathogens susceptible to low temperatures

Selection of Culture Media

• Feces (stool) is cultured for the isolation of common gastrointestinal bacterial pathogens

• Thus in selecting media it is important that you use media appropriate for isolating the common etiologic agents

Common Etiologic Agents of Gastrointestinal Infections

Pathogens
• Salmonella species
• Shigella species
• Campylobacter species

Media to use
• MacConkey agar (lactose non-fermenter), & HE agar or XLD agar
  • Campy-blood agar

Less Common Etiologic Agents of Gastrointestinal Infections

Pathogens
• Plesiomonas species
• Aeromonas species
• Escherichia coli O157:H7
• Yersinia species
• Vibrio species

Media to use
• Blood agar (BAP)
  • Sorbitol-MacConkey agar
  • CIN agar
  • TCBS agar

Less Common Etiologic Agents of Gastrointestinal Infections

• Plesiomonas and Aeromonas can be detected on a routine stool culture.
  – Sheep Blood agar
• Escherichia coli O157:H7, Yersinia species, Vibrio species
  – May be part of the routine culture based on patient age, geographic location or gross specimen appearance
  – If not part of the routine culture, they require a special request by the physician in order to set up the special media.
Selection of Culture Media

• Most laboratories will routinely setup a BAP, MAC, HE (or XLD), and Campy-BAP
  – Please refer to procedure manual for specifics on what media your clinical site sets up
• Keep in mind normal fecal flora of the gastrointestinal tract will also grow on these media

MacConkey Agar

• Selective, differential
  – Selects for Enterobacteriacea and other GNR’s
  – Differentiates between lactose and non-lactose fermenters
  • Lactose sole carbohydrate source
  • Normal enteric flora will ferment lactose
    – Pink colonies
  • Salmonella and Shigella species will not ferment lactose
    – Colorless or transparent colonies

HE (Hextoen Enteric) Agar

• Selective, differential for isolation of Enteric pathogens
  – Selective ingredients – high concentration of bile salts that inhibit gram positive and many gram negative organisms that are part of the normal enteric flora
  – Differential ingredients
    • Lactose and sucrose - Fermentation patterns
    • Ferric salts - H2S production
    • Non-pathogens will ferment one or both carbohydrates
      – Appear bright orange to salmon-pink in color
    • Salmonella and Shigella species will produce green to blue-green colonies
      – Salmonella species will have black precipitate within the colony

XLD (Xylose-Lysine-Desoxycholate) Agar

• Selective, differential for isolation of Enteric pathogens
  – Selective ingredients
    • Sodium desoxycholate
      – Inhibits gram positive and some gram negative organisms that are part of the normal enteric flora
  – Differential ingredients
    • Lactose, sucrose, and xylose - Fermentation patterns
    • Sodium thiosulfate - H2S production
    • Non-pathogens will ferment lactose or sucrose
      – Appear as yellow colonies
    • Shigella species will produce colorless or red colonies
    • Salmonella species will produce colorless or red colonies with or without black precipitation

Campylobacter Blood Agar Campy-BAP

• Selective enrichment for isolation and cultivation of Campylobacter species
  – Brucella agar is base
    • Enhances isolation of microaerophilic organisms
  – Antibiotic mixture
    • Most formulations include vancomycin to inhibit gram positives
    • Inhibits most normal enteric flora
  – Campylobacter species
    • Flat, gray, non-hemolytic colonies or sometimes tan or slightly pink
      – May be raised or mucoid

Why is a HE or XLD agar plate used to setup a stool culture?

Some of the common potential pathogens (Salmonella and Shigella) will grow on HE /XLD agar and can be differentiated from other normal fecal flora GNRs because the non-pathogens ferment the carbohydrates in the media.
MacConkey Sorbital Agar

- Selective and differential
  - Same ingredients as MacConkey but D-sorbital is substituted for lactose
  - Commonly used to isolate E. coli 0157:H7
    - Does not ferment sorbitol
    - Colonies will appear clear or colorless
    - Most other clinical isolates will appear pink to red in color

CIN (Cefsulodin-Irgasan-Novobiocin) Agar

- Selective and differential
  - Ingredients to inhibit normal enteric flora
  - Differential ingredient
    - Mannitol
      - Yersinia species will ferment mannitol, forming clear colonies with a red center ("Bull’s eye")

TCBS Agar (Thiosulfate Citrate Bile Salts Sucrose)

- Selective and differential
  - Sodium citrate, sodium thiosulfate and oxgall
    - Inhibit gram positive and gram negatives normally found in the stools
    - High pH in media encourages growth of Vibrio species while inhibiting other organisms
  - Differential ingredient
    - Sucrose – differentiates between Vibrio species
      - Blue-green colonies – do not ferment sucrose
      - Yellow colonies – ferment sucrose
      - Black centers – Hydrogen sulfide production

Inoculation of Media

- Use a swab to inoculate plates obtain representative areas of the specimen
  - Sample areas that are purulent or bloody
  - Make primary streak with swab
  - Streak for isolation using sterile loop
  - Non inhibitory media (BAP) should always be inoculated first

Why is a colony count NOT performed on a stool culture?

Fecal material is not sterile – it contains lots of normal fecal flora; thus a colony count would be of no value in determining which organisms are pathogenic and which are normal enteric flora.

Why is a gram stain not included in culture set-up of a stool?

Due to the large amount of fecal bacterial flora, a gram stain smear to evaluate the presence of bacteria is not clinically useful. However, gram stain or other tests to evaluate the presence of leukocytes or leukocyte esterase are appropriate. These are performed upon physician request and are not part of a routine stool culture.
Incubation of Media

- **BAP, MAC, HE, XLD**
  - Incubate at 35°C in ambient air
  - Full 48 hours
  - First read at 24 hours
- **Campy-BAP**
  - Incubate at 42°C in a microaerophilic incubator (or bio-bag)
  - Full 48 to 72 hours
  - First read at 24 to 48 hours

Incubation of Media

- **MacConkey Sorbital agar**
  - Incubate at 35°C in ambient air
  - Full 48 hours
  - First read at 24 hours
- **CIN agar**
  - Incubate at 25°C in ambient air (bench top)
  - Full 72 hours
  - First read at 24 hours
- **TCBS agar**
  - Incubate at 35°C in ambient air
  - Full 48 hours
  - First read at 24 hours

Review

- Select culture media
  - BAP, MAC, HE (or XLD), and Campy-BAP
  - Common etiologic agents of gastrointestinal infections
- Inoculation of media
  - Streak for isolation
- Incubation of media