

# **Stool examination for bacterial and viral infections**

CBU School of Medicine  
MBS 240

# Introduction

- Stool: human feces
  - Waste residue of indigestible material from the GIT
- Composition:
  - $\frac{3}{4}$  water and  $\frac{1}{4}$  undigested and unabsorbed material
  - Intestinal secretions- mucous
  - Bile salts and pigments
  - Bacterial and inorganic material
  - Epithelial cells, leukocytes

# **Stool Examination**

## **Aim of examination**

- Detect pathogenic organisms in the stool

## **Types of specimen**

- Stool (fresh random) or rectal swab

## **Who will collect the specimen**

- The patient
- If stool is unobtainable, nursing staff or physician will collect rectal swab.

# Sample collection

- Stool should be collected in a dry, sterile wide mouthed container
  - It should be uncontaminated with urine or any other body fluid
- Rectal swab should only be collected when it is not possible to collect stool
  - Swab should be inserted in the rectum for 10s
  - Care should be taken to avoid unnecessary contamination of specimen with bacteria from anal skin
- Sample should be properly labelled and fresh sample should be tested

# Cont...



# Cont...

## Criteria of specimen rejection

- Specimen contaminated with urine, residual soap, or disinfectants
- Specimens received in leaking transport containers
- Dry specimens
- Specimens submitted in fixative or additives

**Quantity of specimen:** at least 5g of faeces

## 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.*

# Macroscopic Examination

Macroscopic appearance of the specimen. Report the following:

- Color of specimen
- Whether formed, semi formed, unformed or fluid
- Presence of blood, mucus or pus

Appearance	Possible Cause
Unformed, with pus and mucus, mixed with blood	Shigella E.Coli dysentery, Campylobacter enteritis
Blood diarrhea without pus	E.coli H157
Water stools	E.coli
Rice water stools with mucus flakes	Cholera caused by Vibrio cholerae
Unformed or watery and sometimes with blood, mucus and pus	Salmonella

# Characteristics of bloody vs non bloody diarrhoea

Watery diarrhoea	Bloody diarrhoea
No red blood cells or white blood cells in stool i.e no inflammation	Typically both red and white blood cells in stool i.e inflammatory response
Typically afebrile	Often febrile
Usually large volume	Usually small volume diarrhoea
Infection typically in small intestine	Infection typically in colon



# Definitions

- Diarrhoea: increase in frequency, fluidity or volume of bowel movement
  - $\geq 3$  motions a day
- Dysentery: Passage of blood and mucous stained stools
  - Accompanied by abdominal cramps and tenesmus
- Gastroenteritis: inflammation of mucous membranes of the stomach or intestines
  - Usually results in diarrhoea associated with vomiting

# Common bacterial agents of GIT infections

## Diarrhoea causing

- *Staphylococcus aureus*, *Clostridium perfringens*, *Clostridium difficile*, *Bacillus cereus*, *Vibrio cholerae*, *Escherichia coli* (ETEC, EPEC), *Salmonella*, *Shigella* spp, *Campylobacter jejuni*, *Yersinia enterocolitica*

## Dysentery causing

- *Shigella* spp, *Escherichia coli* (EIEC, EHEC), *Vibrio parahemolyticus*, *Campylobacter jejuni*, *Salmonella* spp

Table 12.5 Gastrointestinal Tract

Infection	Most important pathogens	Laboratory diagnosis
Gastritis type B Gastric ulceration Duodenal ulceration Gastric adenocarcinoma Gastric lymphoma (MALT)	<i>Helicobacter pylori</i>	Direct fecal antigen detection Biopsy and histopathology Urea breath test Culture from biopsy Serology for screening
Gastroenteritis/enterocolitis		
<i>Viruses</i>	<b>Rotaviruses</b> Adenoviruses Rarely: enteroviruses, coronaviruses, astroviruses, caliciviruses, Norwalk virus	Direct virus detection with electron microscopy (reference laboratories) or direct detection with immunological methods (e.g., EIA)
<i>Bacteria</i>	<i>Staphylococcus aureus</i> intoxication (enterotoxins A-E)	Toxin detection (with antibodies) in food and stool

*Bacteria*

	<i>Staphylococcus aureus</i> intoxication (enterotoxins A-E)	Toxin detection (with antibodies) in food and stool
	<i>Clostridium perfringens</i> (foods)	Culture (quantitative) from food and stool
	<i>Vibrio parahaemolyticus</i> (food, marine animals)	Culture from stool
	<i>E. coli</i> (EPEC, ETEC, EIEC, EHEC, EAggEC)	No simple tests available; if necessary: culture from stool and identification of pathovars by means of DNA assay; serovar may provide evidence
	<i>Campylobacter jejuni</i>	Culture from stool
	<i>Yersinia enterocolitica</i>	Culture from stool
	<i>Bacillus cereus</i>	Culture from stool
Pseudomembranous colitis (often antibiotic-associated)	<i>Clostridium difficile</i>	Toxin detection (cell culture) in stool. DNA assay for toxin possible
Shigellosis (dysentery)	<i>Shigella</i> spp.	Culture from stool

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Table 12.5 Continued: Gastrointestinal Tract

Infection	Most important pathogens	Laboratory diagnosis
Salmonellosis		
Enteric form	<i>Salmonella enterica</i> (enteric serovars)	Culture from stool
Typhoid form	<i>Salmonella enterica</i> (typhoid serovars) (or possibly enteric salmonellae in predisposed persons)	Culture from blood and stool; serology (Gruber-Widal results of limited significance)
Cholera	<i>Vibrio cholerae</i>	Culture from stool, possibly also from vomit
Whipple's disease	<i>Tropheryma whipplei</i>	Microscopy and DNA detection from small intestine biopsy. Culture not possible

# Shigella species

- Found in food and water contaminated with stool, and from infected-person to person when careful sanitation is not observed
- Dysentery caused by *Shigella spp* is called shigellosis
- *S.dysenteriae*, *S.flexneri*, *S.boydii* and *S.sonnei* cause bacillary dysentery
  - *S.sonnei*- mild infection
  - *S.flexneri*, *S.bodyii*- more severe disease
  - *S.dysenteriae*- most serious

## Cont...

- *S. dysenteriae* type 1 is particularly virulent-epidemic and pandemic dysentery
- Highly infectious and resistant to common antibiotics

# Salmonella species

- Acute gastroenteritis- usually self limiting characterized by fever and diarrhoea
- Incubation is 12-36 hours
- Majority of the outbreaks are caused by *S. typhimurium* and *S. enteritidis*
- Infection is caused by consumption of animal foods or food products
- Infection leads to *Salmonella* food poisoning or sometimes septicemia



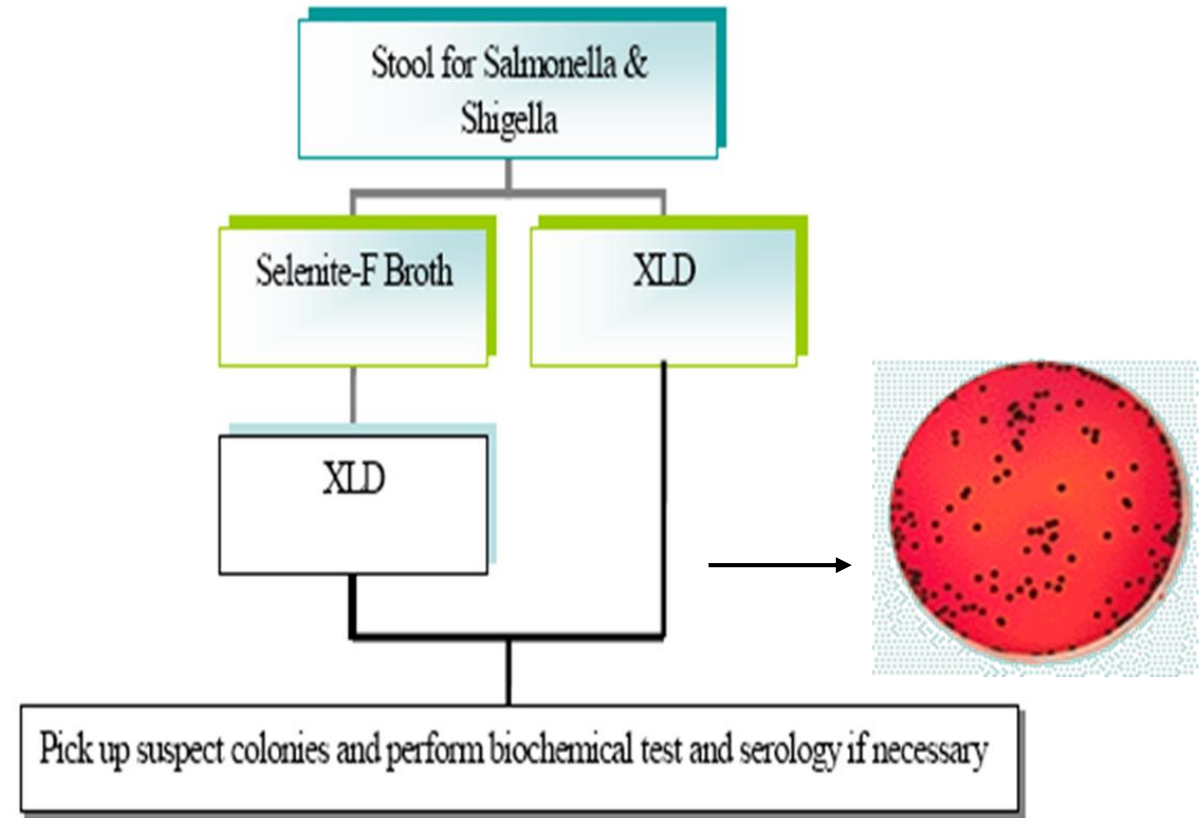
## Cont...

### **Culture (done after macroscopic examination)**

- The stool culture is a test that detects and identifies bacteria that cause infections of the lower digestive tract
- The test distinguishes between the types of bacteria that cause disease (pathogenic) and the types that are normally found in the digestive tract (normal flora)
- The test helps to determine if pathogenic bacteria are the cause of gastrointestinal symptoms (gastroenteritis).

# Routine Stool culture for Salmonella and Shigella

- Selenite-F broth or tetrathionate.
- SSA, XLD and HEA.
- Subculture on Blood agar and MacConkey agar

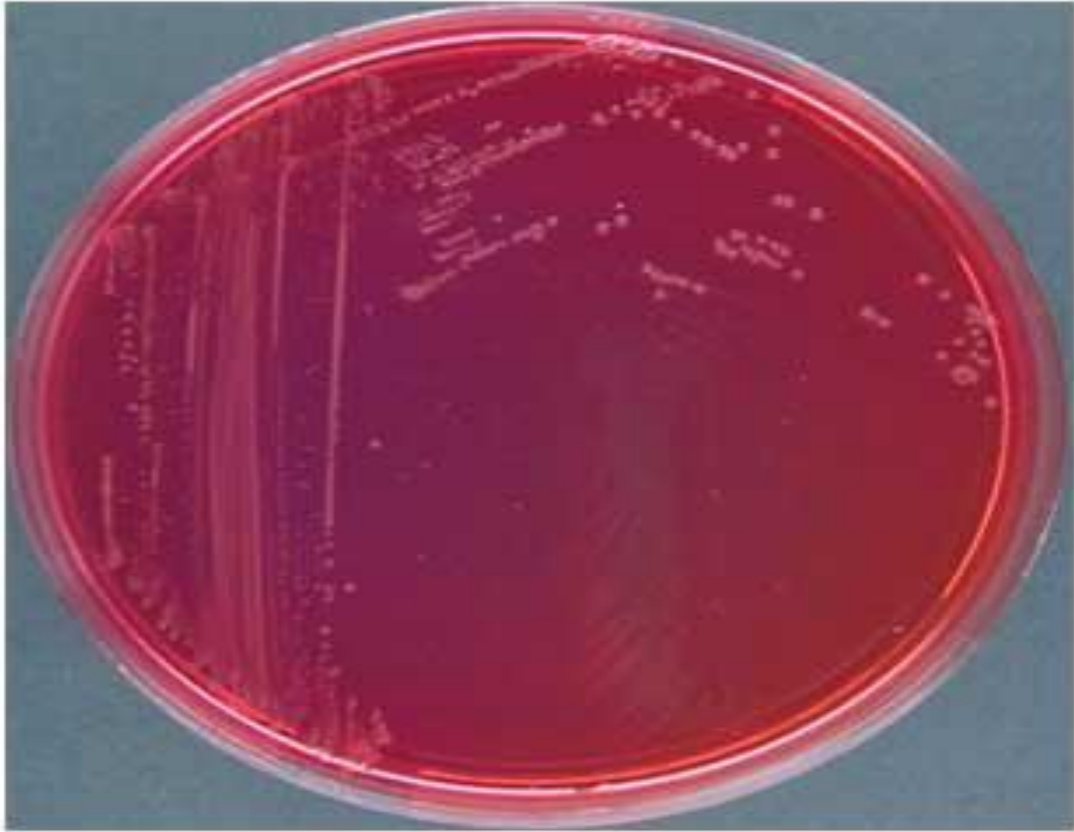


# Culture on XLD

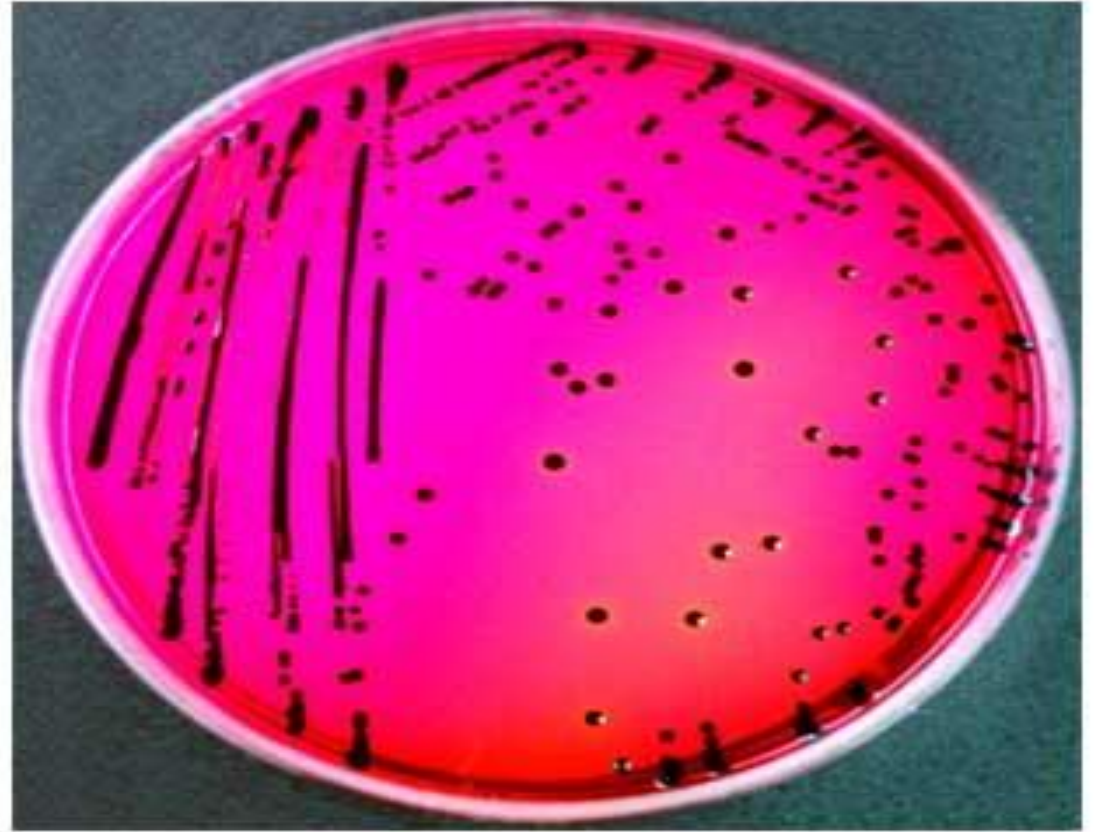
- Selenite-F Broth is used as an enrichment medium for the isolation of *Salmonella* from feces, urine, water, foods and other materials
- Xylose lysine Deoxycholate (XLD) is a selective and differential medium for the recovery of *Salmonella* and *Shigella* species.

Organism	Color of colony
Salmonella	Red colonies, Black centre
Shigella	Red colonies
E.coli	Yellow

Cont...



***Shigella on XLD.***

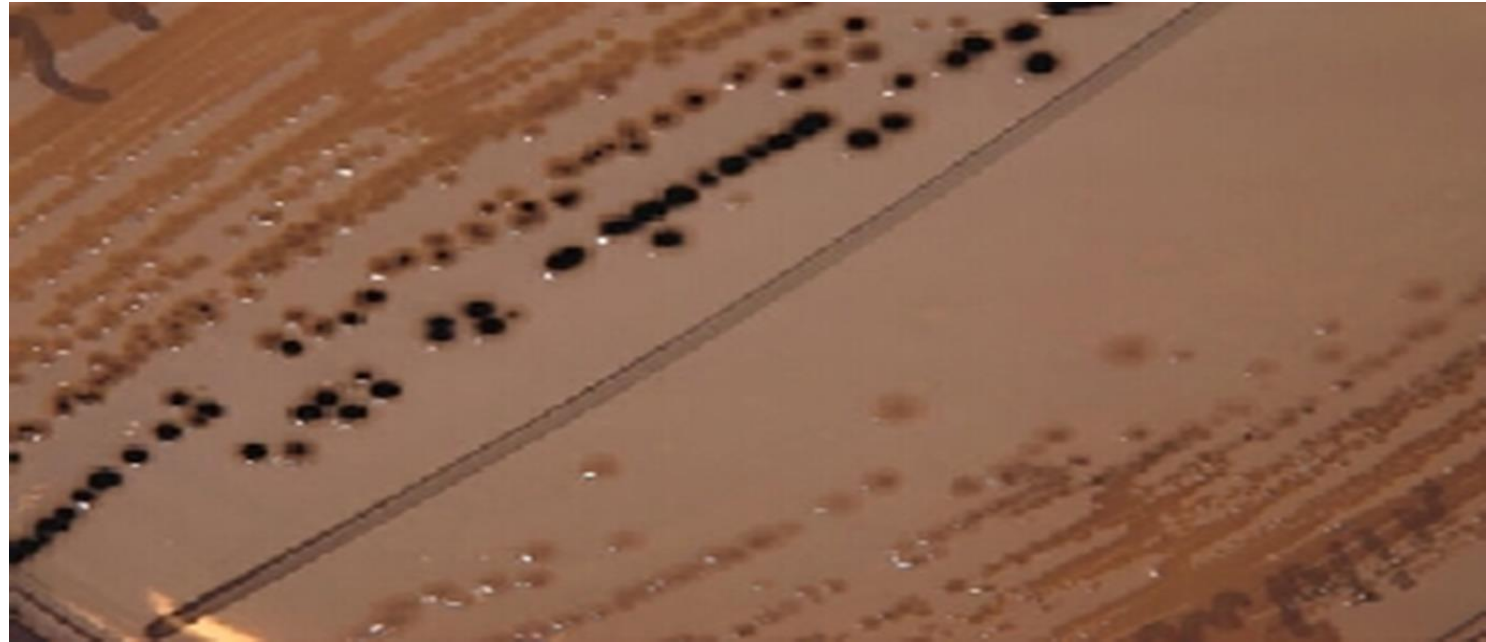


***Salmonella on XLD.***

Image Source: Faculty of Health and Medical Sciences - University of Copenhagen, Denmark

# Culture on Salmonella Shigella Agar

- Salmonella Shigella Agar (SSA) is moderately selective and differential media for the isolation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella* and *Shigella*.



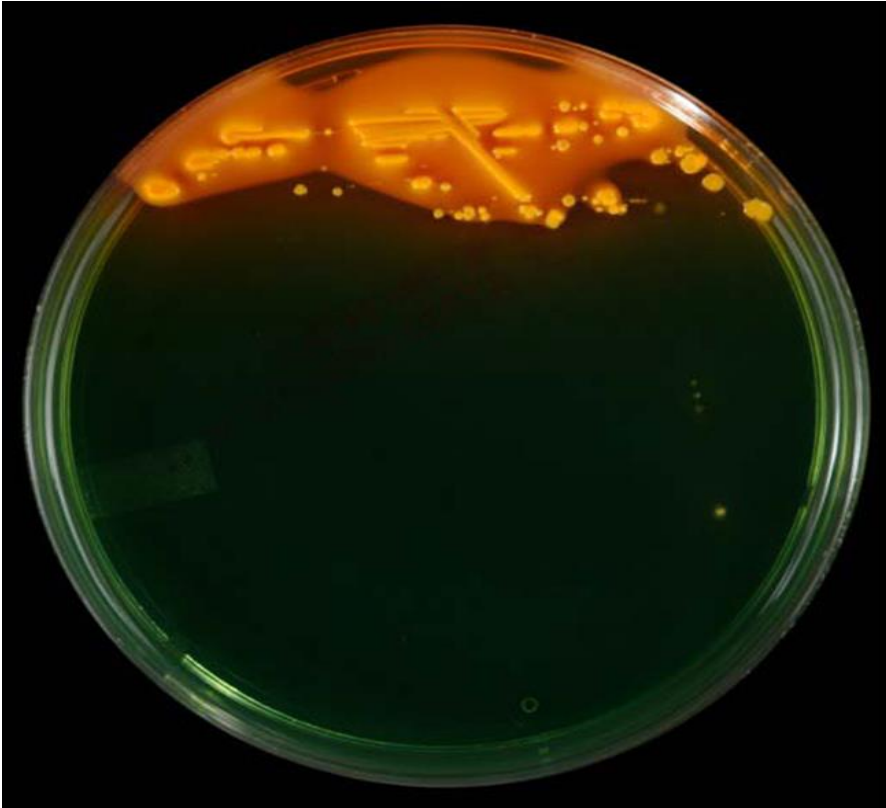
# Culture on HEA

- Hektoen Enteric Agar (HEA) is used for the isolation and cultivation of gram-negative enteric microorganisms, especially *Salmonella* and *Shigella*

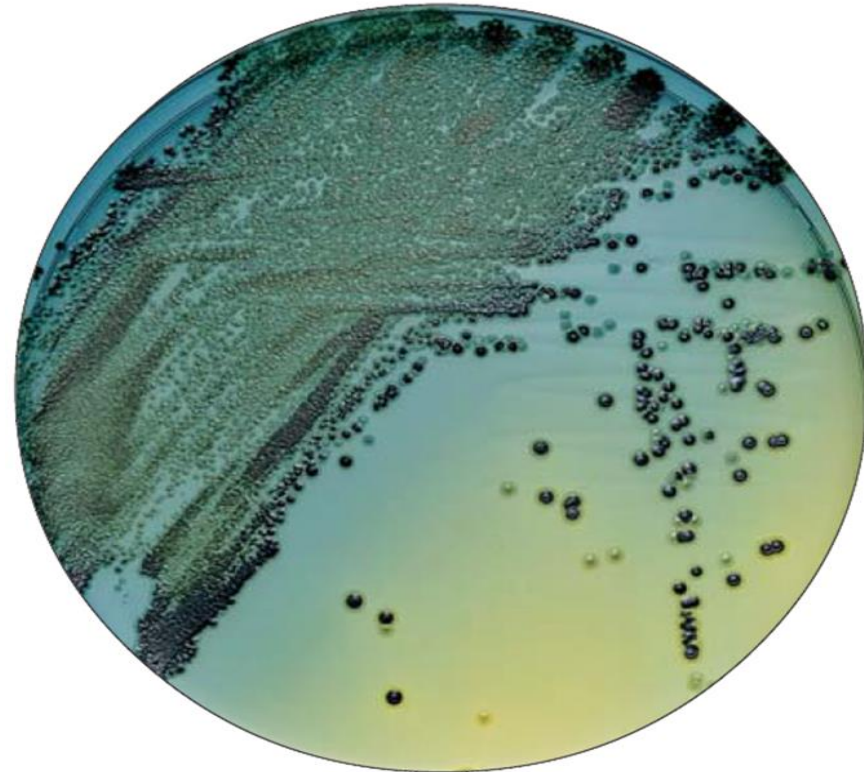
Organism	Colony color
Salmonella and Shigella	Blue to green blue
E.coli	Yellow to salmon



Cont...



*E. Coli* on HEA



Salmonella and Shigella

# Biochemical and Sensitivity tests

- After culture, growths are subjected to biochemical tests to confirm the actual bacteria isolated between *Salmonella* and *Shigella*
  - Urease test
  - Indole test
  - Lysine decarboxylase (LDC)
  - Kliger iron agar (KIA)
  - Motility
- Sensitivity tests are also done after culture for antibiotic sensitivity for treatment purposes



# Widals test and PCR

- Widals test is a serological test used in diagnosing of typhoid fever
- It measures the patient's antibodies against *Salmonella typhi* "O" (somatic) and "H" (flagella) antigens
- In acute typhoid fever, "O" agglutinins can usually be detected 6-8 days after the onset of fever and "H" agglutinins after 10-12 days
- PCR can also be used to diagnose typhoid fever, but it is expensive

# ***Escherichia coli***

- Most *E. coli* strains are considered normal flora and do not cause disease
- Strains of *E. coli* which are recognized to cause diarrhoeal disease include enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC)
  - Mostly implicated in under five children
- ETEC produces heat-labile enterotoxin and heat stable enterotoxin
- EPEC has been implicated in diarrhoea in infants-no toxin or gut invasion

# Cont...

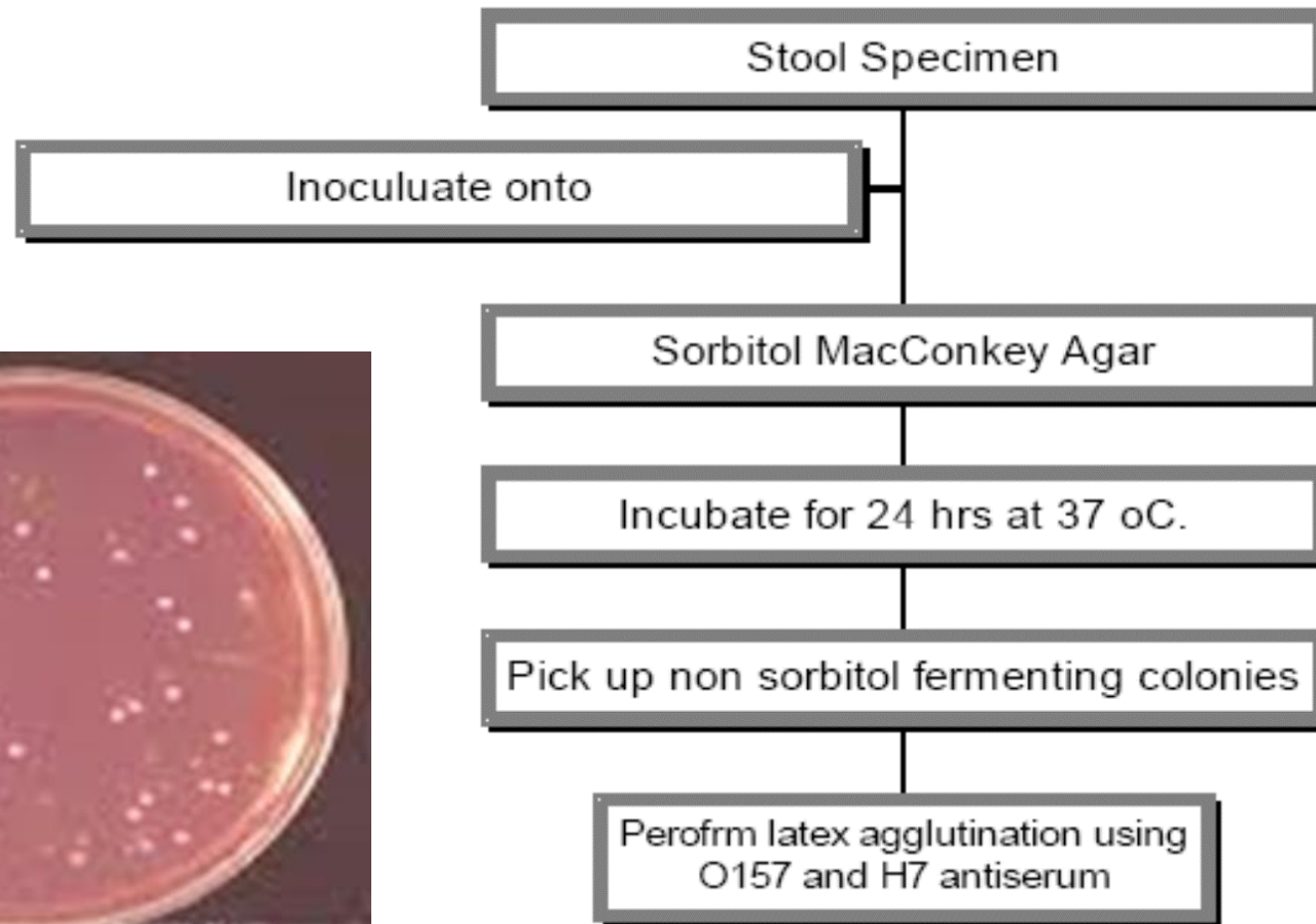
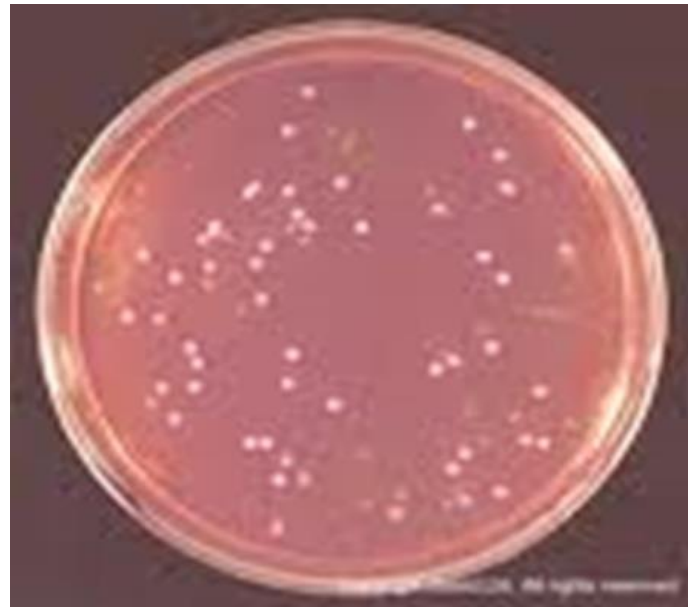
- EIEC and EHEC- invasive organism (causes dysentery)
- Following infection by EHEC, HUS (hemolytic uremic syndrome) is characterized by acute renal failure, anaemia and thrombocytopenia
- *E. coli* O157:H7 is the most commonly recognized serotype
  - Cause outbreaks and sporadic cases
  - Found in raw or undercooked hamburger/beef, spinach, or unpasteurized cider

# ***E.coli* O157:H7**

## **Media**

- Sorbitol MacConkey Agar (SMAC)
- A loopful of stool is streaked on Sorbitol MacConkey agar
- Incubate at 37°C. Under aerobic conditions. Examine plates for non-sorbitol fermenting colonies(NSF)

## Cont...



## Cont...

- 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.
- Negative result - no agglutination of the Test latex.
- Non-interpretable result - clumping of the Control latex.

**Cont...**



**Positive**

**Negative**



**Ag-Ab complex**

# ***Vibrio cholerae***

- Serogroups- O1 and O139
- Biotypes- Classical and El Tor
- Causes cholera: severe dehydration, vomiting, abdominal pain and acidosis
- Due to exotoxin CT cAMP leading to the outpouring of fluid and electrolyte-diarrhoea
- In severe cases, rice water stools (without feacal matter) are passed



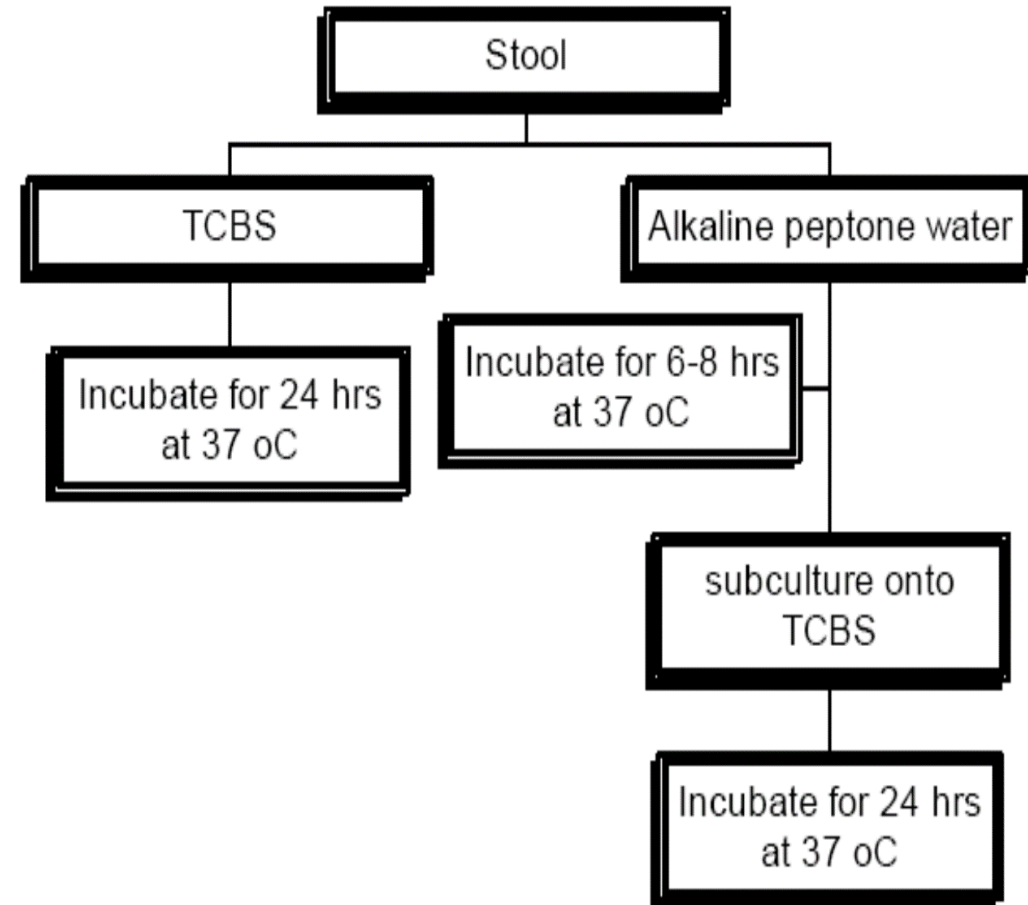
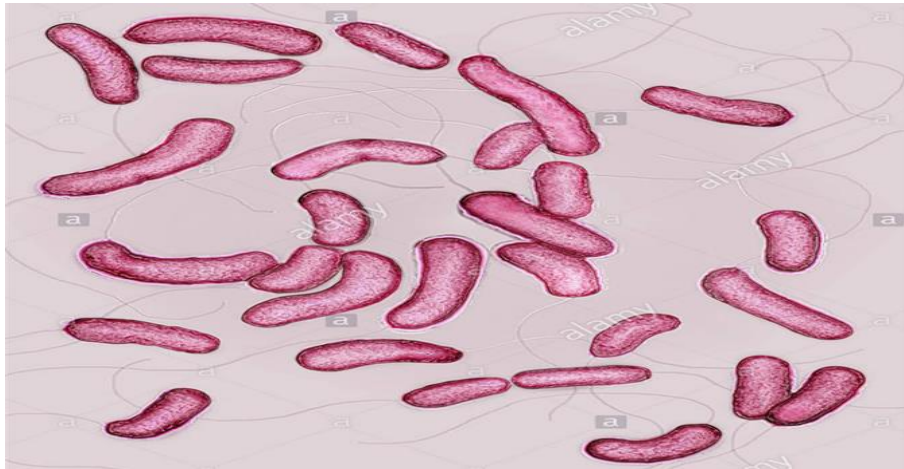
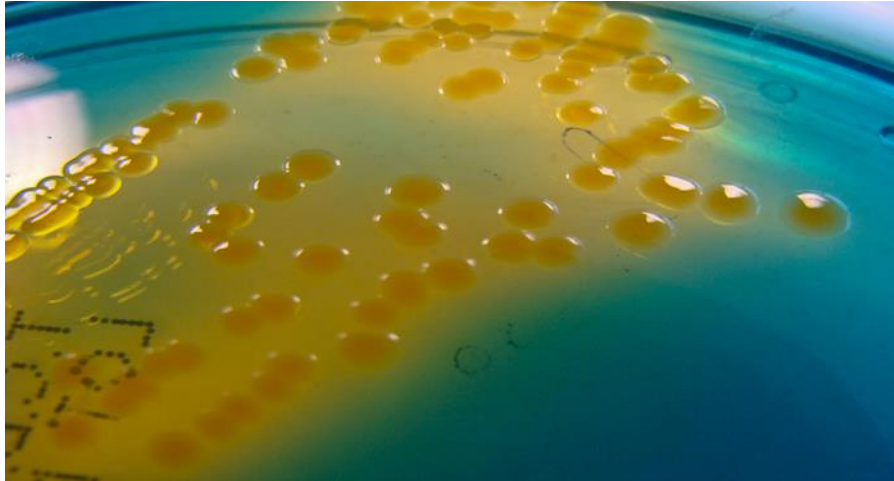
# Laboratory Diagnosis

- Microscopy
  - Gram-negative curved rod
- Culture
  - Alkaline peptone water for enrichment
  - Thiosulfate citrate bile salts (TCBS) agar- yellow colonies
  - MacConkey agar- Non lactose fermenter
- Biochemical tests
  - Oxidase positive
  - Citrate positive

# Cont...

- Serology
  - O1/O139 serogroups
  - Ogawa/Inaba/Hikojima
- Rapid tests
  - SD BIOLINE Cholera O1/O139

# Cont...



*The*

*End...*