Microbiology Lab

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As a brief intro: In GI microbiology lab we will be only concerned about 3 types of bacteria: Salmonella, Shigella, and Vibrio cholera, also we'll talk a bit about Proteus. These are the main GI pathogenic bacteria, They are all gram negative bacteria "2 membranes, Endotoxins (AKA: Lipopolysaccharides) on the outer membrane " .

- When we have a sample of **infected stool**, we culture it in a broth (a liquid media مرق).
We use selenite (type of Gypsum جبس that occurs in transparent crystals or crystalline masses) broth for 2 reasons:

1. So that the sample becomes homogenous and as a result we can culture it in an appropriate media.
2. Selenite broth will inhibit the Coliforms (normal flora present in the sample) which I don't want to study, so this **will enhance the growth of the pathogenic bacteria**.

** This will take ±16 hrs. Then, we cultivate the bacteria in a selective media.

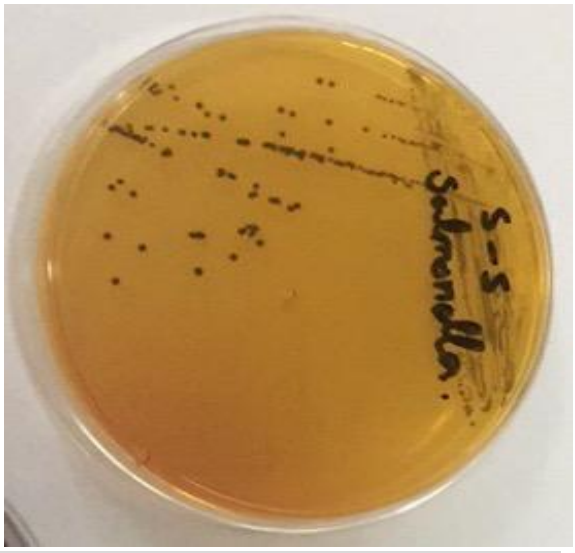
As a refresh, Types of media :

1. Enrichment media: Allows the growth of all types of bacteria present.
2. Selective media: Has inhibitory factors which allow the growth of a specific type of bacteria and inhibits that of other types.
3. Differential media: allows the growth of 2 types or groups of bacteria and present each with a different color.

- After the selenite broth step, we grow the bacteria in a proper media.

1. **SS agar media (yellow):** Called **SS** because it's selective for **S**almonella and **S**higella "both are gram –ve rods from Enterobacteriaceae family". So when we grow bacteria in this media, the manifested colonies will be either that of salmonella or Shigella.

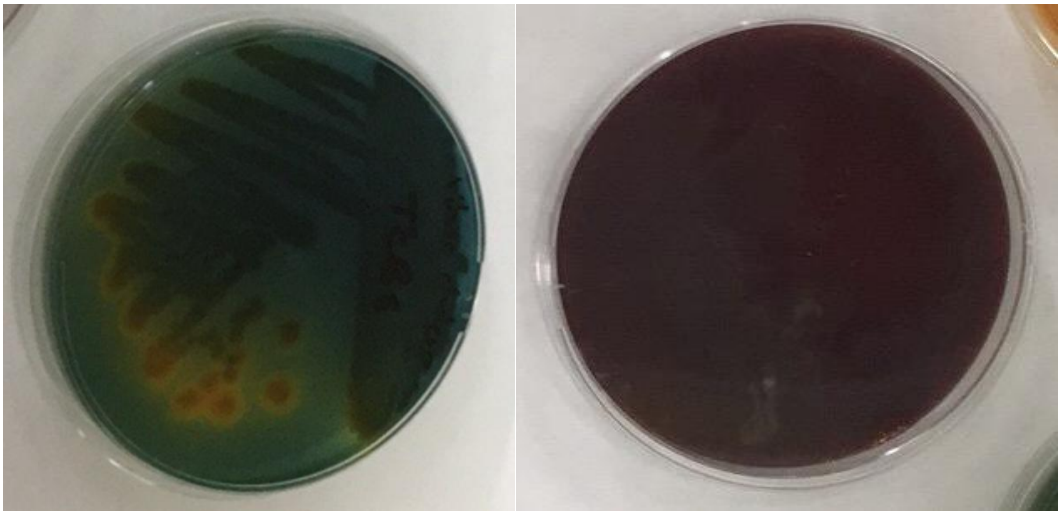
- Difference here is built Upon Hydrogen Sulfide H_2S production:
1. If the bacteria was H_2S **+ve** : Its colonies will appear with black dots (due to the release of H_2S (a -black compound)) → The bacteria will be identified as **SALMONELLA.**
2. If the bacteria was H_2S **-ve** : Colonies with transparent dots → **SHIGELLA.**



2. **Hektoen enteric agar (green) media:** Selective for Salmonella and Shigella; works in the same principle of SS agar media.
   -Green with black dots→ Salmonella
   -Green with transparent dots → Shigella

3. **TCBS** (**T**hiosulfate-**C**itrate-**B**ile salts-**S**ucrose) **agar media:** Selective for Vibrio cholera " comma shaped, Flagellated gram negative bacteria " .
   - In the picture below, A: If the bacteria grew in the way shown; that is having a yellow color (due to fermentation of sucrose in media) then it's vibrio cholera.

4. **Blood agar for Proteus , Seen in B**: Proteus is isolated from a stool or urine sample. It is highly mobile because it's flagellated, so if it's cultured in a blood media it will show swarming phenomena انتشار in which we just do a small streak and it <u>will spread and grow in wave like shape</u>. It has a sharp smell.



A **B**

For further identification, after using any of the media mentioned previously, we go for **biochemical tests**. We take 2 to 3 colonies from the media used before, and culture them in a number of test tubes with different media than the original.
** We incubate them for 24 hrs.

- Biochemical tests:

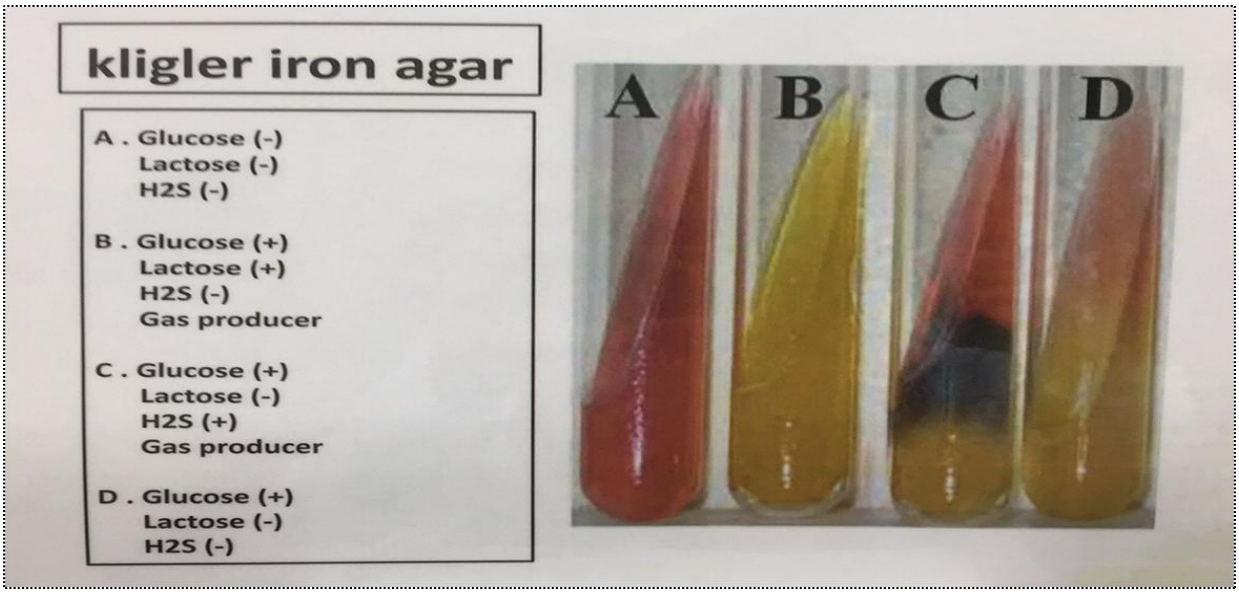
1. Kliglar sugar iron agar (Red): <u>First</u> test to be done. We can tell 4 things about the cultured bacteria:
1) is it a glucose fermenter or not?
2) Is it a lactose fermenter or not?
3) Is it Gas +ve or Gas –ve (does it produce CO_2 or not)?
4) Is it H_2S +ve or H_2S –ve (do we see a black color or not, respectively)?

**** We're supposed to know the 4 things mentioned above for Salmonella, Shigella, and Vibrio Cholera from the table mentioned in page 5**.

- Procedure: We pour the media in the test tube in a way so that the bottom of the test tube (the butt) is filled <u>completely</u> and the upper part (the slant) takes a slant shape مائل as we saw in the lab. Accordingly, the bottom part will have anaerobic conditions (not exposed to air oxygen) for <u>glucose</u> fermentation, and the upper slant part will have aerobic conditions for <u>lactose</u> fermentation. We take 2 to 3 colonies with a loop, insert the loop into the bottom of the media in the test tube, then pull it up and make a streak on the slant part. So: Lactose up ↑↑ , Glucose below↓↓ !

- The next day after incubation, if:
1. Bottom part turned from **red** into **yellow** → **Glucose** fermenter bacteria was isolated
2. Upper slant part turned from **red** into **yellow** → **Lactose** fermenter bacteria was isolated.
3. Bottom part remained red → Glucose **non-fermenter** bacteria was isolated.
4. Upper part remained red → Lactose **non-fermenter** bacteria was isolated.

** If the bacteria released gas, the gas will be collected below the media.
Salmonella and Shigella are glucose fermenters and lactose non-fermenters. So we'll be seeing a **yellow** bottom part and a **red** slant upper part. (In the picture below)



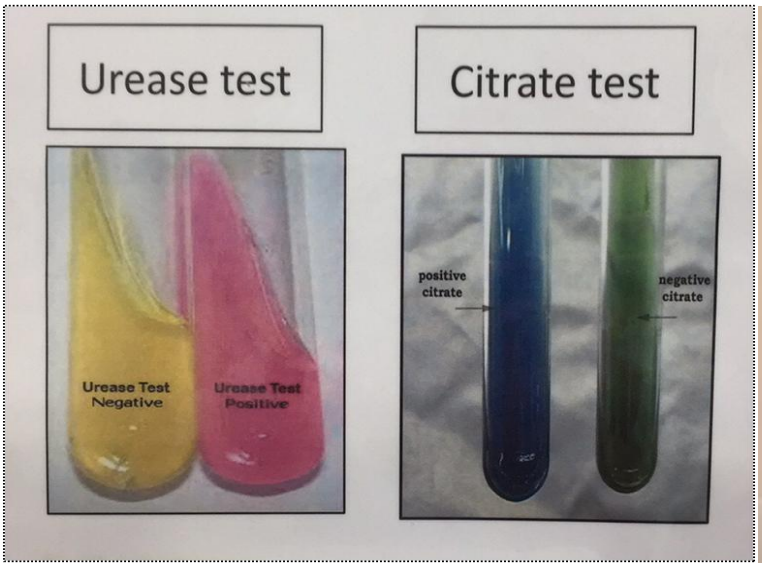
NOTE : You'll see symbols like Y; R; K; A, and things like Y/Y or A/A; R/R or K/K .
1 . Y refers to yellow , A refers to acidic : these results refer to a positive result which means fermentation took place , fermentation of sugars to acids to be precise , which resulted in acidic environment .

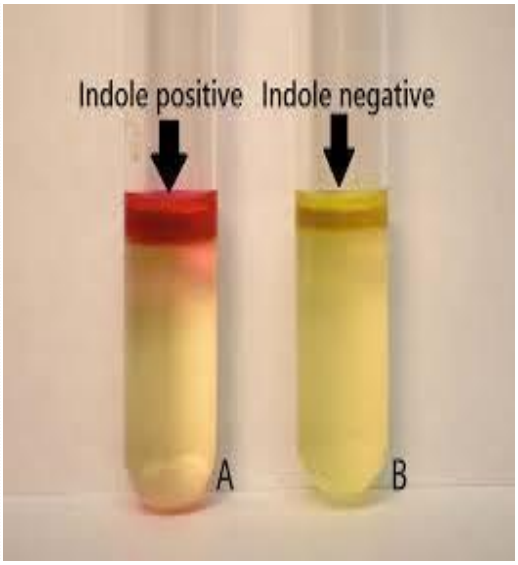
2 . R refers to red , K refers to alkaline : these results refer to a negative result which means fermentation didn't take place, which resulted in alkaline environment .
3. Color/color or Env./ Env. Refers to result in SLANT / result in BUTT .
EX. K/A : first , it is same as R/Y; second, it refers to acidic in slant /alkaline in butt, which tells that the bacteria is lactose non-fermenter / glucose fermenter; then, by referring to the table in the last page we find that the type of bacteria isolated could be : salmonella; shegella; vibrio ; and much more , so we need further tests to identify the species !
2. Urease test (Yellow): Second to be done; picture below, A1. Urease is an enzyme that helps some bacterial species in dissociating Urea into Ammonia and Carbon dioxide, and so increasing pH (especially in the stomach) for more survive and pathogenesis to develop.
- Results: If the color changed from yellow to **pink** → Bacteria isolated is urease **+ve** , while If the color remained yellow → Bacteria isolated is urease **–ve.**

3. Citrate test (Green): Third test to be done; Picture below, A2. It detects the ability of an organism to use citrate as the sole source of carbon and energy.
Use of citrate involves the enzyme citritase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and carbon dioxide (CO2). Production of sodium bicarbonate (NaHCO3) as well as ammonia (NH3) from the use of sodium citrate and ammonium salts results in alkaline pH. This results in a change of the medium's color from green to blue.
So, If the color changed from green to blue → citrate **+ve**, while If the color remained green → citrate **–ve**.

4. SIM test; S for **S**ulfide (H_2S), **I** for **I**ndole, and **M** for **M**otility**: Fourth and last test done**; Picture below, B.

1- If we saw a black color → H_2S +ve " as previous tests " .
2- After incubation, I get Kovac's reagent (transparent in color) and put some on the surface. If the medium gave a pink colored layer "OR a red to red- violet ring "at the top → indole " aromatic compound" +ve
3- If the whole media became turbid معكرة → bacteria is **motile**.
If the turbidity just occurred at the site of loop insertion → the bacteria is considered **immotile**





A 1 **A 2** **B**

** Please make sure to study the results of the four biochemical tests with each of Salmonella, Shigella, and Vibrio cholera from the table below.

Tentative differentiation of commonly isolation clinical aerobic enteric bacilli by means of Kligler's iron agar and other biochemical tests during 24-hours incubation at 37°C

Organisms	Slant	Butt	Gas	H_2S	Urease	Citrate	Indole	Motility	Oxidase
E.coli	Y*	Y	±	–	–	–	+	±	–
Citrobacter spp.	Y*	Y	+	+	W	+	–	±	–
Enterobacter-serratia	Y*	Y	±	–	–	+	±	±	–
Klebsialla spp.	Y*	Y	±	–	+	+	–	–	–
Proteus spp.	R	Y	+	+	+	±	±	+	–
Morganella spp.	R	Y	–	–	+	–	+	+	–
Providencia spp.	R	Y	±	–	+	+	+	+	–
Salmonella spp.	R	Y	+	+	–	+	–	±	–
Shigella spp.	R	Y	–	–	–	–	±	–	–
Pseudomonas spp.	R	R	–	–	–	–	–	+	+
Vibrio cholera	R	Y	–	–	–	±	+	+	+
Acinetobacter	R	R	–	–	–	+	–	–	–

Y= YELLOW , Y* =Few strains may be fermented after 24 hours , R= RED , W= WEAK.

Best of luck *_* ♥