Stool Examination

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Introduction

Stool (faeces) is an important body substance which has to be checked for the presence of disease-causing microorganisms

• **Diarrhoea:** Increase in the frequency, fluidity or the volume of the bowel movement, which is relative to the usual habit of an individual. The passage of three or more motions a day is considered as diarrhoea.

• **Dysentery:** Passage of blood and mucus stained stools, which is often associated with abdominal cramps and tenesmus.

• **Gastroenteritis:** Inflammation of the mucus membranes of the stomach and the intestines, resulting in diarrhoea which is associated with vomiting.
Examination of the stool for bacteria

• Bacterial agents which are responsible for diarrhoea

1. **Gram positive**: (Staphylococcus aureus, Clostridium perfringens, Clostridium difficile, Bacillus cereus)

2. **Gram negative**: (Vibrios (i) Vibrio cholera (ii) Vibrio parahemolyticus (iii) Other halophlic vibrios, Escherichia coli (Enterotoxigenic Escherichia coli ETEC, EnteropathogenicE. coli EPEC), Salmonella (i) S.enteritidis (ii) S.typhimurium, Shigella spp, Campylobacter jejuni, Yersinia enterocolitica

• Bacterial agents which are responsible for dysentery

Shigella spp (sh. dysentriae, sh.flexneri, sh.boydii and sh.sonnei), Escherichia coli (EIEC and EHEC), V.parahemolyticus, Campylobacter jejuni, Salmonella spp.
Laboratory examination of feces

Role of microbiological laboratory in investigating infective diarrhoeal disease:

With most patients, diarrhoea is self-limiting and can be treated with rehydration and other supportive therapy without the need for antimicrobials and microbiological investigations. The microbiological examination of fecal specimens is mainly undertaken:

• To investigate outbreaks of dysentery (mainly shigellosis), cholera, and other acute bacterial infective diarrhoeal disease of public health concern.

• To assist the central public health laboratory in the surveillance of endemic shigellosis and salmonellosis (including susceptibility of pathogens to antimicrobials).
Specimen collection and transport

1- Give the patient a clean, dry, disinfectant-free bedpan or suitable wide-necked container in which to pass a specimen. The container need not be sterile. Ask the patient to avoid contaminating the faeces with urine.

2- Transfer a portion (about a spoonful) of the specimen, especially that which contains mucus, pus, or blood, into a clean, dry, leakproof container.

3- Write on the request form the colour of the specimen and whether it is formed, semiformed, unformed, or fluid. Report also if blood, mucus, worms, or tapeworm segments are present.

4- Label the specimen and send it with a request form to reach the laboratory within 1 hour (if a delay longer than 1 hour is anticipated, collect the specimen in Cary-Blair medium.)
• Feces for microbiological examination should be collected during the acute stage of diarrhoea.

• If an etiologic agent is not isolated with the first culture or visual examination, two additional specimens should be submitted to the laboratory over the next few days. Because organisms may be shed intermittently, collection of specimens at different times over several days enhances recovery.

• Rectal swabs: Only when it is not possible to obtain faeces, collect a specimen using a cotton wool swab. Insert the swab in the rectum for about 10 seconds. Care should be taken to avoid unnecessary contamination of the specimen with bacteria from the anal skin.
Macroscopic examination

Various points which have to be noted are:

• Consistency: formed, unformed (soft), loose or watery.
• The presence of blood, mucus or pus.
• Colour (white, yellow, brown or black).
• Normal faeces appear brown and formed or semifomed. Infant faeces are yellow-green and semifomed.
Microscopic examination

1- Methylene blue preparation

Place a small fleck of the stool specimen or the rectal swab together with a small flake of mucus in a drop of 0.05% methylene blue solution on a clean glass slide and examine it for cellular exudates as follows:

• Clumps of pus cells of > 50 cells per high power field along with macrophages and erythrocytes are typical of shigellosis.

• A smaller number of pus cells of <20 per high power field are found in salmonellosis and in infections which are caused by invasive *E.coli*.

• Few leucocytes (< 5 cells per high power field) are present in cholera, EPEC and ETEC and viral diarrhea.
Microscopic examination

2- Wet mount

• The simplest way of examining a bacterial suspension for motile bacteria is by doing a wet mount. Place a small drop of suspension on a slide, cover it with a coverslip and examine microscopically for motile organisms by using the 10X and the 40X objectives.

3- Hanging drop preparation

• Placing a drop of suspension on a cover glass and inverting this over a cavity slide or a normal slide which is supported on a ring of plasticine can also be used for observing motile organisms.
Type of motility organisms

- Sluggish
- Actively motile
- Dartingly motile
- Tumbling
- Falling leaf
- Actively motile with pseudopodia

E.coli
Salmonella spp
Vibro cholera
Campylobacter spp
Giardia lamblia
Entamoeba histolytica
Microscopic examination

4- Basic fuschin smear

Make a thin smear of the specimen on a slide, stain it with Basic fuschin and examine the slide by using a 100X objective. This has been shown to be a sensitive method for the presumptive diagnosis of *Campylobacter spp*. It appears as small, delicate, spiral curved bacteria or s-shaped forms.
Direct detection of agents of gastroenteritis in faeces

1- **Wet Mounts**: is the fastest method for detecting intestinal parasites

2- **Stains**: Feces may be **Gram stained** for detection of certain etiologic agents. E.g., many thin, comma-shaped, gram-negative bacilli may indicate Campylobacter infection (if vibrios have been ruled out). In addition, polymorphonuclear cells may also be detected.

3- **Antigen Detection**: Enzyme immunoassays (EIAs) can detect numerous microorganisms capable of causing GI tract infections. For example, EIAs are commercially available to detect E. coli O157:H7 and Campylobacter spp., the presence of the Shiga toxins produced by EHEC, or the presence of C. difficile toxins A or A and B.
Direct detection of agents of gastroenteritis in faeces

4- Molecular Biologic Techniques :

The development of amplification techniques has led to numerous publications for the direct detection of many enteric pathogens, including all major organism groups bacteria, viruses, and parasites. A disadvantage with probe technology is that the organism itself is not available for susceptibility testing, which is important for certain bacterial pathogens (e.g., Shigella) for which susceptibility patterns vary
Culture of Faecal specimens

Fecal specimens for culture should be inoculated to several media for maximal yield, including solid agar and broth.

- **Enrichment Broths:** They are sometimes used for enhanced recovery of Salmonella, Shigella, Campylobacter, and Y. enterocolitica.
- **Gram-negative broth (Hajna GN) or selenite F broth** yields good recovery.
- **Enrichment broths for Enterobacteriaceae** should be incubated in air at 35°C for 6 to 8 hours and then several drops should be subcultured to at least two selective media.
- **Stool** would be inoculated to broth initially; those broths that test negative could be discarded without subculturing.
Culture of Faecal specimens

• Specimens received for detection of the most frequently isolated Enterobacteriaceae and Salmonella and Shigella spp. should be plated to a supportive medium, a slightly selective and differential medium, and a moderately selective medium.

• Blood agar is an excellent general supportive medium. Blood agar medium allows growth of yeast species, staphylococci, and enterococci, in addition to gram-negative bacilli.

• The slightly selective agar should support growth of most Enterobacteriaceae, vibrios, and other possible pathogens; MacConkey agar works well.
MacConkey’s Agar

- It inhibits most of the gram positive organisms and the swarming growth of Proteus, which may pose a problem in mixed cultures.

- Salmonella, Shigella and Vibro form colourless colonies on this medium as these are nonlactose fermenters. E.coli which are lactose fermenters, form pink coloured colonies
Xylose lysine deoxycholate (XLD) agar

- It is recommended for the isolation of Salmonella and particularly Shigella from faecal samples.
- Shigella forms pink-red colonies because it does not ferment xylose and lactose.
- Salmonella also forms pink coloured colonies with black centres because of hydrogen sulphide production.
Thiosulphate citrate bile salt sucrose (TCBS) agar

- An excellent, selective medium for the primary isolation of V.cholerae. Prior enrichment in alkaline peptone water is recommended unless the specimen contains large number of Vibrio bacteria in the acute stage. On TCBS, Vibrio produces large yellow coloured colonies because of sucrose fermentation.
This MacConkey’s agar contains sorbitol instead of lactose. E.coli 0157 produces colourless colonies on this medium because it does not ferment sorbitol. Most of the other E.coli strains and other enterobacteria ferment sorbitol and produce pink colonies. So, this medium is useful for screening 0157 E.coli.
References


