Lab 5: Biochemical Tests in Microbiology

Faculty of Medicine
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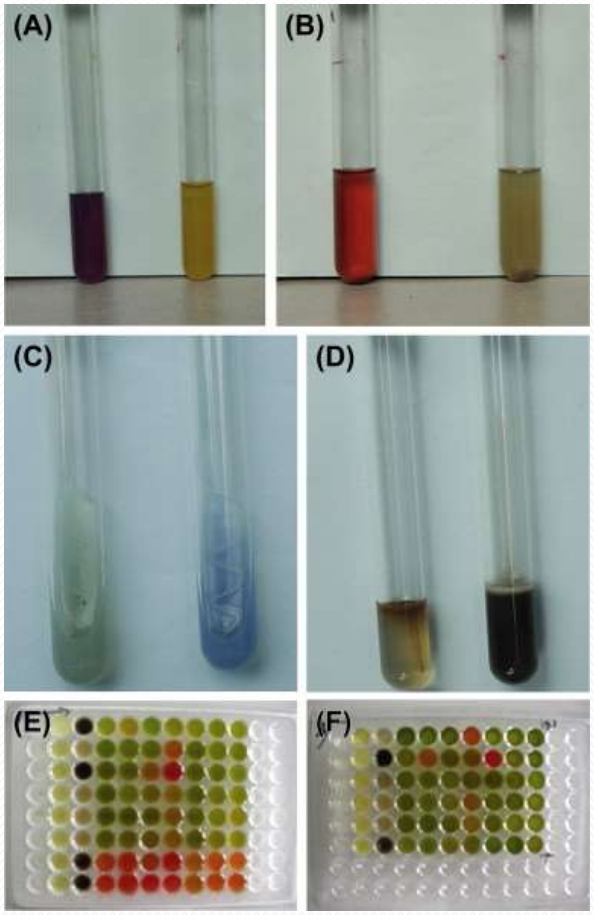
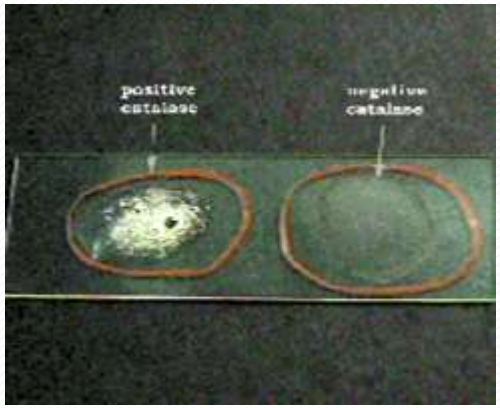
Introduction

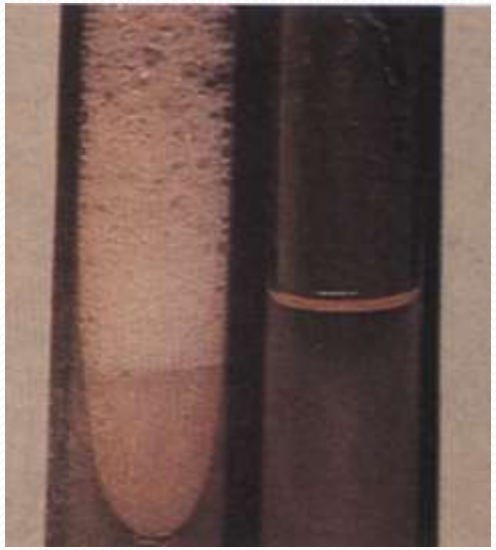
- Biochemical tests are the tests that are performed on different bacteria for their identification on the basis of their biochemical activities towards different biochemical compounds
- Tests include:
  - Enzymatic reaction
  - Metabolism
  - Carbohydrate fermentation
  - Motility tests



1. Catalase Test

- This test is used to identify organisms that produce the enzyme, catalase
- This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas
- Place a drop of H_2O_2 on the culture. A positive reaction show gas bubbles
- Often used to differentiate *Streptococcus* (catalase -) from *Staphylococcus* (catalase +)

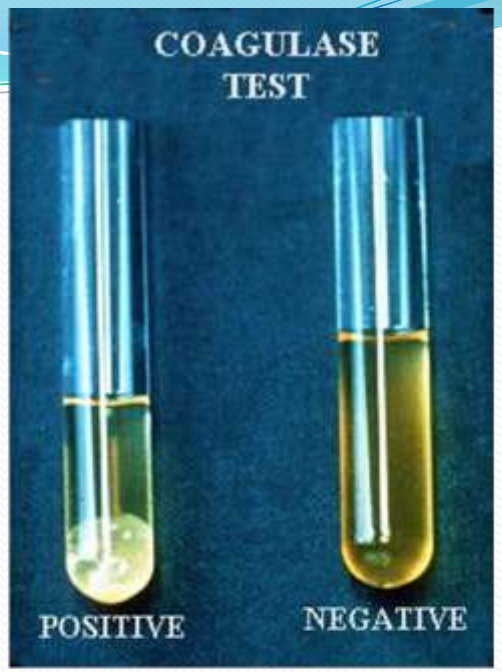




2. Coagulase test

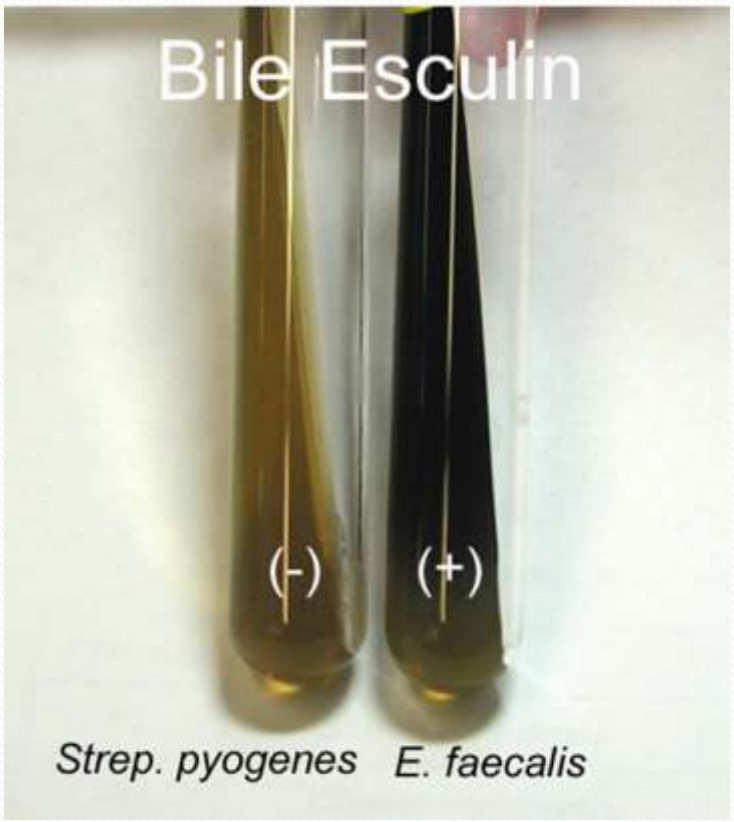
- The ability to clot plasma. This test used to differentiate between S. aureus & other Staphylococcus species
- Methods:

1. The tube coagulase test (Free):
   - Mix 0.1 ml of culture + 0.5 ml of plasma
   - Incubate at 37C for 4 h
   - Observing the tube for clot formation
2. Slide method:
   - Mix one drop of plasma with bacterial growth in a clean slide
   - Read result within 10-15 seconds



3. Bile esculin

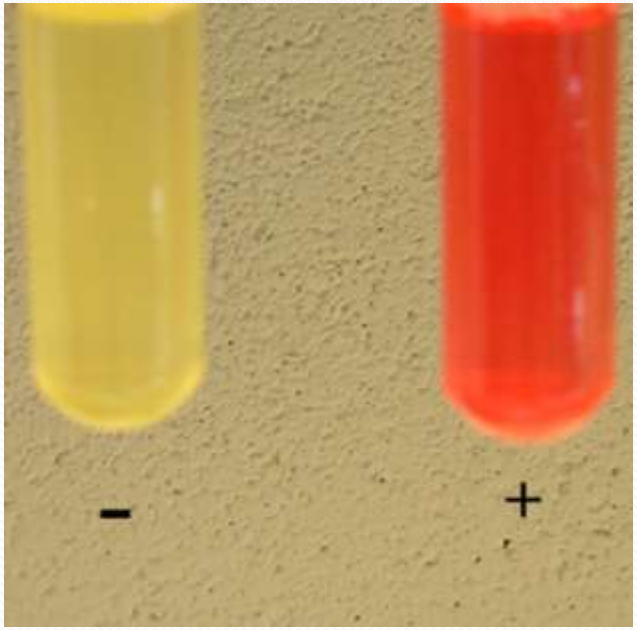
- To detect beta glucoside which breaks down esculin to form a black precipitate due to the presence of ferric ions
- Used to identify members of the genus Enterococcus
- Enterococcus hydrolyze esculin to products that react with ferric citrate in the medium to produce insoluble iron salts, resulting in the blackening of the medium



4. Urease

- Detects urease production
- Procedure:
  1. Inoculate a urea tube with 3 loopfuls of slant culture.
  2. Incubate 24 hours, observe for reaction.
  3. A pink color formation indicates the breakdown of urea to ammonia and CO_2

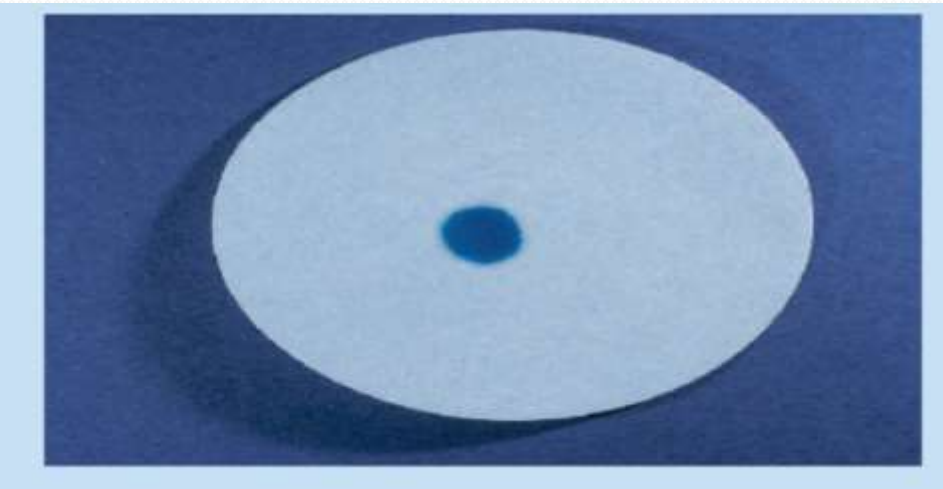
- Proteus +ve
- Salmonella -ve



5. Oxidase

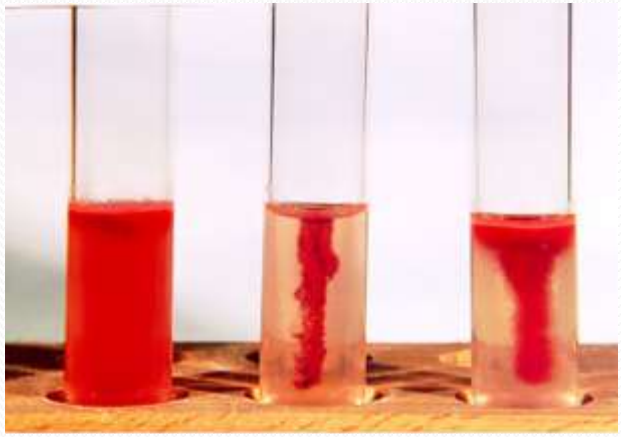
- To test for the production of oxidase
- spot inoculate organism on to a filter paper soaked with 1% tetramethylphenylene diamine dihydrochloride - positive is purple, negative is yellow

- Pseudomonas aeruginosa +
- Escherichia coli -



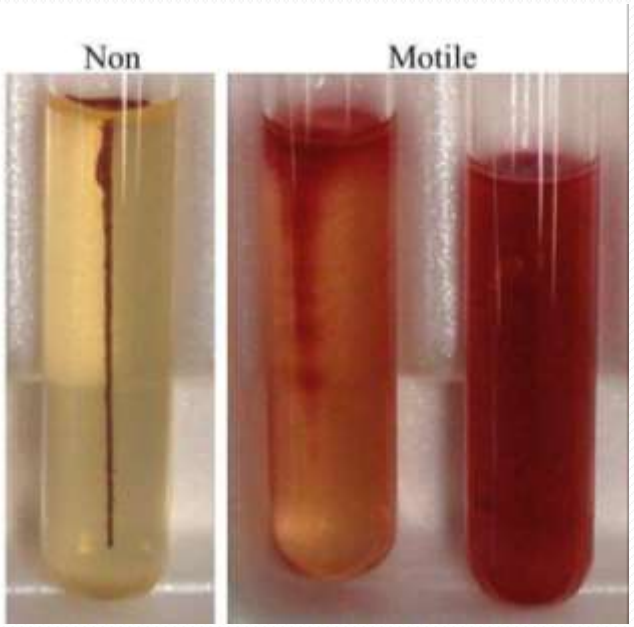
6. Motility test

- This test is done to help differentiate species of bacteria that are motile.
- Media: semisolid media.
- How to Perform Test: Stab motility media with inoculating needle.
- Reading Results: If bacteria is motile, there will be growth going out away from the stab line, and test is positive. If bacteria is not motile, there will only be growth along the stab line. A colored indicator can be used to make the results easier to see.



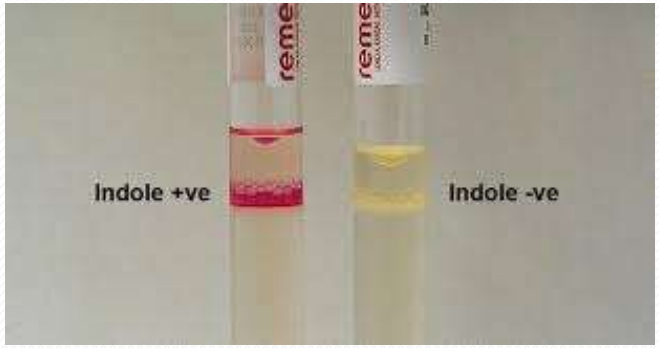
From left to right:

\+ − +



7. Indole test

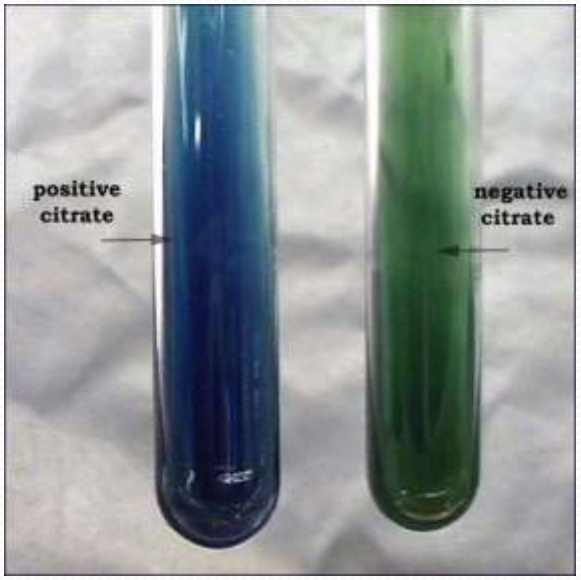
- Inoculate the tryptophan broth with the test organism and incubate at 37°C for 24-28h.
- Add 0.5mL of the Kovac's reagent
- Examine the upper layer of liquid Positive result red colour (occurring within a few seconds) negative result yellow colour

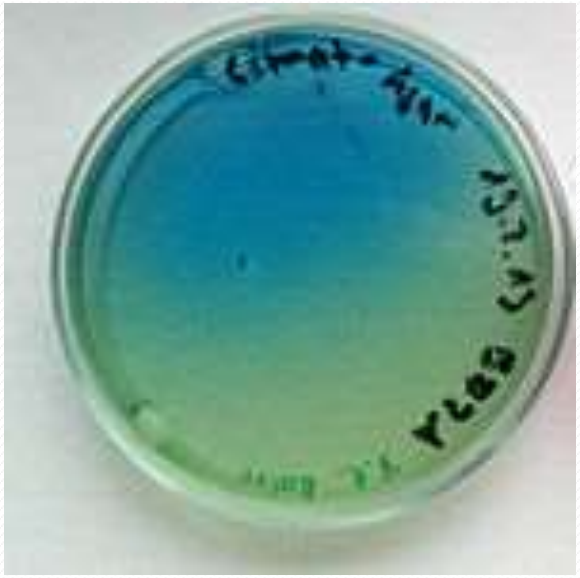


8. Citrate test

- Principle: Test the ability of bacteria to produce citritase which breaks down citrate to oxaloacetate and acetate that end up by producing sodium bicarbonate ($NaHCO_3$) as well as ammonia (NH_3) that produce alkalin medium
- Procedure:

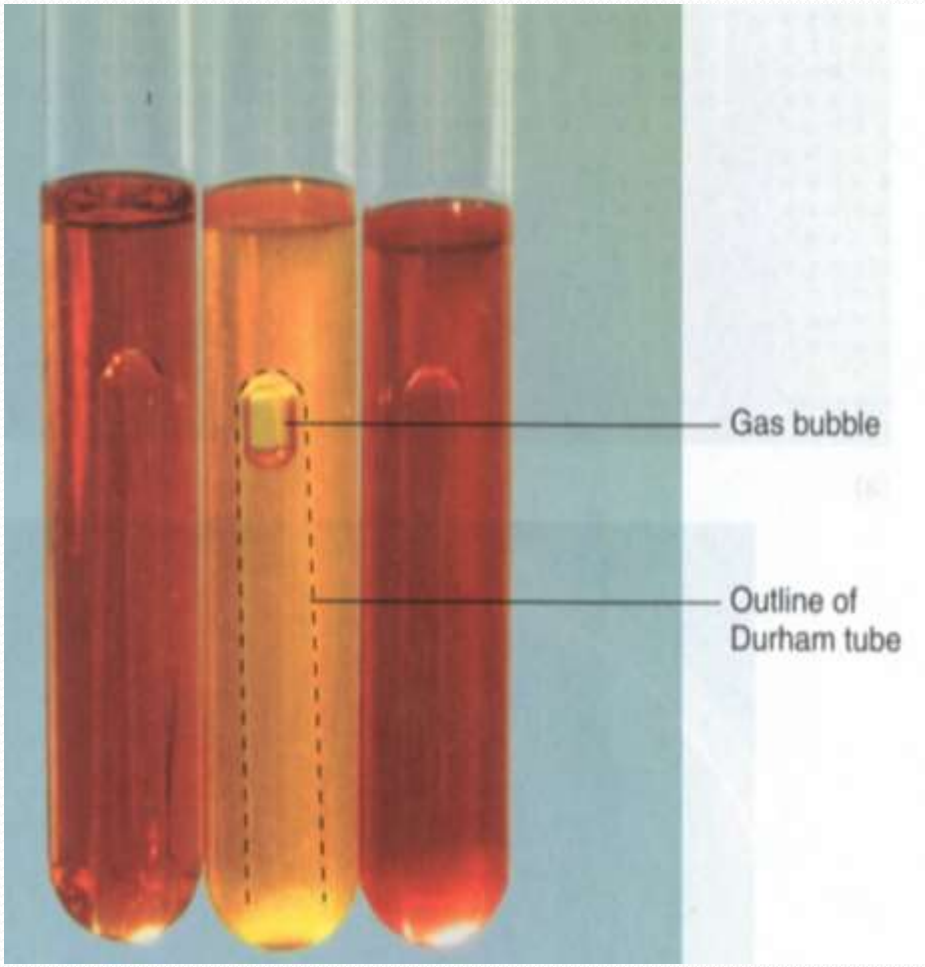
1. Streak up the slant with the inoculum.
2. Incubate at 25 or 37 degrees C
3. Observe color change from green to blue





9. Carbohydrate Fermentation

- This medium show fermentation (acid production) and gas formation
- The small Durham tube for collecting gas bubbles
- Positive for acid (yellow) and gas (open space)



10. Triple sugar iron agar (TSI)

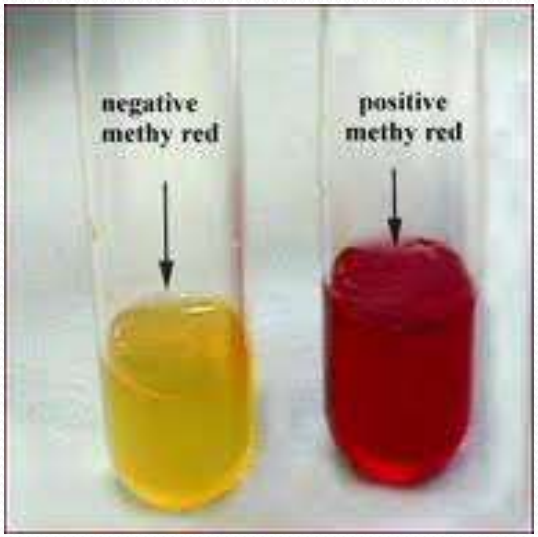
- The TSI slant is a test tube that contains agar, a pH-sensitive dye (phenol red), 1% lactose, 1% sucrose, 0.1% glucose, as well as sodium thiosulfate and ferrous sulfate
- An alkaline slant-alkaline butt (red/red) (K/K) indicates no fermentation
- An acid slant-acid butt (yellow/yellow) (A/A) indicates Glucose and lactose and/or sucrose fermentation
- An alkaline slant-acid butt (red/yellow) (K/A) indicates glucose fermentation only
- Cracks, splits, or bubbles in medium indicate gas production.
- A black precipitate in butt indicates hydrogen sulfide production.

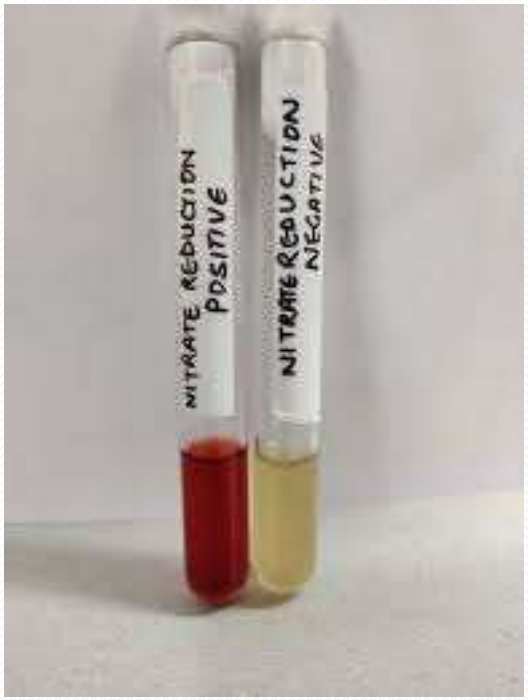
Triple sugar iron agar (TSI)

	Tube a	Tube b	Tube c	Tube d
Slant	–	A	K	K
Butt	–	A	K	A
Gas	–	+	–	–
H_2S	–	–	+	+

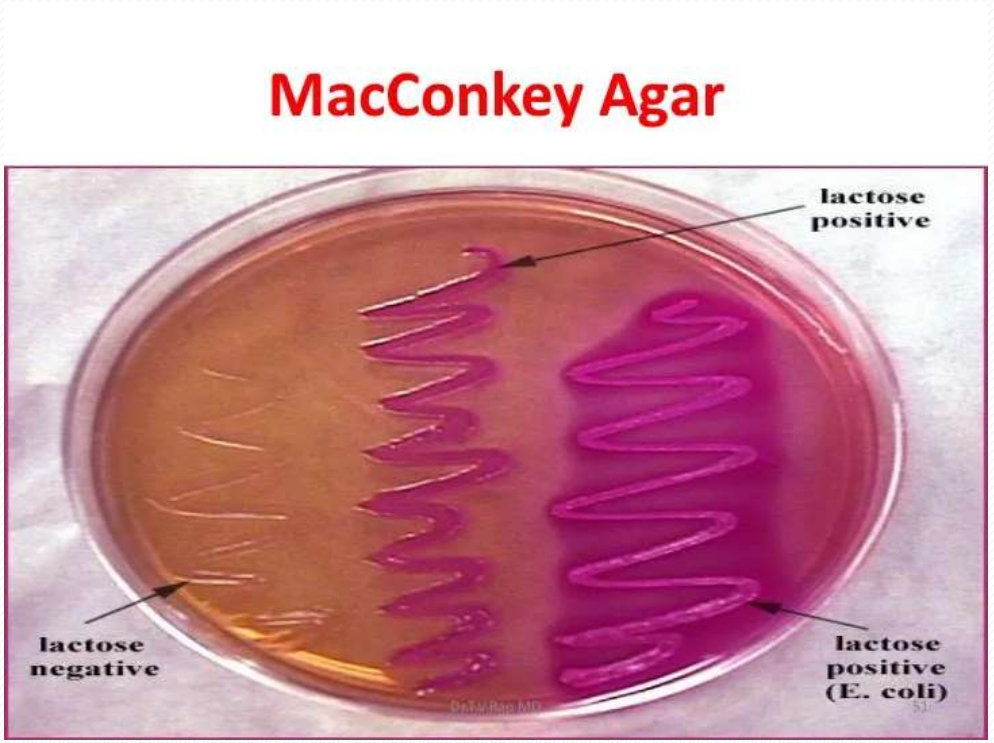
11. Nitrate Reduction

- It is used to determine if an organism is capable of reducing nitrate (NO_3^-) to nitrite (NO_2^-) or other nitrogenous compounds via the action of the enzyme nitratase
- This test is important in the identification of both Gram-positive and Gram-negative species
- After 24-48 hrs of incubation, nitrate reagents are addedRed colour is positive for nitrate reduction to nitrite



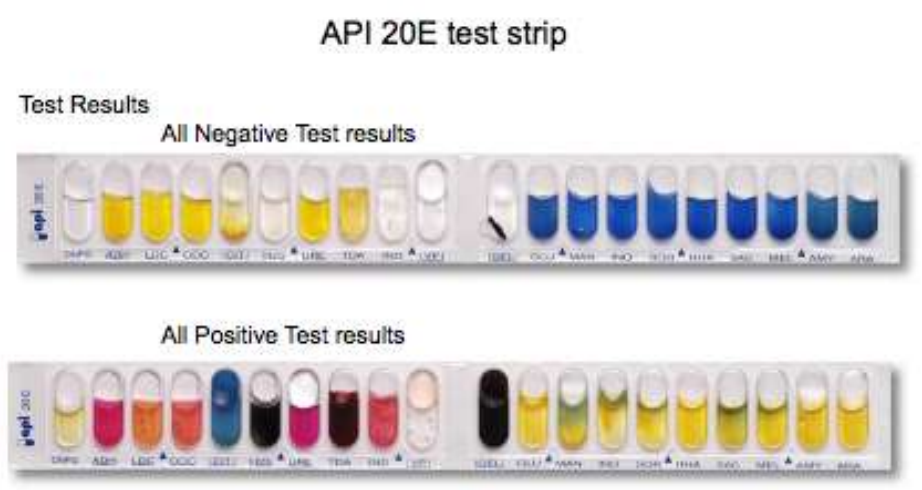


Biochemical tests and agars



API (Analytical Profile Index) 20E

- Commercial biochemical test panels - Cover a significant number of clinically-important groups of bacteria
- Different test panels are prepared in **dehydrated forms** which are reconstituted upon use by addition of bacterial suspensions. After incubation, positive test results are scored as a seven-digit number (profile). Identity of the bacterium is then easily derived from the database with the relevant cumulative profile code book.



Automatic Machines

- Automated system that performs bacterial identification using biochemical tests and advanced database and software
- Identification cards:
  - Gram negative bacterial identification
  - Gram positive bacterial identification
  - Yeast identification
  - Neisseria, Haemophilus and other fastidious Gram negative bacteria identification
  - Anaerobic bacteria and coryneform bacteria identification