MICROBIOLOGY

بسم الله الرحمن الرحيم

FINAL | Lecture #1

Micro Lab

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا ولا تستبدلنا

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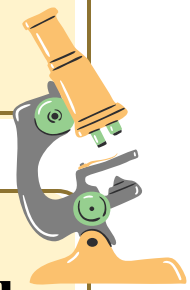


Gastro Intestinal System

Pathogens in the GI system are due to either bacteria/ virus/ parasite
In this lecture we are going to discuss most common bacteria

Stool Sample Collection

- What is the difference between stool and urine?
  The stool is non sterile, while urine is sterile.

- When a stool sample is requested, we should not give them a sterile container however at the same time the container should be clean and suitable for stool collection (not used).

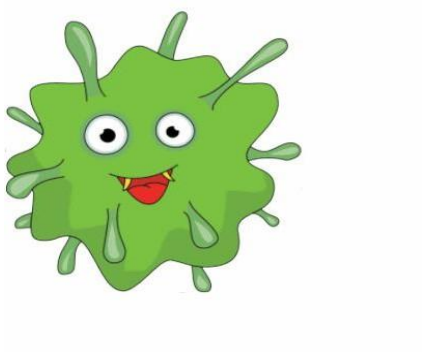
- In a government hospital where there are many patients waiting with their samples, and the samples should be handled **within 30 minutes**, but we need more time to process them, what is the ideal solution?
  We use a medium **salanite broth** which inhibits the growth of normal flora, even though normal flora isn't harmful inside the human body, once it's cultured on media, it can become dangerous in the lab.

Stool Sample Collection

- Stool samples should be handled within 30 minutes because extended exposure to room temperature can kill some bacteria. This may lead to **false-negative results**, where no pathogens are detected even though they are present.

- When a patient comes from another governorate (far location) their hospital should place the sample in medium **(salanite broth)** and it should be stored in refrigerator before the sample is transported.

Stool Collection & culture



- Stool should be collected in clean wide mouth container not sterile

Stool should be added to Selenite broth

Why? ?

- ❑ Inhibits the growth of coliforms/normal flora
- ❑ Enhances the growth of Pathogen

Urine vs Stool Culture Agar

- What is the most important medium we use to culture urine?
  Blood agar, because it is an **enrichment media**, we **don't** use it for stool because it contains normal flora we use **selective media** instead.

- We used selenite broth for stool because it provides inhibition, but it doesn't kill the normal flora (it only reduces their number) so once you subculture it from the broth back onto blood agar, the bacteria will grow and multiply.

We will discuss stool culture in the next slides

❖ Most common pathogens (Bacteria) :

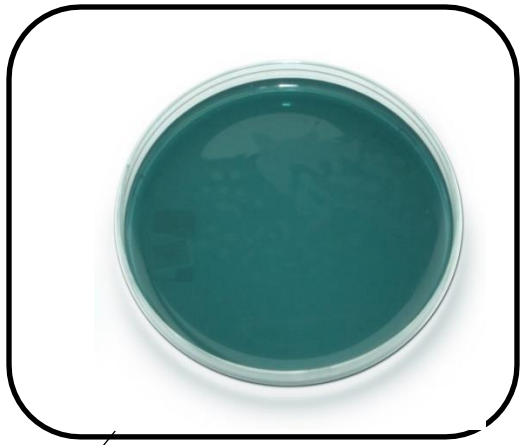
- E.coli
- Salmonella
- Shigella
- Vibrio
- Proteus
- Yersinia , Campylobacter , Clostridium, Bacillus …etc

The most common in Jordan's hospitals are the **E.coli** and **Proteus** and the others are less common because Jordan is clean country and food poisoning cases occur only once every five years.

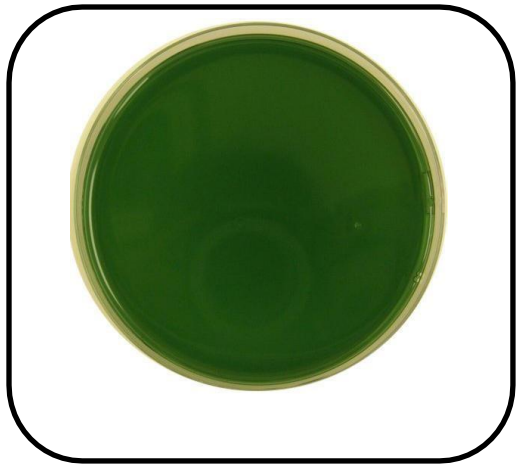
Stool sample should be cultured on the following media using streak plate method

S-S agar

Salmonella – Shigella agar



Hekton agar



T.C.B.S

They are the same. They are considered selective and differential media not enriched media.
Selective because only two types of bacteria grew on the agar.

It is selective media for **vibrio** species

S-S agar

The media is pink

SS Agar Plate
(Salmonella–Shigella Agar)

Salmonella

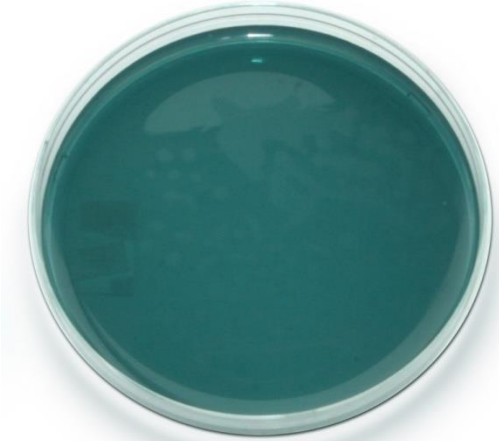
The color is pinkish-yellow with black dots in the middle, indicating H2S production

Shigella

The same color appears but without H2S production

Hekton enteric agar

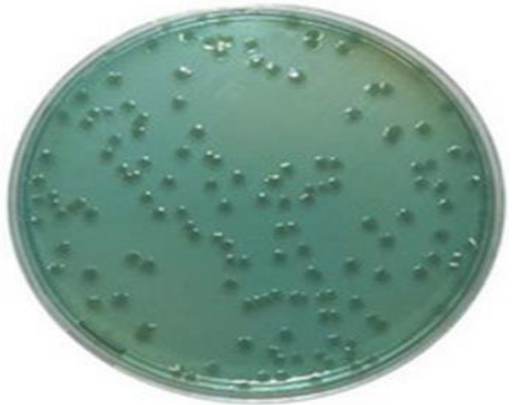
Similar concept to S-S agar



Salmonella

The color is green with black dots in the middle, indicating H2S production

Shigella

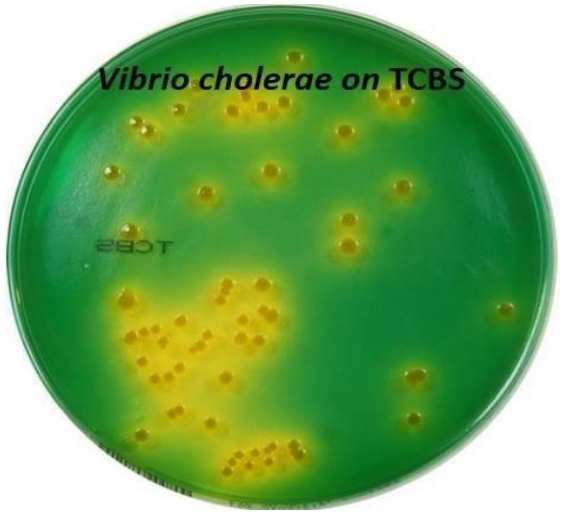


The same color appears but without H2S production

T.C.B.S media

T.C.B.S is an abbreviation for Thiosulfate Citrate Bile Salts Sucrose media

- Selective for Vibrio Spp.
- Ph (8.5-10) It has high PH because vibrios prefer alkaline conditions, while most media are neutral or slightly acidic.
- **When Vibrio ferment sucrose it turns the media from green to Yellow**



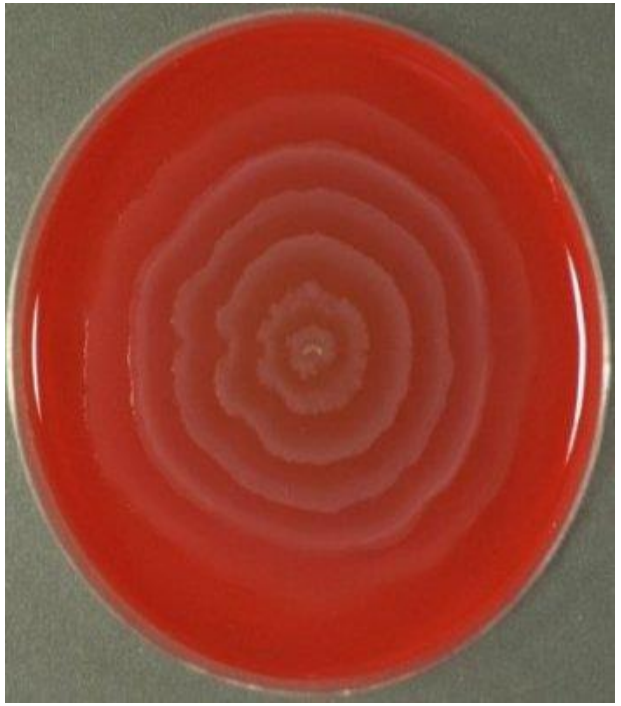
Proteus

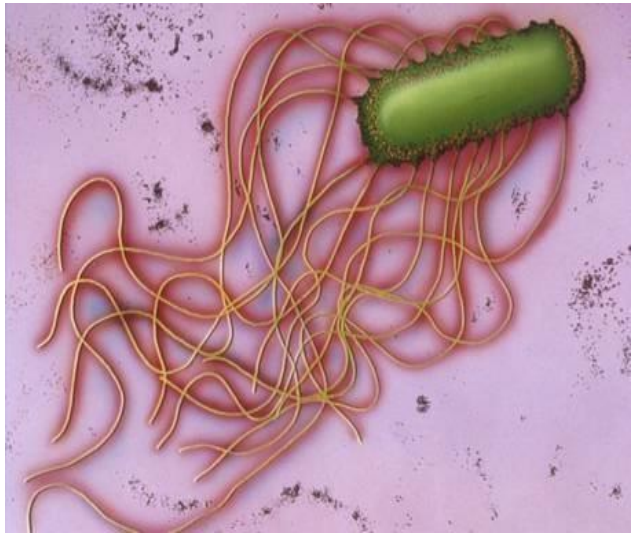
- Gram negative rods , non lactose fermenter

  From the Enterobacteriaceae family

- **Swarming motility (flagellated)**

- Prevent swarming by culturing it on CLED or MacConkey media





Proteus Culture

- We also culture the stool sample on Mac Conkey agar to look for Proteus
- If we culture it on blood agar, Proteus tends to spread rapidly across the plate within the first 6 hrs. What is the impact of leaving it to spread like that?

It overgrows and masks other bacteria on the plate , making it difficult to detect or isolate them.

- So, if stool is cultured on blood agar, it is considered an error, and a new sample should be collected . This spreading behavior is called ‘swarming’.
- How do we prevent swarming?

By culturing on MacConkey agar or on CLED media that contains salts, which inhibits the movement of flagellated bacteria. This way, Proteus no longer spreads across the plate, and grows instead as discrete colonies like other bacteria.

Salmonella

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

Urease test

citrate test

SIM test

Kligler Test

- When we saw colonies on s-s agar with black dots, it's presumptively 80% salmonella but to confirm, we need to perform four biochemical tests :

1) Kligler: the media is poured into tubes while still in liquid form, immediately after being removed from the autoclave. It must be dispensed within five minutes, before it begins to solidify. Before the media sets, the tubes are placed at a 40-degree angle, why?

This step is helpful during fermentation tests. When the medium is inoculated with bacteria, the inoculating needle is inserted to the bottom of the tube and withdrawn upward through the slant. This technique allows for distribution of bacteria in both aerobic (upper) and anaerobic (lower) environments within the same tube.

- It has been found that lactose fermentation requires oxygen, whereas glucose fermentation doesn't require oxygen.

Result Interpretation

The media remains pink in the absence of growth.
In the figure:

- Upper part (the slant) remains pink: indicates no lactose fermentation
- Lower part: color change to yellow indicates glucose fermentation
- Between them : the black color indicates H2S production

If it was shigella the results would be:

- Upper part: pink indicating no lactose fermentation
- Lower part: yellow indicating glucose fermentation
- No H2S formation in between

Urease and Citrate Test

2) Urease Test: it is originally yellow in color. After inoculation:

If it remains yellow : it indicates a negative result

if it turns pink : it indicates a positive result

In Salmonella we would get a negative result

3) Citrate test: the color of the media before inoculation is green. After inoculation, the next day:

If it turns to blue : indicates (+ve) for citrate test

It is a positive result for salmonella

SIM Test

- S: is for H2S production, the color of media is yellowish-transparent before inoculation. (Positive result for salmonella).
- I: indicates indole test , this is a kovac's reagent. On the second day ,we add a drop of kovac's reagent to the surface of tube. A ring forms on the top

  If it turns red: the result is (+ve) for indole test

  If it remains yellowish to brown : it is a (-ve) result

  (Negative result for salmonella)

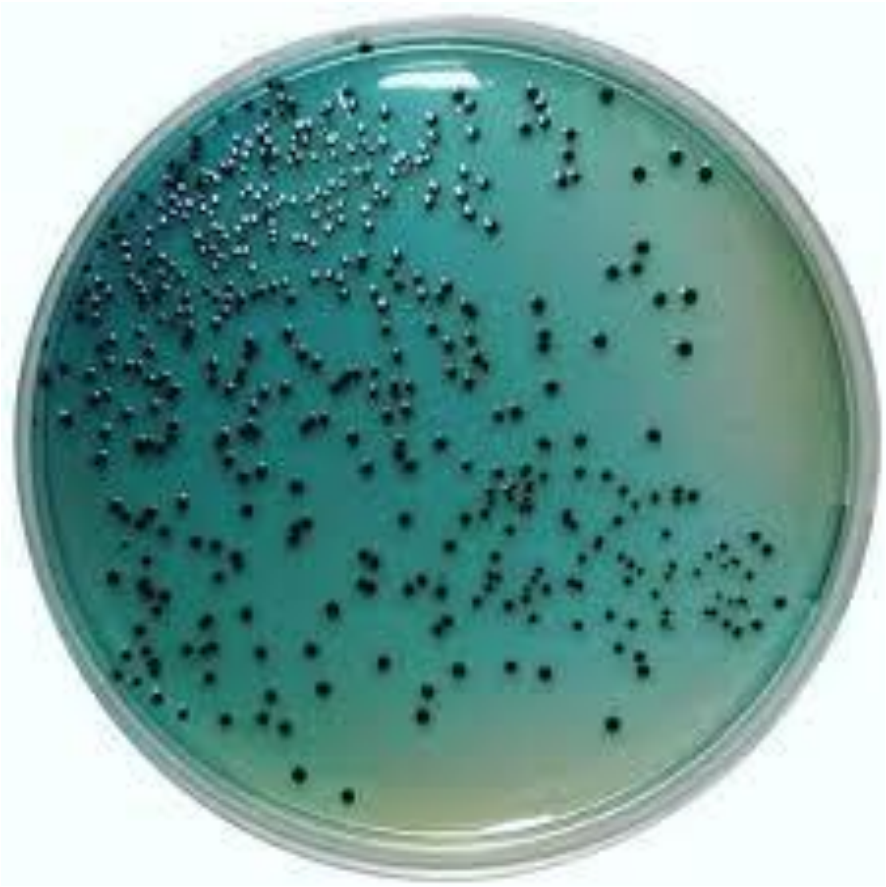
- M: indicates motility

  In this tube ,it is motile because the medium appears turbid not clear. Most H2S producing bacteria are usually motile.

- There is also a special motility test can be done by placing the bacteria on a slide with coverslip and observing under the microscope if it is motile.

Did you spot the pathogen?

Click to test your knowledge!



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

اللهم إنا نسألك أن تحفظ أهل غزة، وأن ترفع عنهم البلاء، وتفرج همهم، وتنفس كربهم. اللهم كن لهم عونًا ونصيرًا، اللهم احفظهم بحفظك، واكلأهم برعايتك. اللهم اجعل لهم من كل ضيق مخرجًا، ومن كل هم فرجًا، وارزقهم الأمن والأمان والسلامة والإيمان.

اللهم إن أهل غزة في حاجة إلى نصرك ورحمتك، فارحم ضعفهم، وقوي عزيمتهم، وثبت أقدامهم. اللهم اشف جرحاهم، وارحم شهداءهم، واربط على قلوبهم، وأعد لهم الأمن والاستقرار.

اللهم كن لهم وليًا ونصيرًا ومعينًا، ولا تكلهم إلى أنفسهم طرفة عين. اللهم فرج كربهم، وادفع عنهم البلاء، وارزقهم الصبر والاحتساب، واجعل لهم من كل ضيق فرجًا ومن كل هم مخرجًا. اللهم انصرهم نصراً مؤزراً، واجعل لهم من لدنك سلطانا نصيراً. اللهم آمين. اللهم منزل الكتاب، سريع الحساب، هازم الأحزاب، اهزمهم وزلزلهم. اللهم إنهم طغوا وبغوا، فشتت شملهم، وفرق جمعهم، ورد كيدهم في نحورهم. اللهم اجعل تدبيرهم تدميرًا عليهم، واجعلهم عبرةً لمن يعتبر.