

# Microbiology lab

Done by Mohammed Nawaiseh

ft. Mohammed Darwesh.

- Salmonella, shigella and vibrio cholera.
- Estimated time to read: 10 - 20 mins .

# Salmonella, shigella and vibrio cholera only

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

+ Black      orange ⊖      blue ⊕      ⊕ pinking  
 ⊕ pink      green ⊖

Organisms	Slant	Butt	Gas	H <sub>2</sub> S	Urease	Citrate	Indole	Motility	Oxidase
<i>E. coli</i>	Y*	Y	±	-	-	-	+	±	-
<i>Citrobacter spp.</i>	Y*	Y	+	+	W	+	-	±	-
<i>Enterobacter-serratia</i>	Y*	Y	±	-	-	+	+	-	-
<i>Klebsiella spp.</i>	Y*	Y	±	-	+	±	±	+	-
<i>Proteus spp.</i>	R	Y	+	+	+	-	+	+	-
<i>Morganella spp.</i>	R	Y	-	-	+	+	+	+	-
<i>Providencia spp.</i>	R	Y	±	-	+	+	-	±	-
<i>Salmonella spp.</i>	R	Y	+	+	-	+	-	±	-
<i>Shigella spp.</i>	R	Y	-	-	-	-	-	+	+
<i>Pseudomonas spp.</i>	R	R	-	-	-	-	-	+	+
<i>Vibrio cholera</i>	R	Y	-	-	-	±	+	+	+
<i>Acinetobacter</i>	R	R	-	-	-	+	-	-	-

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

SIM

H<sub>2</sub>S + Indol + Motility

Fenol red  
 6-8  
 API → API  
 Fermentation, Lactose  
 glucose

- **Salmonella, shigella and vibrio cholerae** → lactose –ve (**red** slant), glucose +ve (**yellow** butt), urease –ve (**orange**).
- **Salmonella, shigella** → oxidase –ve.
- **vibrio cholerae** → oxidase +ve, indole +ve
- **Salmonella** → gas production, H<sub>2</sub>S production (black), citrate +ve (**blue**).
- **Shigella** → not motile + indole (+, -).

- **Klegler tube:**

1) **RED** colored Tube → بتميله بزاوية ( slightly rotated )

2) butt (Tube ال اسفل) → you have to observe if it's a glucose fermenter or non glucose fermentor

# glucose fermenter → **yellow** in color (forms acidic medium)

# glucose non-fermenter → stays red (no change in color) .

3) slant (Tube ال اعلى) → know if lactose fermenter or not lactose fermentor

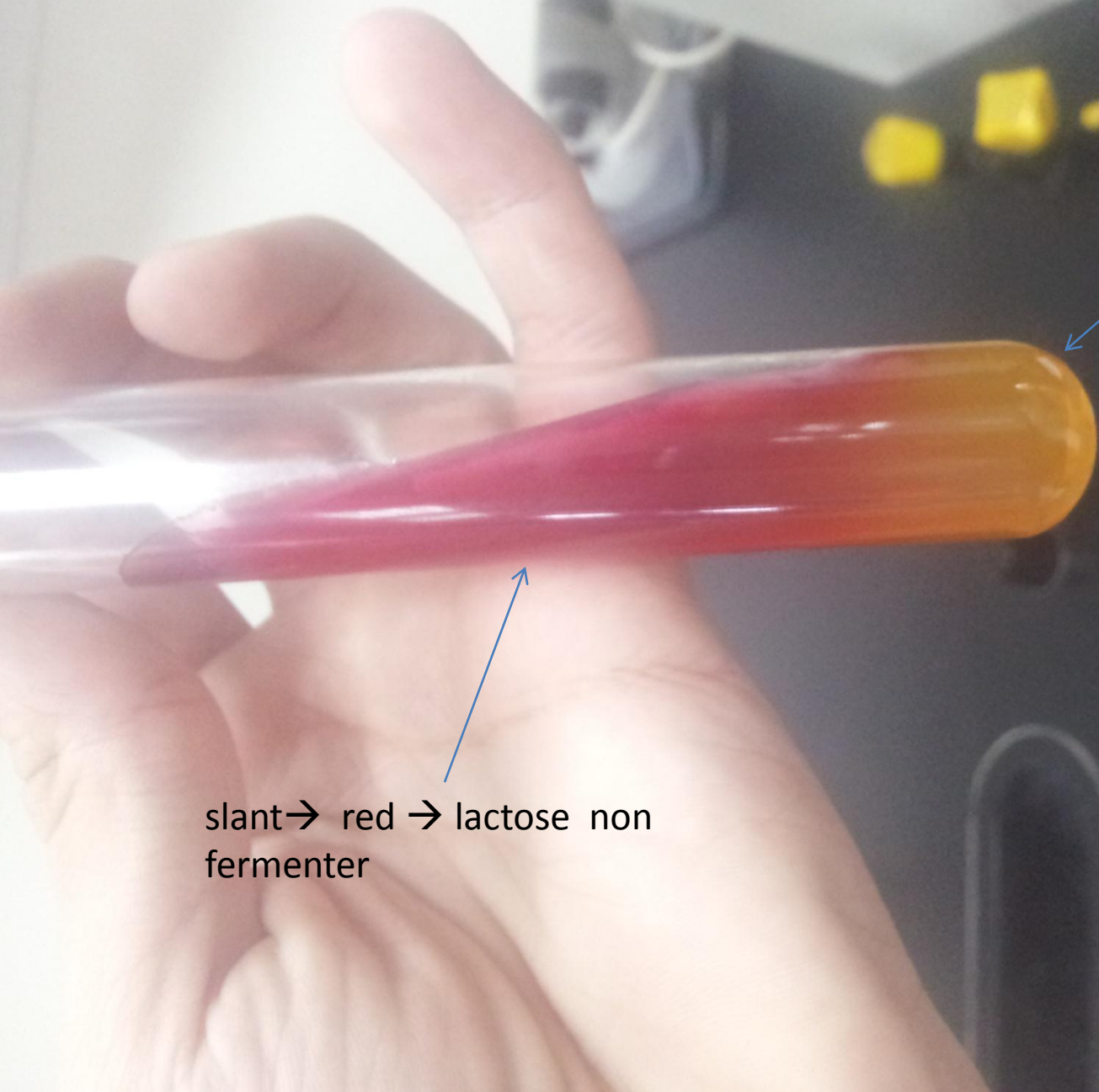
# lactose fermenter → **yellow** in color (forms acidic medium)

# lactose non-fermenter → stay red (no change in color) .

- There is no oxygen In the lower part of the tube (butt) ,which tell us that the medium there is **anaerobic** ;so the test there indicates if the bacteria is glucose fermenter or not.
- What about the upper part of the tube ? (slant) there is oxygen ,so the medium there is **aerobic** ;so the test there indicates if the bacteria is lactose fermenter or not.
- If the color changes to yellow (acidic medium) that indicates that the rxn happened and the bacteria is glucose \ lactose fermenter .

Butt → yellow (acidic medium) → glucose fermenter.

slant → red → lactose non fermenter



shigella

lactose non-fermenter, glucose fermentor, no  
black dots, no gas production → shigella







Gas production test →  
bubbles in the tube.



- **S-s agar** : salmonella-shigella ;if there are black dots--->salmonella .  
#if the lines (not the whole dish ) are transparent ---->shigella.
- **Heckton-enteric agar** : green agar #if there are (black + green) dots--->salmonella .  
#if the lines (not the whole dish ) are transparent , or green with no black dots (no H<sub>2</sub>S production)---->shigella .
- **Widal test → salmonella typhi (not gastro enteritis) : Titer > 160**  
**Antigens** : O (cell wall) , H (flagellar), Vi (virulence) .
- **Salmonella** → because it grows in the bottom of the culture (non aerobic)(glucose fermenter) →lactose non fermenter→yellow colour ; but because Salmonella→produce H<sub>2</sub>S →black colour ,this black colour will remove the yellow colour from the glucose fermenter rxn (only black will appear)→that means Salmonella produces H<sub>2</sub>S and its glucose fermentor

Salmonella → s-s agar → black dots (H<sub>2</sub>S)



Salmonella → s-s agar → black dots (H<sub>2</sub>S)



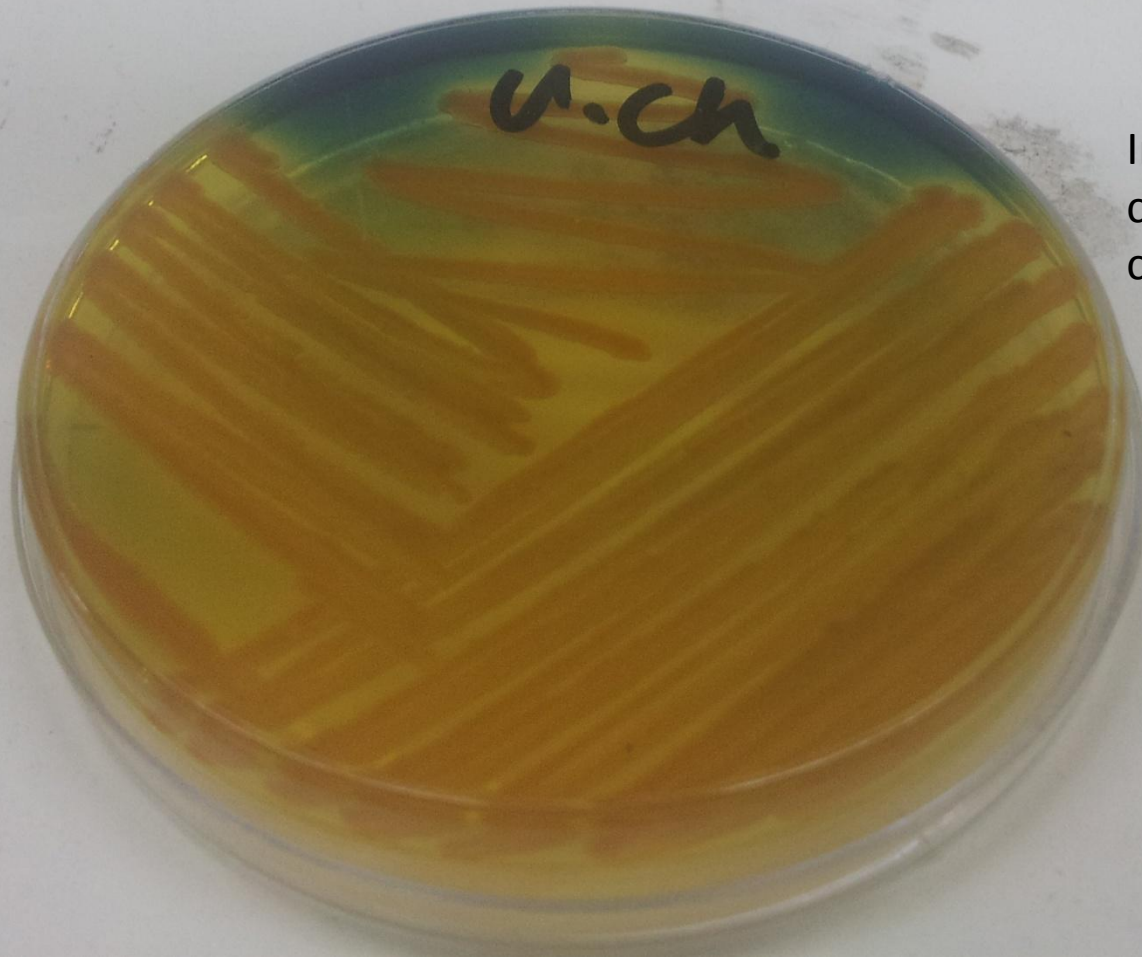
shigella → s-s agar → transparent lines (not the whole dish )



**Vibrio cholera** → s-s agar → transparent lines (not the whole dish) → can't be distinguished from shigella, UNLESS we know further information, which is :

#Vibrio cholera → motile (long flagellum), **oxidase +ve**.

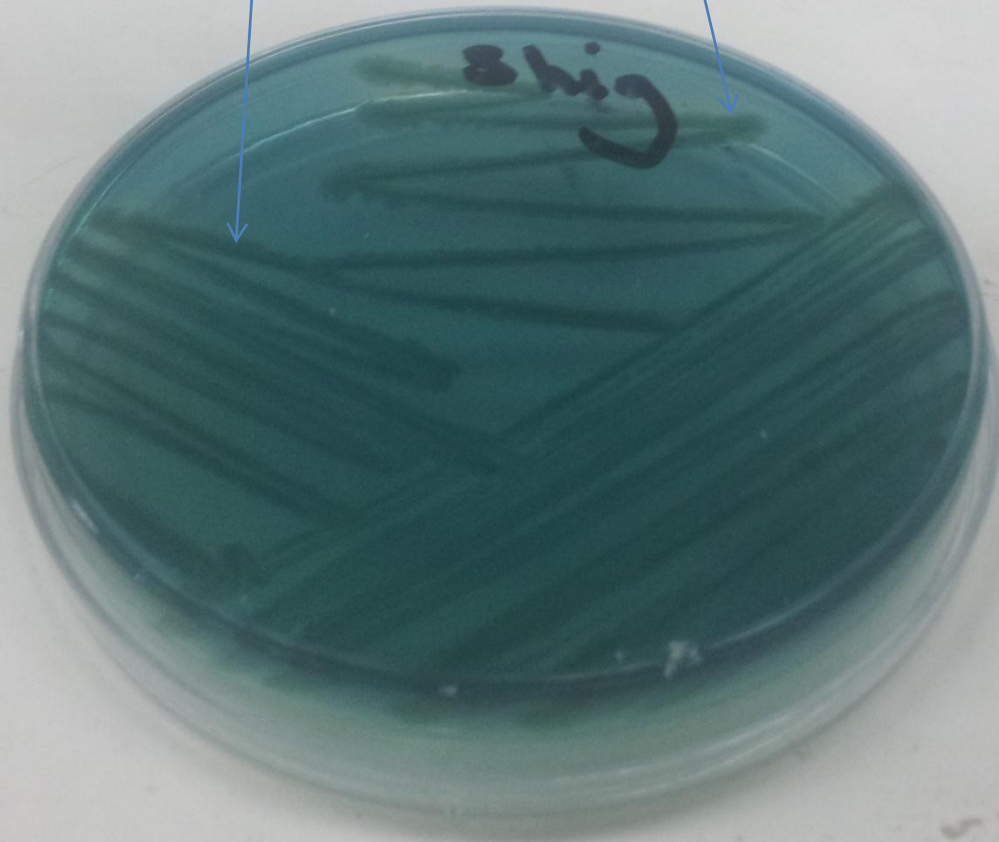
#Shigella → non motile, **oxidase -ve**.



In TCBS medium → vibrio cholera → yellow golden colonies

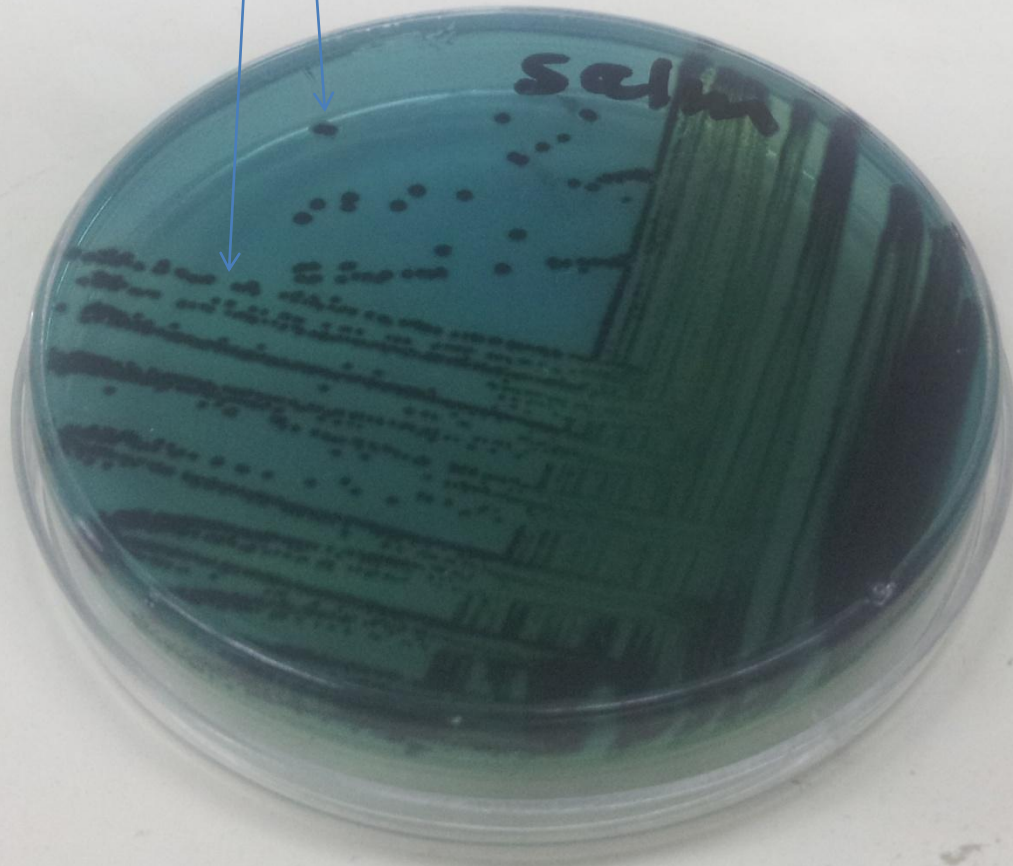
- Gas production test → bubbles observed in the tube.

**Shigella** → Heckton-enteric agar : a green agar  
#if the lines (not the whole dish ) are transparent or  
green with no black dots (no **H<sub>2</sub>S production**) →  
**shigela** , transparent lines (not the whole dish )





salmonella → Heckton-enteric agar : green agar  
#if there are ( black + green ) dots ---> salmonella .

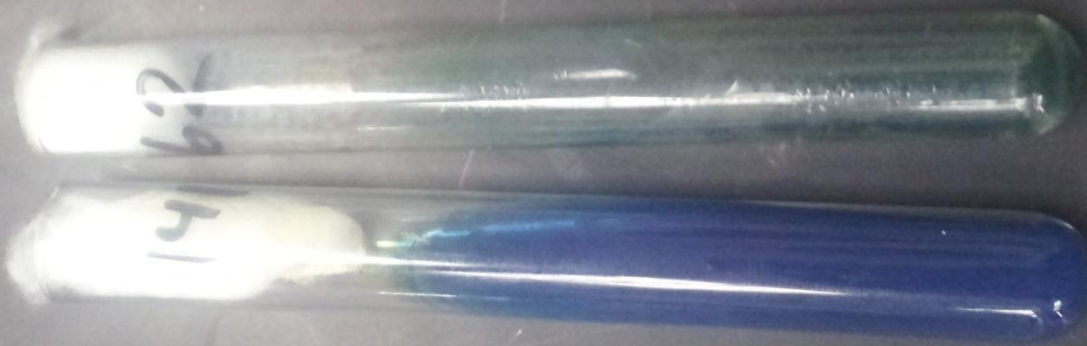


- Citrate test:
  - ve → green
  - +ve → blue
- Urease:
  - +ve → pink
  - ve → orange
- SIM:S → H<sub>2</sub>S production.
  - I → indole → pink ring.
  - M → motility.

# Citrate test:

-ve → green

+ve → blue



-ve → green

+ve → blue

Urease:

+ve → pink

-ve → orange



-ve → orange



+ve → pink

SIM:S  $\rightarrow$  H<sub>2</sub>S production.

I  $\rightarrow$  indole  $\rightarrow$  pink ring.

M  $\rightarrow$  motility.



H<sub>2</sub>S  
production.



M  $\rightarrow$  motility.

I  $\rightarrow$  indole  $\rightarrow$  pink ring.

NO H<sub>2</sub>S  
production.

SIM:S → H<sub>2</sub>S production.

I → indole → pink ring.

M → motility.



This picture is most likely to be shigella because its not motile.

In this picture there is indole ring ,there is no motility and there is no H<sub>2</sub>s production

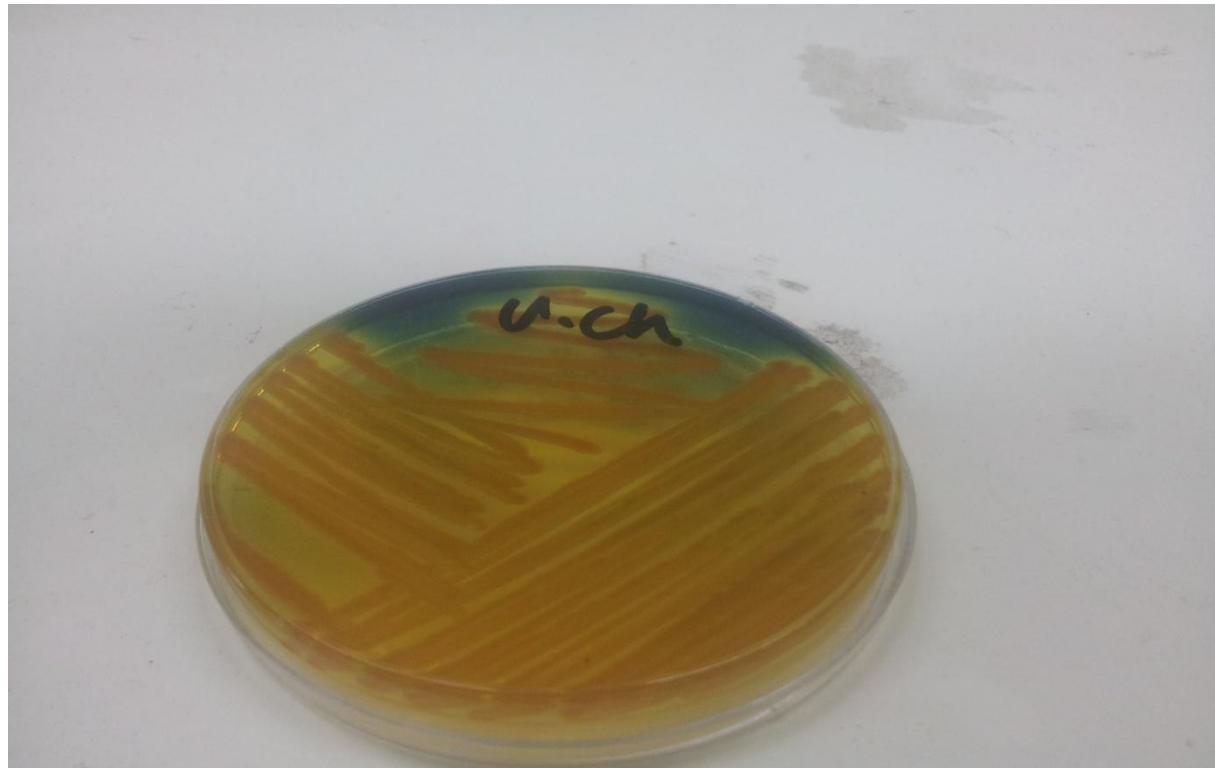
In this picture there are (1) indole ring (the pink ring in the top) , (2) there is no motility; because the white column in the middle of the tube has the same shape of inoculated needle and if it was motile it will move to all parts of the tube (not only where we put the needle in the tube) and there is no H<sub>2</sub>S production (no black dots)



- *Vibrio cholera* → motile (long flagellum), oxidase +ve.
- *Shigella* → non motile, oxidase -ve .
- *Salmonella* → motile (peritrichous flagella), oxidase -ve .



- TCBS agar → *Vibrio cholera* → yellow ; because its sucrose fermenter.



# Salmonella, shigella and vibrio cholera only

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

+ Black      orang<sup>+</sup> ⊖      blue ⊕      ⊕      pink<sup>+</sup> ⊖      blue ⊕      green ⊖      pink<sup>+</sup> ⊕

Organisms	Slant	Butt	Gas	H <sub>2</sub> S	Urease	Citrate	Indole	Motility	Oxidase
<i>E.coli</i>	Y*	Y	±	-	-	-	+	±	-
<i>Citrobacter spp.</i>	Y*	Y	+	±	W	+	-	±	-
<i>Enterobacter-serratia</i>	Y*	Y	±	-	-	+	±	±	-
<i>Klebsiella spp.</i>	Y*	Y	±	-	±	+	+	-	-
<i>Proteus spp.</i>	R	Y	+	+	+	±	+	+	-
<i>Morganella spp.</i>	R	Y	-	-	+	-	+	+	-
<i>Providencia spp.</i>	R	Y	±	-	+	+	+	+	-
<i>Salmonella spp.</i>	R	Y	+	+	-	+	-	±	-
<i>Shigella spp.</i>	R	Y	-	-	-	-	±	-	-
<i>Pseudomonas spp.</i>	R	R	-	-	-	-	-	+	+
<i>Vibrio cholera</i>	R	Y	-	-	-	±	+	+	+
<i>Acinetobacter</i>	R	R	-	-	-	+	-	-	-

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

SIM

H<sub>2</sub>S + Indol + Motility

Fenol red  
 6-5  
 +1 → sp1  
 Fermentation Lactase

- Sorry for any mistake(s) .

# Dedication

- Mo2nes badaenah, mohammed darwesh, ahmad masri , mo3taz el ibraheem, abduallah shurman , osama shakhaterh, mohammed salameh, mohammed abu alia, ali khreasat , muhand haddaden, 7amzeh ma7afdah, baha2 shrayedh, murad khderat, ahmad tarawneh, yousef abdeh(abu 9arah), abduallah awad, rami afifi, jameel sa7ori, 3ala2 sha3ban, sura quaqazeh, elaf bataenh, ola zaqebah, naderah turk , lujian 9arrar, deemah nsour(abu zuhair), bara2h mal7as, rand khawldeh.
- to my awesome group 6b and off course wont forget the spectacular correction team ! , el classic and abu yamen a7la dedication .