Gastro Intestinal System

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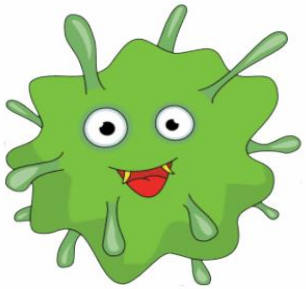
Stool Collection & culture



- When do we need stool samples ?

If the patient has diarrhea , abdominal pain , fever , bloody stool, and vomiting, all these signs confirm the presence of digestive problems.

- The most important difference between urine and stool samples is the sterility. Urine is a sterile sample, but the stool is not sterile.

❑ **Stool should be collected in clean wide mouth container not sterile** (sterile تكون بنفضل العينة culture طلب حال في الا)

□ The stool sample shouldn't still be in the container for more than 30 minutes .Either we inoculate it directly, or we put it in the refrigerator (it can still be for 24 hours without any problems).

Stool should be added to Selenite broth

Why? ?

□ We inoculate the sample in Selenite broth because the stool sample has many normal flora , so if there is a pathogen in the sample , the selenite broth will:

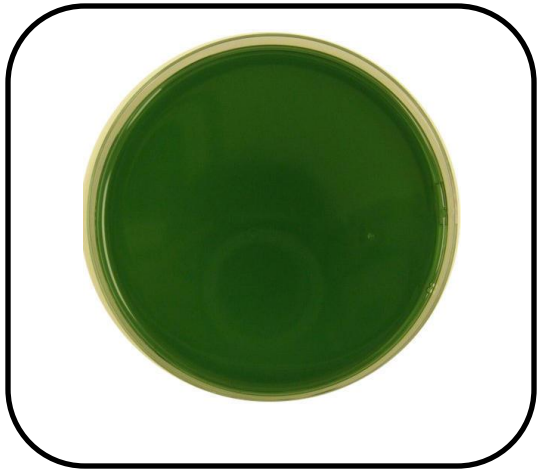
1. ❑ **Inhibits the growth of coliforms** → normal flora
2. ❑ **Enhances the growth of Pathogen**

❖ Most common pathogens (Bacteria) :

- **E.coli** (Macconkey agar) — most common
- **Salmonella** — most common
- **Shigella**
- **Vibrio**
- **Proteus**
- Yersinia , Campylobacter , Clostridium, Bacillus …etc

Stool sample should be cultured on the following media using streak plate method

- once we inoculate the sample in the selenite broth , there are 3 types of media we can plate the sample on :



① S-S agar ② Hekton agar ③ T.C.B.S

- S-S and Hekton agar are considered selective and differential media for Salmonella and Shigella species.

- Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) : selective media for Vibrio species.

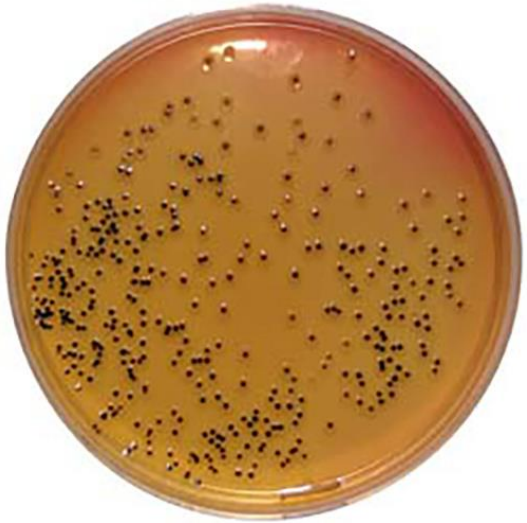
S-S agar

This agar is selective for Salmonella and Shigella spp.

- Yellow colonies with black spots due to H2S production >> Salmonella.
- No black spots >> Shigella

SS Agar Plate
(Salmonella–Shigella Agar)

Salmonella



Shigella

Hekton enteric agar

- Same as S-S agar, but here the color is blue green.

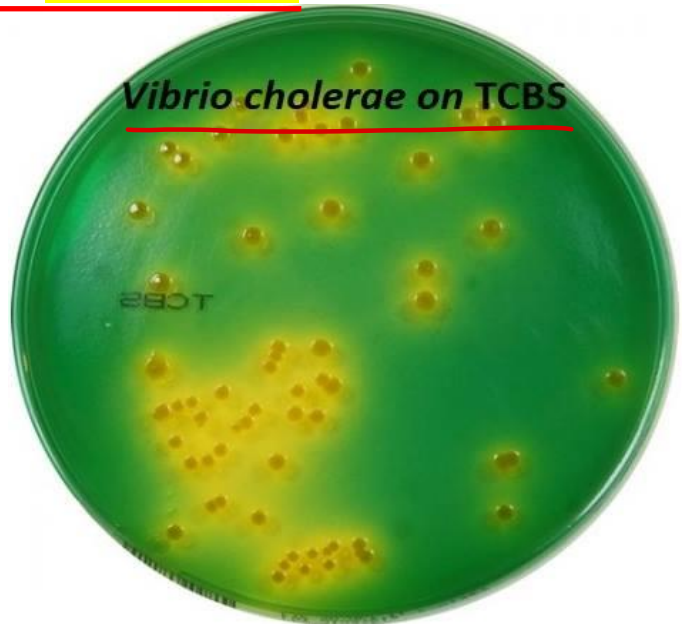
Salmonella

Shigella

- bule green colonies with black spots due to H2S production >> Salmonella.
- No black spots >> Shigella

T.C.B.S media

- Highly Selective for Vibrio Spp. because it is highly alkaline (suitable for vibrio) while other media are neutral .
- Ph (8.5-10)
- **When Vibrio ferment sucrose it turns the media from green to Yellow**



- It is also differential media , it differentiates between vibrio cholera and other vibrio spp.
- vibrio cholera >> ferment sucrose >> yellow colonies.
- other vibrio spp. >> don't ferment sucrose >> green colonies .

ركزوا على الألوان(مهم)

Salmonella

(Red over Yellow).

These are biochemical tests

- **Kligler : red/Yellow + H2S**
- **Urease : Negative**
- **Citrate : Positive**
- **SIM : Positive / Negative / Positive**

H2S Indole Motility

1 kligler

its original color is yellow . If the test is:
positive >> will be pink.
negative >> remain yellow.

2 Urease test

Its original color is green . If the test is:
positive >> will be blue.
negative >> remain gree.

3 citrate test

4 SIM test

■ kligler test : its original color is red , the upper part of the kligler is called "slant," and the lower part is called " botttom".

☆ we inoculate the sample and put it in the incubator for 24 hours, if the :

slant still pink >> non lactose fermenter

slant becomes yellow >> lactose fermenter

bottom still red >> non glucose fermenter

bottom becomes yellow >> glucose fermenter

- In between slant and bottom >> H2S production >> black
- If there are other gases, we will see bubbles.
- 90% of H2S production bacteria are glucose fermenter.

- Lactose fermentation test in the slant because it needs oxygen, while glucose fermentation test in the bottom because it doesn't need oxygen.

So Salmonella is a glucose fermenters, lactose non fermenters, and produce H2S.

■ SIM test : Its original color is transparent.
S (H2S) , I (INDOLE) , M (MOTILITY).

- H2S >> positive >> black color

- Indole >> We add kovacs reagent >> it makes ring on the surface if this ring
turns into red >> indole positive
remains brown (the color of the reagent) >> indole negative

- Motility >> Turbidity
- 90% of H2S production bacteria are motile.

SIM test

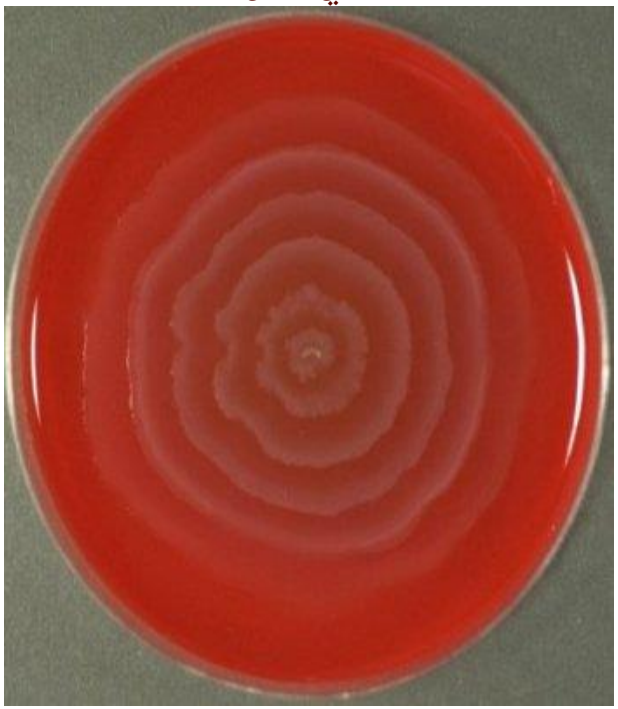
اذا البكتيريا كانت non motile رح تضل مكانها ، بس اذا كانت motile اللون الاسود رح ينتشر بكل ال tube و بكون عامل زي شكل الشجرة

■ Why don't we plate the sample on blood directly?? Because:
1. blood agar is considered as enriched media and cultured , and we said the stool sample has many normal flora , and if we put it in blood media directly, there will be thousands of types of bacteria.

2. 80% of samples are Proteus, which is highly motile (بتفرش على كل الصحن و بتغطي كل اشي تحتها)

Proteus

عاملة زي شكل الوردة



- Gram negative rods , non lactose fermenter
- **Swarming motility (flagellated)**
- Prevent swarming by culturing it on CLED or MacConkey media