Stool sample processing

By

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The specimen of choice

- The specimen of choice is diarrhea stool (fresh random) in fecal transport system.
- A rectal swab is only recommended for infants.
- Visible pus, mucus, or blood should be included in sample specimens.
- A single stool specimen cannot be used to rule out bacteria as a cause of diarrhea. More than two specimens should only be submitted from patients for whom there is a high degree of suspicion.
Pre specimen processing

Patient preparing

- Instruct the patient on how the specimen should be collected and transferred to the container; provide him/her with sticks or plastic spoon and containers.
How to collect the sample

- Faecal material is placed into **sterile container** with the aid of the **plastic spoon**
• **Quantity of specimen**

  The specimen should contain *at least 5 g of faeces*
Labelling the specimen

- Label the specimen with patient information (Name, age, department and diagnosis at admission)

- Very importantly give a good history on the request form:
  1. symptoms of gastroenteritis as bloody stool, vomiting, fever, abdominal pain and dehydration.
2. **Food habits** (drinking raw/unpasteurized milk, eating undercooked meat)

3. **Exposure to farm** environment

4. Medical **disease** as hepatitis, colitis, protozoal infection, and renal impairment.

5. Any **medical therapy** especially antimicrobial therapy
Transport

- **Time relapse before processing the sample**
  
  Stool samples should be examined and cultured as soon as possible after collection. As the stool specimen cools, the drop in pH will inhibit the growth of most *Shigella* spp. and some *Salmonella* spp.

- **In case of delay longer than 1 hour in transportation of specimens to the laboratory**, part of specimen will be transferred to cotton swab and inserted into a container of Cary-Blair transport medium.
Criteria of specimen rejection

- specimen contaminated with urine.
- residual soap, or disinfectants.
- Specimens received in grossly leaking transport containers;
- dry specimens
- specimens submitted in fixative or additives;
Processing of fecal sample

- The color and consistency of samples described, whether they were semisolid or fluid and whether it contained blood, mucus and pus.
- Direct microscopic examination of a fecal sample in physiologic saline
When to order stool culture?

- According to the Centers for Disease Control and Prevention (CDC), doctors are most likely to order a stool culture for patients with any of the following characteristics:
  - Bloody diarrhea
  - High Fever
  - Tenesmus (is the constant feeling of the need to empty the bowel, with passage of blood & mucus accompanied by pain, and cramping)
  - Severe or persistent symptoms >3 days
  - Recent travel to a third world country
  - Known exposure to a bacterial agent
  - Presence of fecal leukocytes
  - Severe dehydration
Results

- Some bacteria that are normal inhabitants of the digestive tract are known as the enteric bacteria.
- *Escherichia coli, Klebsiella, Enterobacter,* and *Pseudomonas* are members of this group.
- When only normal flora are found the results are reported as "no enteric pathogens found."
True gastrointestinal pathogens

The following bacteria are not normal inhabitants of the digestive tract, and are known to cause gastrointestinal infection:

- *Campylobacter*
- *Shigella*
- *Salmonella*
- *Yersinia*
- *Enterohemorrhagic E. coli*
- *Vibrio*
- *Aeromonas*
If bacteria are not the cause of an intestinal infection, a **fungal** or **viral culture** might be necessary.
Patients with AIDS, or other immune system diseases, sometimes have gastrointestinal infections caused by fungal organisms such as Candida, or by viral organisms including Cytomegalovirus (CMV).

Candida can also become an opportunistic intestinal pathogen when antibiotics or radiation have destroyed the normal stool flora.
Several intestinal parasites, such as *Giardia lamblia*, also cause gastrointestinal infection and diarrhea.

Parasites are not cultured, but are identified by **Direct microscopic examination** of a fecal sample in physiologic saline and **parasites test**.
SO Routine stool culture is used to detect

- *Campylobacter*
- Enterohemorrhagic *E. coli* (O157:H7)
- *Shigella*
- *Salmonella*.

- Less frequently isolated bacterial causes of diarrhea are *Vibrio spp.*, *Yersinia enterocolitica*, and *Aeromonas spp.*
Culture the specimens

When a stool specimen is submitted to the laboratory, various procedures are conducted.
Routine culture for Salmonella & Shigella

Media

- Selenite-F broth or tetrathioniate.
- SSA, XLD and HEA.

Reagents

- API 20 E Kit.
- Salmonella and Shigella antiserum (polyvalent and monovalent).
Stool for Salmonella & Shigella

Selenite-F Broth

XLD

XLD

Pick up suspect colonies and perform biochemical test and serology if necessary
Selenite-F broth

- **Selenite Broth** (Selenite-F Broth) is used as an enrichment medium for the isolation of Salmonella from feces, urine, water, foods and other materials of sanitary importance.

- **Sodium selenite** inhibits the growth of gram-positive and many gram-negative bacteria including enterococci and coliforms, whereas the salmonellae are not affected.
Sodium selenite is **highly toxic** at near-neutral pH.

**Buffer salts** are present to help maintain the pH which may rise as the toxicity decreases. A rise in pH decreases selective activity of Selenite.

A fermentable carbohydrate (**lactose**) is also present to provide acid to neutralise the alkali produced when the selenite is reduced by bacteria.

The function of the **phosphate** is two-fold; it serves to maintain a stable pH and lessens the toxicity of the selenite, thus increasing the capacity of the medium.
Tetrathionate Broth

- **Tetrathionate Broth base**, with added iodine-iodide solution, is used as a selective enrichment medium for the isolation of Salmonella from feces, urine, foods and other materials of sanitary importance.
Routine culture for Enterohemorrhagic *E. coli*
Stool Specimen

Inoculate onto

Sorbitol MacConkey Agar

Incubate for 24 hrs at 37 oC.

Pick up non sorbitol fermenting colonies

Perform latex agglutination using O157 and H7 antiserum
Interpretation

a) **Positive result**
   - Agglutination of the Test latex occurs within 1 minute.
   - No agglutination of the Control latex.
   Perform biochemical tests to confirm that the organism is an E. coli strain.

b) **Negative result**
   - No agglutination of the Test latex.

c) **Non-interpretable result**
   - Clumping of the Control latex.
Vibrio spp.

Media

- Alkaline peptone water
- TCBS (Thiosulfate Citrate Bile salt Sucrose Agar)
<table>
<thead>
<tr>
<th>CULTURE MEDIA</th>
<th>Purpose</th>
<th>Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mac Conkey agar</td>
<td>To recover <em>Enterobacteriaceae</em> and other fastidious Gram negative organisms</td>
<td>Salmonella, shigella (NLF)</td>
</tr>
<tr>
<td>Hektoen enteric agar (HE)</td>
<td>A highly selective media to recover <em>salmonella</em>, <em>shigella</em>; contain indicator to detect H2S production</td>
<td><em>Salmonella</em>: blue green with black centre</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Shigella</em>: green without black centre</td>
</tr>
<tr>
<td>Xylose-lysine deoxycholate (XLD)</td>
<td>A differential media for isolation of <em>shigella</em> and <em>salmonella</em> from stool</td>
<td><em>Salmonella</em>: red with black centre</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Shigella</em>: red or clear</td>
</tr>
<tr>
<td>Campylobacter blood agar (CAMPY-BA)</td>
<td>Selective media to isolate <em>campylobacter</em> from stool</td>
<td>Appears pink grey moist when incubated at 42°C</td>
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Selective Media Commonly Used to Recover Enteropathogen
### Selective media commonly used to recover diarrheal agents

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<tr>
<th>CULTURE MEDIA</th>
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<tr>
<td>Thiosulphate bile salt sucrose agar (TCBS)</td>
<td><em>Vibrio</em> species</td>
<td>Yellow colonies; sucrose fermenting vibrio sp. SUCH as <em>V. cholera</em></td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas</em> species</td>
<td>Blue green colonies: non sucrose fermenters as <em>V. vulnificus, parahaemolyticus</em></td>
</tr>
<tr>
<td>Cycloserine cefoxitin fructose agar (CCFA)</td>
<td>selective media for <em>clostridium difficile</em></td>
<td>Appears yellow from fructose fermentation</td>
</tr>
<tr>
<td>Sorbitol macconkey agar (SMAC)</td>
<td>A differential media to detect sorbitol negative <em>E.coli</em> (contain sorbitol instead of lactose)</td>
<td><em>E.coli O157: H7</em> appear colorless</td>
</tr>
</tbody>
</table>
*E. Coli* O157:H7 on Sorbitol MacConkeys’ agar (SMAC)
Campylobacter colonies on campy blood agar
Salmonella colonies on Hektoen enteric agar
Salmonella colonies on XLD
Thank you