# Examination of faecal specimens

## Possible pathogens

_ BACTERIA

| ***Gram positive*** | ***Gram negative*** |
|---|---|
| *Clostridium perfringens* types A and C | *Shigella* species |
| *Clostridium difficile* | *Salmonella* serovars |
| *Bacillus cereus* (toxin) | *Campylobacter* species |
| *Staphylococcus aureus* (toxin) | *Yersinia enterocolitica* |
| | *Escherichia coli* (ETEC, EIEC, EPEC, VTEC) |
| | *Vibrio cholerae* 01, 0139 |
| | Other *Vibrio* species |
| | *Aeromonas* species |

Also *Mycobacterium tuberculosis*

### Commensals

The normal microbial flora of the gastrointestinal tract is greatly influenced by diet. Microorganisms which may form part of this normal flora include:

– Coliform bacilli and species of *Proteus*, *Pseudomonas*, *Clostridium*, *Bacteroides*, *Enterococcus*, and lactobacilli.

– Also *Mycoplasma*, *Candida* species and a variety of protozoa and viruses.

## COLLECTION AND TRANSPORT OF FAECES

Faeces for microbiological examination **should be collected during the acute stage** of diarrhoea.

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, leak proof container.

**Worms and tapeworm segments:** When the specimen contains worms or tapeworm segments, transfer these to a separate container and send them to the laboratory for identification.

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

4- Label the specimen and send it to a laboratory within 1 hour (if a delay longer than 1 hour is anticipated, collect the specimen in **Cary-Blair medium**).

***Note*** *;Salmonella* serovars, *Shigella*, *Vibrio* and *Yersinia* species survive well in Cary-Blair medium for up to 48 hours, and *Campylobacter* for up to 6 hours.

## LABORATORY EXAMINATION OF FAECES

**Role of microbiological laboratory in investigating infective diarrheal disease**

With most patients, diarrhea 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 faecal specimens is mainly undertaken:

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

## Day 1

**1- Describe the appearance of the specimen**

– Color of the specimen.

– Whether it is formed, semiformed , unformed or fluid.

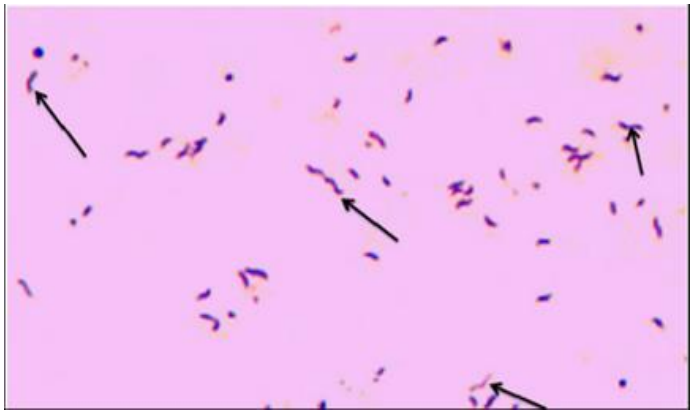
– Presence of blood, mucus or pus

– Presence of worms, e.g. *Enterobius vermicularis*, *Ascaris lumbricoides*, or tapeworm segments e.g. *Taenia* species.

***Normal faeces***: Appear brown and formed or semiformed. Infant faeces are yellow-green and semiformed.

**2- Examine the specimen microscopically *Saline and eosin preparations to detect E. histolytica and other parasites*** .

3- ***Basic fuchsin smear to detect campylobacters***



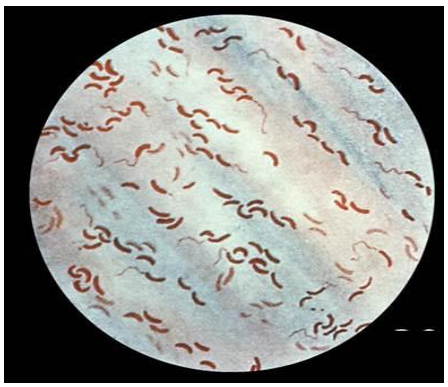
Prepare when the specimen is unformed and, or, contains mucus, pus, or blood.

– Make a *thin* smear of the specimen on a slide. When dry, *gently* heat-fix. Stain by covering the smear with basic fuchsin for 10–20 seconds. Wash well with water and allow to air dry.

– Examine the smear for campylobacters using the 100_ oil immersion objective.

*Campylobacter organisms* : Look for abundant small, delicate, spiral curved bacteria , S-shapes, and short spirochaetal forms as shown in picture below.

***4- Motility test and Gram stained smear when cholera is suspected***



Examine an alkaline peptone water culture (sample from the surface of the culture) for vibrios showing a rapid and darting motility. The preparation is best examined using dark-field microscopy but the vibrios can also be seen using transmitted light.

## 5- Culture the specimen

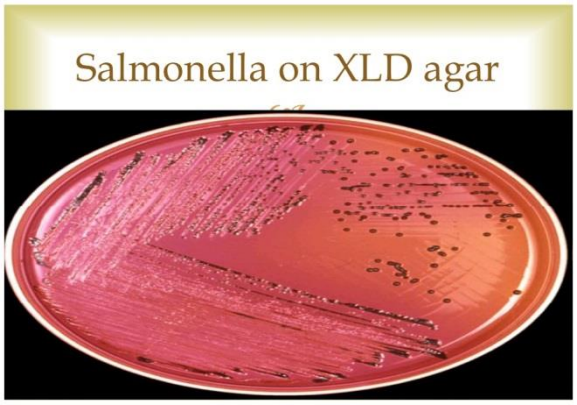
When the specimen is formed or semi formed, make a thick suspension of it in about 1 ml of sterile peptone water.

- ***Xylose lysine deoxycholate (XLD) agar***

– Inoculate a loopful of fresh emulsified faeces or a fluid specimen on XLD agar.

– Incubate the XLD agar plate aerobically at 35–37 °C overnight.

**XLD agar: This selective medium is recommended for the isolation of salmonellae and particularly shigellae from faecal specimens. It contains the indicator phenol red which is red at an alkaline pH (medium is pH 7.4) and yellow at an acid pH. Shigellae form pink-red colonies because they do not ferment xylose, lactose, or sucrose. Salmonellae also form pink-red colonies even though they ferment xylose with acid production. This is because they break down the amino acid lysine which gives an alkaline reaction. Hydrogen sulphide (H2S) producing salmonellae form red colonies with black centres.**





- ***Alkaline peptone water and TCBS agar when cholera is suspected***

– Inoculate several loopfuls of specimen in alkaline (pH 8.6) peptone water, and incubate at 35–37 °C for 5–8 hours.

**Note:** Prior enrichment in alkaline peptone water is not necessary if the specimen is likely to contain large numbers of vibrios (e.g. in acute cholera). Alkaline peptone water is a useful transport medium for *V. cholerae*.

Subculture several loopfuls of the peptone water culture (taken from the surface) on (TCBS) agar. Incubate aerobically at 35–37 °C overnight.

**TCBS medium:** The choice of TCBS agar as a primary selective medium for isolating *V. cholerae* and other *Vibrio* species

- ***Sorbitol MacConkey agar, when an outbreak of E. coli 0157 is suspected***

– Inoculate a loopful of specimen on sorbitol MacConkey agar

– Incubate the plate aerobically at 35–37 °C overnight.

**Sorbitol MacConkey agar**

This MacConkey medium contains the carbohydrate sorbitol instead of lactose. *E. coli* 0157 produces colourless colonies on the medium because it does not ferment sorbitol. Most other *E. coli* strains and other enterobacteria ferment sorbitol, producing pink colonies. Sorbitol MacConkey agar is therefore a useful way of screening for *E. coli* 0157.

## Day 2

### 6- Examine and report the cultures *XLD agar culture*

***On MacConkey agar***, shigellae, and salmonellae and other non-lactose fermenting organisms, produce colourless colonies. *E. coli* and other lactose fermenting organisms produce pink colonies.

*Identification of suspect Salmonella and Shigella isolates* Perform a urease test using urea broth or agar. A positive urease test within 2–4 h indicates that the organism is probably *Proteus*.

When the urease test is *negative* at 4 hours proceed as follows:

1- Perform indole and lysine decarboxylase (LDC) tests.

2- Inoculate a tube of Kligler iron agar.

## *Results*

***LDC:*** *Shigella* are LDC negative. *Salmonella* serovars are LDC positive except *S. Paratyphi* A which is LDC negative.

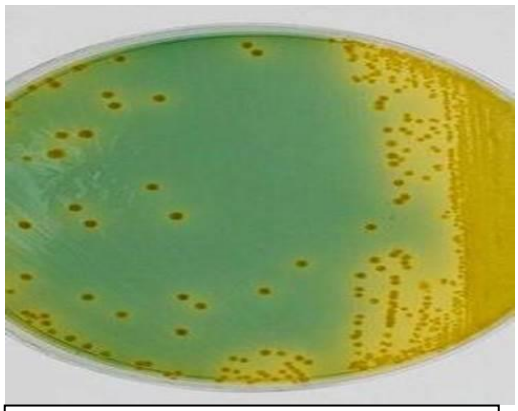
***Indole*** *S. sonnei* is indole negative. Other shigellae give variable indole reactions *Salmonella* serovars are indole negative.

***KIA*** *Salmonella* and *Shigella* organisms produce a pink-red slope and yellow butt. Many salmonellae also produce blackening due to hydrogen sulphide production and cracks in the medium due to gas production from glucose fermentation. *Salmonella* Typhi produces only a small amount of blackening and no cracks in the medium.

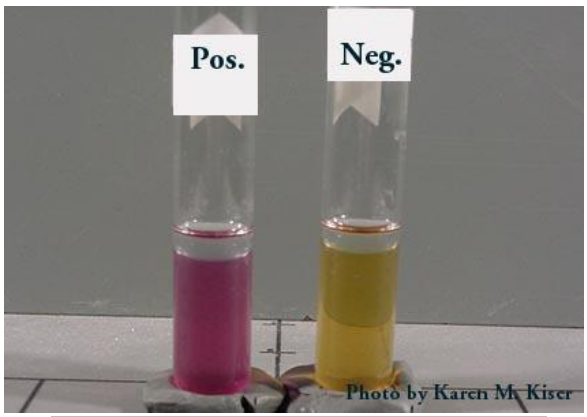
***TCBS agar culture***

*V. cholerae* is sucrose fermenting and therefore produces yellow 2–3 mm in diameter shiny colonies on TCBS agar with a yellow colour in the medium, as shown in.

***Note*:** With prolonged incubation (48 h or more) the colonies may become green.



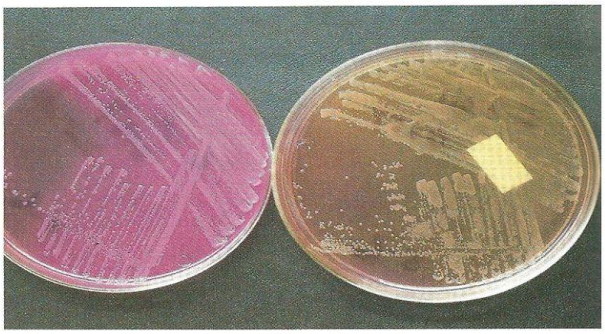
*V. cholera on TCBS agar*




(LDC) tests

## Sorbitol MacConkey (SMAC) Agar

Appearance of *E. coli* 0157:H7 on sorbitol-MacConkey agar *(right)* and a control strain of *E. coli* that ferments sorbitol *(left)*.



## Summary of the Microbiological Examination of Faecal Specimens

### Day 1

| | | ADDITIONAL INVESTIGATIONS |
|---|---|---|
| **1 Describe Specimen** | ■ ***Report***<br>– Colour<br>– Whether formed, semi-formed, unformed, fluid<br>– Presence of blood, mucus, pus<br>– Presence of worms | |
| **2 Examine Microscopically** | ■ ***Saline and eosin***: To detect parasites | ■ ***Methylene blue:*** To detect WBCs when the specimen is unformed<br>■ ***Basic fuchsin smear:*** When *Campylobacter* enteritis is suspected<br>■ ***Motility test and Gram smear:*** From alkaline peptone water culture when cholera is suspected |
| **3 Culture Specimen** | ■ ***XLD agar*** Incubate aerobically<br>■ ***MacConkey agar*** Incubate aerobically | ■ ***Alkaline peptone water and TCBS agar:*** When cholera is suspected<br>■ ***Sorbitol MacConkey agar:*** When infection with VTEC 0157 is suspected |

### Day 2 and Onwards

| | | |
|---|---|---|
| ■ **Examine and Report Cultures** | ■ ***XLD and MacConkey agar cultures*** Look for: *Salmonella* and *Shigella*:<br>– Exclude *Proteus* using urease test<br>– Identify presumptively:<br>● LDC and indole<br>● Motility<br>● KIA<br>– Identify serologically<br>– Perform susceptibility testing on *Shigella* isolates | ■ ***Examine TCBS culture***<br>– Look for colonies that could be *V. cholerae*<br>– Gram stain colonies<br>– Subculture on nutrient agar<br>– Perform oxidase test<br>– Identify serologically<br>■ ***Examine sorbitol MacConkey agar culture***<br>– Look for colourless colonies that could be VTEC 0157<br>– Perform 0157 latex agglutination test |