Practical 7

Biochemical properties testing

1. part

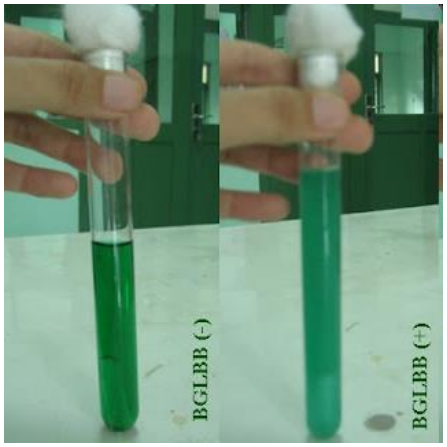
Tests for biochemical properties and metabolic activity testing

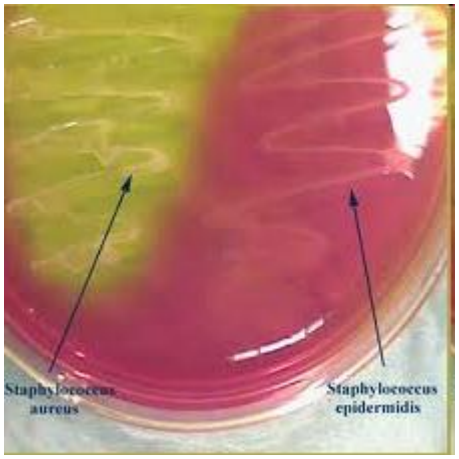
Aim – final identification
Method – subcultivation on the series of testing diagnostic meida

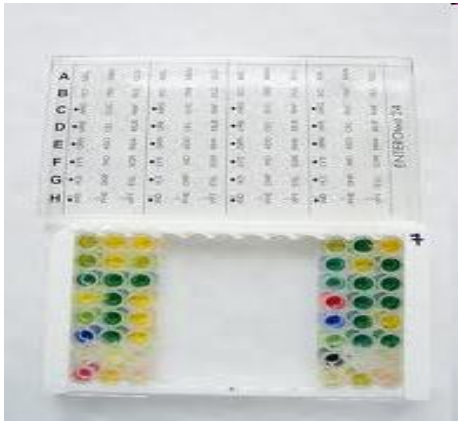
-liquid media – with chemical structure – substrate and indicator,

-solid media with biochemical – metabolic substrate and indicator

-diagnostic disc with substrate, micromethods – liquid media with substrate and indicator in microwells
Algorithm – of chosen procedures







Demonstration

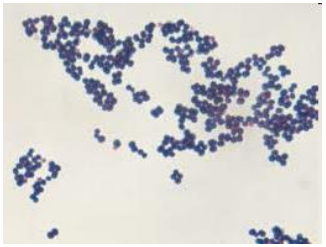
G+cocci:

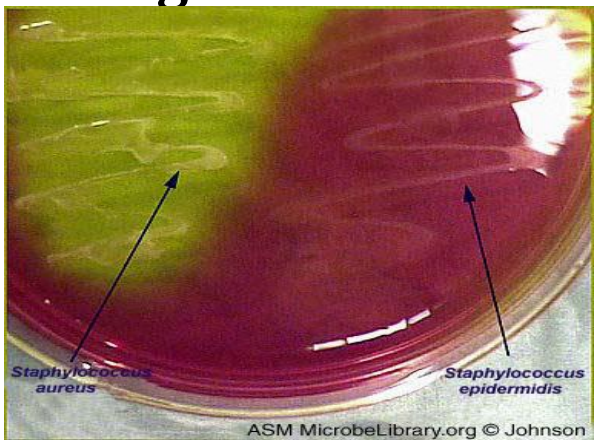
Staphylococcus aureus a Staphylococcus epidermidis on the selective-diagnostic medium Salt mannit: selectively NaCl allowed growing of staphylococci that tolerate it while others do not

mannitol is the diagnostic substrate utilised by St. aureus which metabolised it, formed acid that makes the medium becomming acid and change the pH and indicator color (from pink → yellow)

St. aureus changes the original red color to yellow,

St. epidermidis is growing on the medium, tolerates salt without changing the pH and indicator color - not utilising manitol





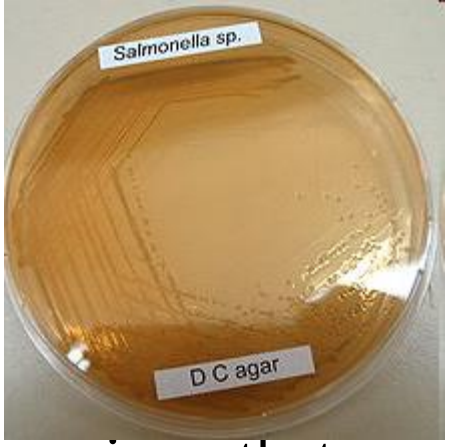
ENDO agar

- differential
- selective
- detection of coliform and other enteric microorganisms

5. ENDO agar

E. coli *Shigella dysenteriae* *Salmonella typhi* *Proteus vulgaris*

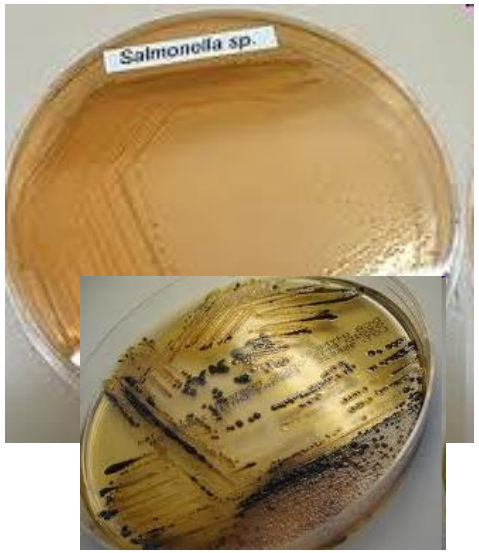
DCA agar

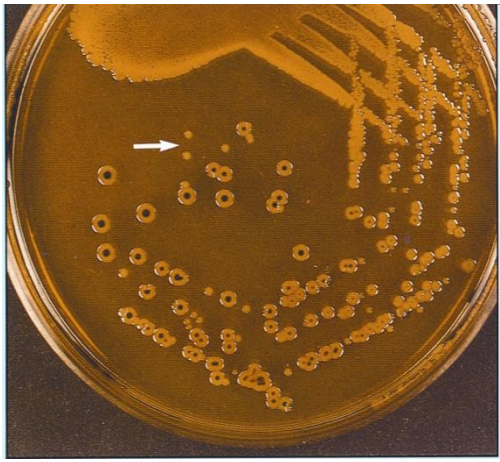


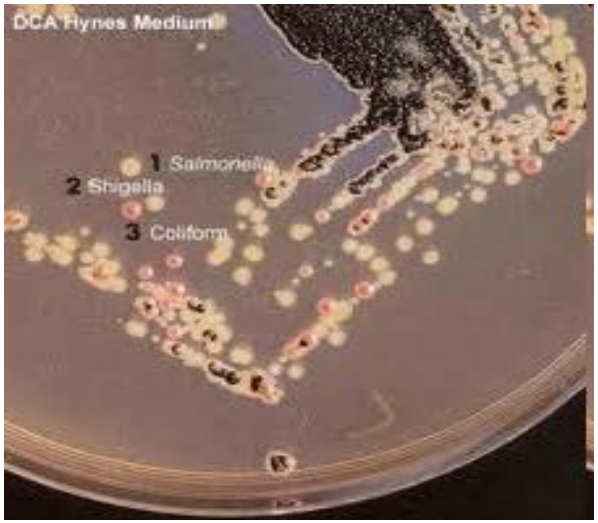
- It is particularly useful for the isolation of organisms that cause bacilliary dysentery, salmonella strains that cause food poisoning and *Salmonella* Paratyphi. It is not so selective for *Salmonella* Typhi. This growth medium is inhibitory to most gut bacteria, in particular species of the genus *Proteus*, although these species do survive on DCA agar. *Salmonella spp* appear to be yellow or colourless colonies, often with a dark centre. As there are many bacteria that also look like Salmonella on DCA, it is widely recommended that more selective agars are used for the identification of *Salmonella*, namely xylose lysine deoxycholate (XLD) agar.

DC agar

E. coli *Shigella dysenteriae* *Salmonella typhi* *Proteus vulgaris*







TSI agar

- The **Triple Sugar Iron** or **TSI**
- to test microorganism's ability to ferment sugars and to produce hydrogen sulfate.
- It is often used in the selective identification of enteric bacteria including Salmonella and Shigella.
- test tube that contains
- agar
- pH-sensitive dye (phenol red),
- 1% lactose, 1% sucrose, 0.1% glucose,
- sodium thiosulfate and ferrous sulfate

Surface of tube medium – aerobe environment, lactose negative bacteria do not ferment, it is alkaline, red

Lower part - in anaerobe environment, enterobacteria ferments glycids – acidic – yellow or black H2S .

1 negative control 2 Ps.aeruginosa : net fermenting - red

3 Shigella sonnei: H2S - negat.,gas – negat., TSI – acid/alcalic red/yellow

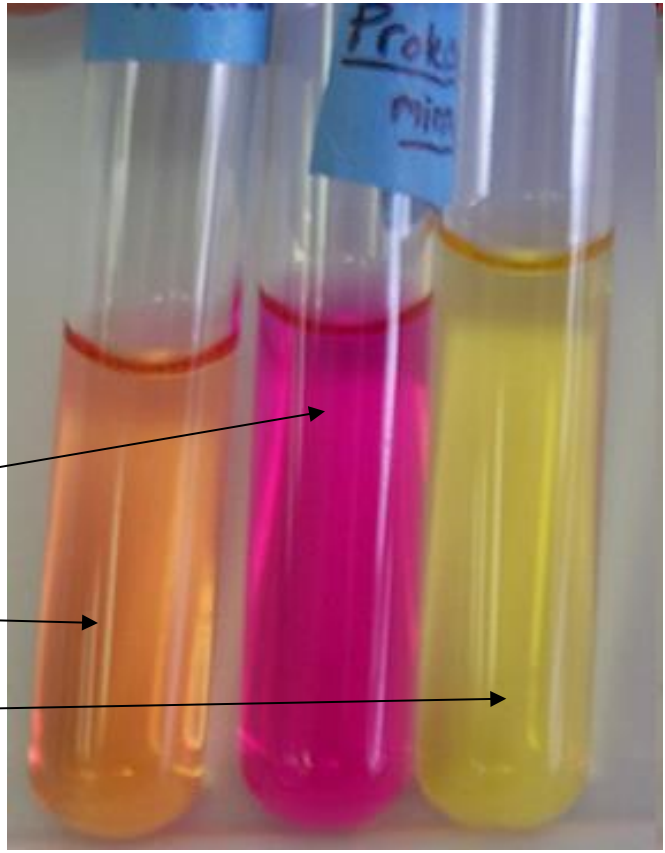
4 Salmonella typhi: H2S – pozit., gas–negat., TSI – acidic/alcalic red/yellow

5 Escherichia coli: H2S – negat.,gas -posit., TSI – acid/acidic red/red

6 Proteus mirabilis: H2S – posit, gas - negat., TSI – acid /acid red/red

Urease test

- This test is used to identify bacteria capable of hydrolyzing urea using the **enzyme urease.**
- It is commonly used to distinguish the genus *Proteus* from other enteric bacteria.
- The hydrolysis of urea forms the weak base, ammonia, as one of its products. This weak base raises the pH of the media above 8.4 and **the pH indicator, phenol red, turns from yellow to pink.**
- *Proteus mirabilis* is a rapid hydrolyzer of urea (center tube pictured here).
- The tube on the far right was inoculated with a urease negative organism and
- the tube on the far left was uninoculated.



Helicobacter pylori

has urease activity that hydrolyse urea (making so a good environment– NH4 – for surviving in acidic stomach

Simmon's Citrate Agar

- This is a defined medium used to determine if an organism can use citrate as its sole carbon source.
- It is often used to differentiate between members of *Enterobacteriaceae*. In organisms capable of utilizing citrate, the **enzyme citrase** hydrolyzes citrate into oxaoloacetic acid and acetic acid. The oxaloacetic acid is then hydrolyzed. If CO2 is produced, it reacts with components of the medium to produce an alkaline compound.
- **The alkaline pH turns the pH indicator (bromthymol blue) from green to blue.**
- This is a positive result, the tube on the right is **citrate positive**. *Klebsiella pneumoniae* and *Proteus mirabilis* are examples of citrate positive organisms.
- *Escherichia coli* and *Shigella dysenteria*e are citrate negative.

THE END