Culture based methods & Biochemical reactions

Learning outcome

- **You should be able to:**

1. Understand the culture methods for diagnosis

1. Explain the important and principle of biochemical reactions

2. Understand the antimicrobial susceptibility test

Media for transport swab

Liquid:

- Skimmed milk transport medium
- Campylobacter transport medium
- Brucella transport medium

Semisolid:

- Stuart transport medium
- Cary and blare with and without charchol
- Amies transport medium

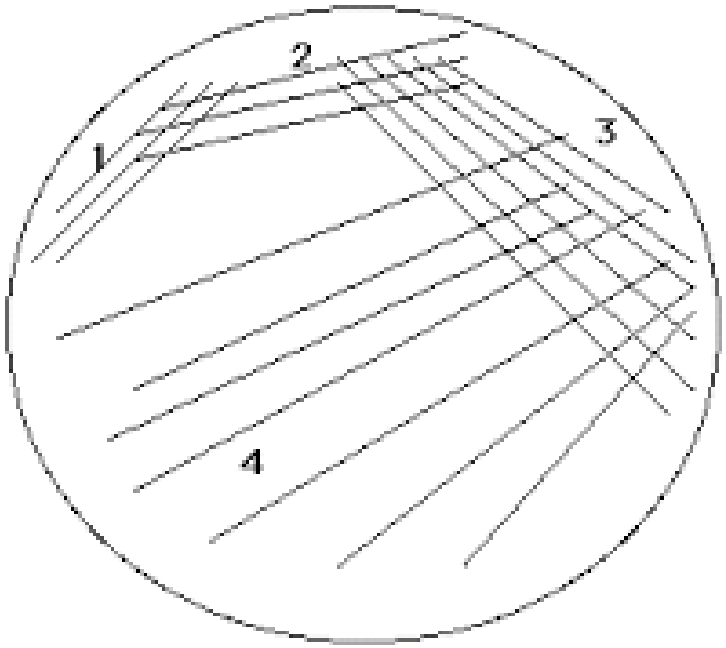
Samples culturing

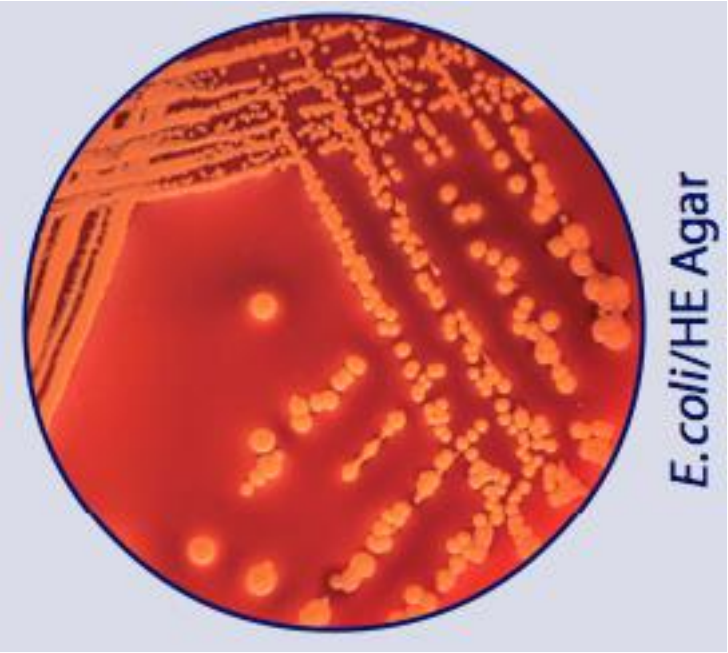
Sample is

1. Inoculated for culture and identification in appropriate media
2. Streaked on agar media (BA, EMB, NA, etc….)

   What is the streaking technique?
3. Incubated at suitable temperature and for specific time.
4. Individual colony is picked and re-cultured for pure colonies.

Streaking technique





Samples culturing

5. Identification based on microscopy examination for:

A. Motility of bacteria: by slide test (hanging drops) or tube test

B. Morphology and staining reactions

- Simple stain
- Gram stain
- Ziehl-Neelsen stain

(acid fast bacilli Vs non acid fast bacilli)

Culture media

1. What are used for?

- ……
- ……

2. Can you tell me what types of culture media are there?

Culture character

Pigment production:

- Endopigment (restricted to colonies)
- Golden yellow: Staphylococcus aureus
- Exopigment (diffuse into medium)
- Green: Pseudomonas aeruginosa

Culture character

Hydrolysis on Blood agar

1- beta- hemolysis (complete):

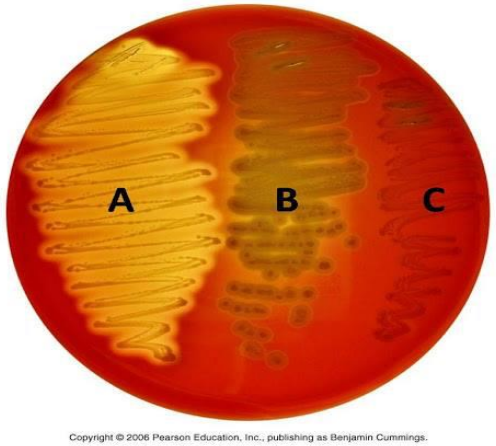
Streptococcus pyogenes

Staphylococcus aureus

2- α- hemolysis (incomplete):

Streptococcus pneumoniae

Streptococcus viridans



3- γ-hemolysis (no hemolysis):

Enterococci

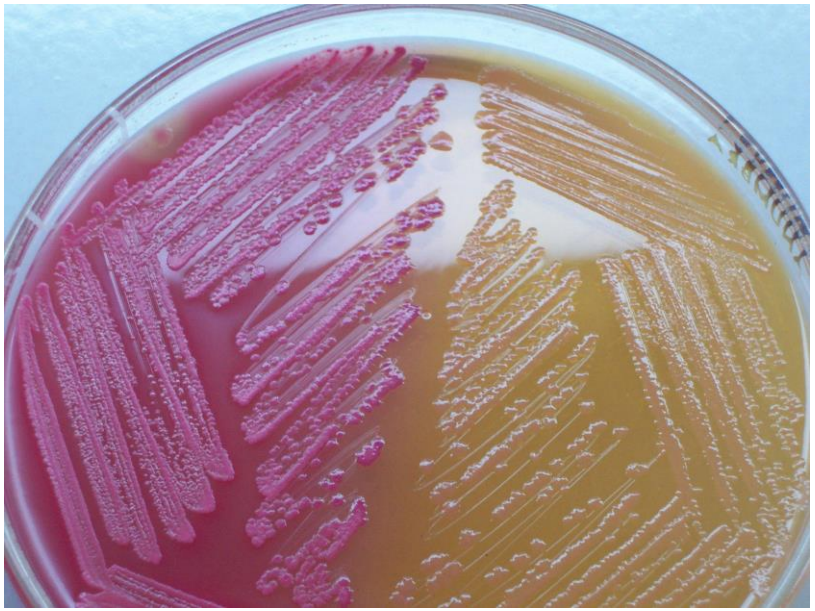
Culture character

MacConkey agar:

Lactose fermenter Vs non lactose fermenter

E. coli & klebsiella Vs Salmonella & shigella

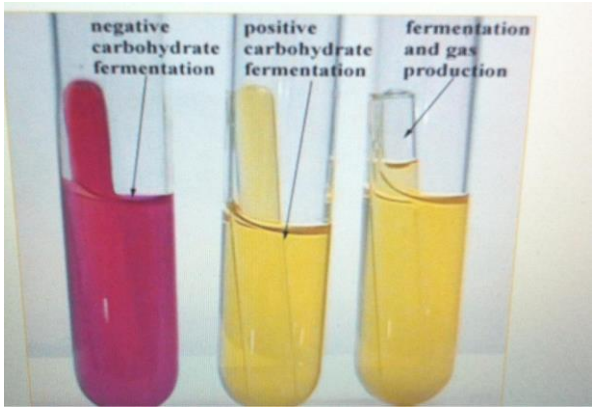
Rose colonies Vs Pale colonies



Biochemical reactions

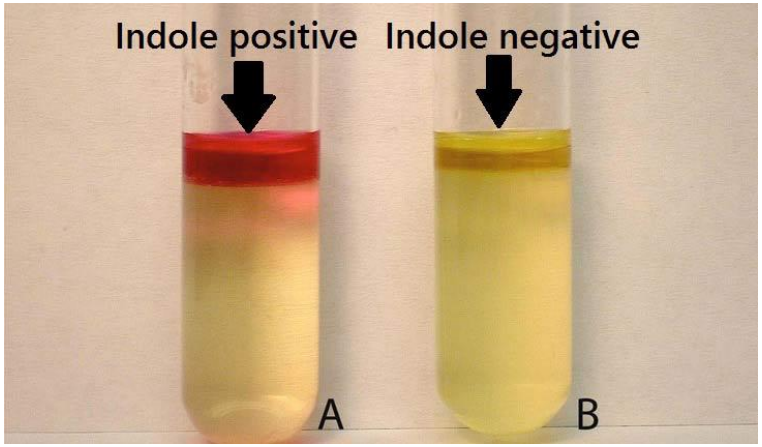
- Physiology characteristics (traditional methods)
- Rapid test method
- Use of substrate and sugar to identify pathogens
- Include: Enzymes, sugar fermentation, capacity to digest or metabolize compounds
- (carbohydrates, proteins lipids)
- Combined test

Biochemical reactions

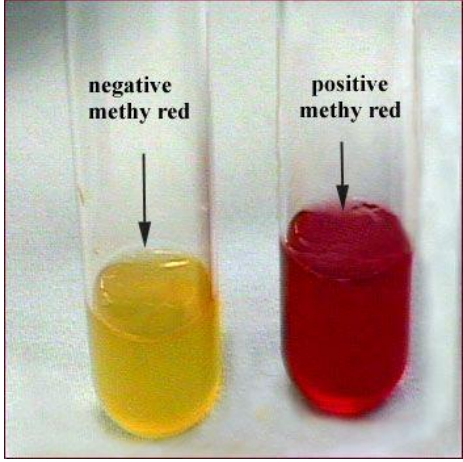


1. Sugar fermentation
2. Indole production

Tryptophan $\xrightarrow[\text{Kovac's reagent}]{}$ indol (red ring)



Biochemical reactions



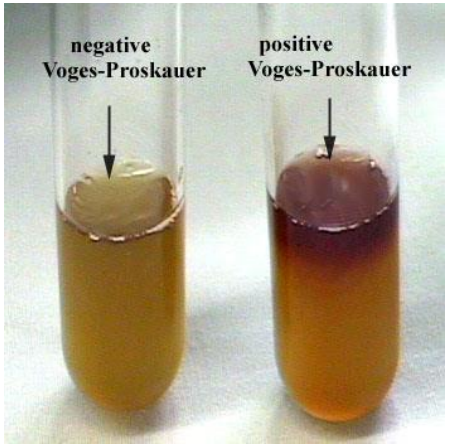
3- Methyl red reaction (MR)

Glucose $\xrightarrow[\text{indicator}]{\text{Methyl red}}$ high acid production

4- Voges proskaur's reaction (VP)

Glucose $\xrightarrow[\text{KOH}]{}$ acetyl methyl carbinol

Media is turned to red brown colour

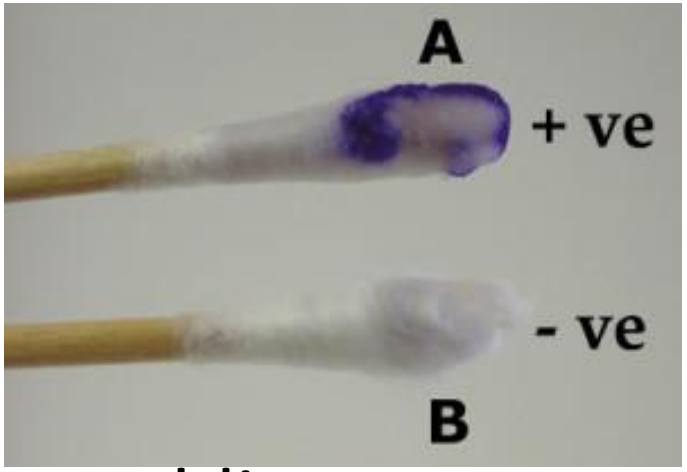


Biochemical reactions

5- Action on milk

Lactose $\xrightarrow[\text{Litmus indicator}]{}$ acid production

Biochemical reactions

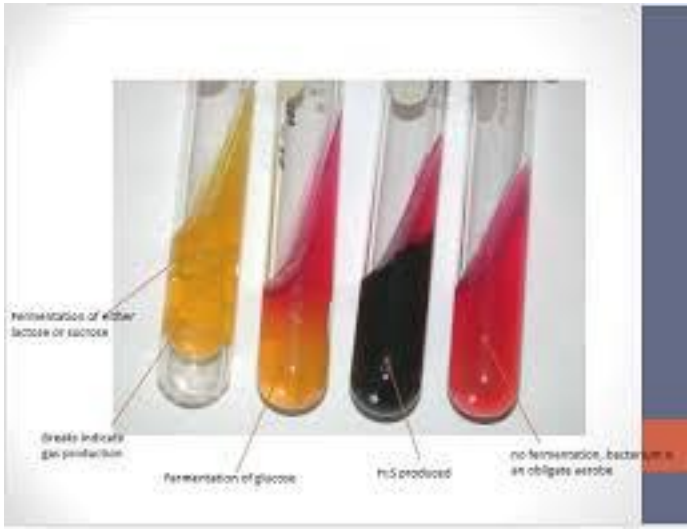


6- Oxidase test:

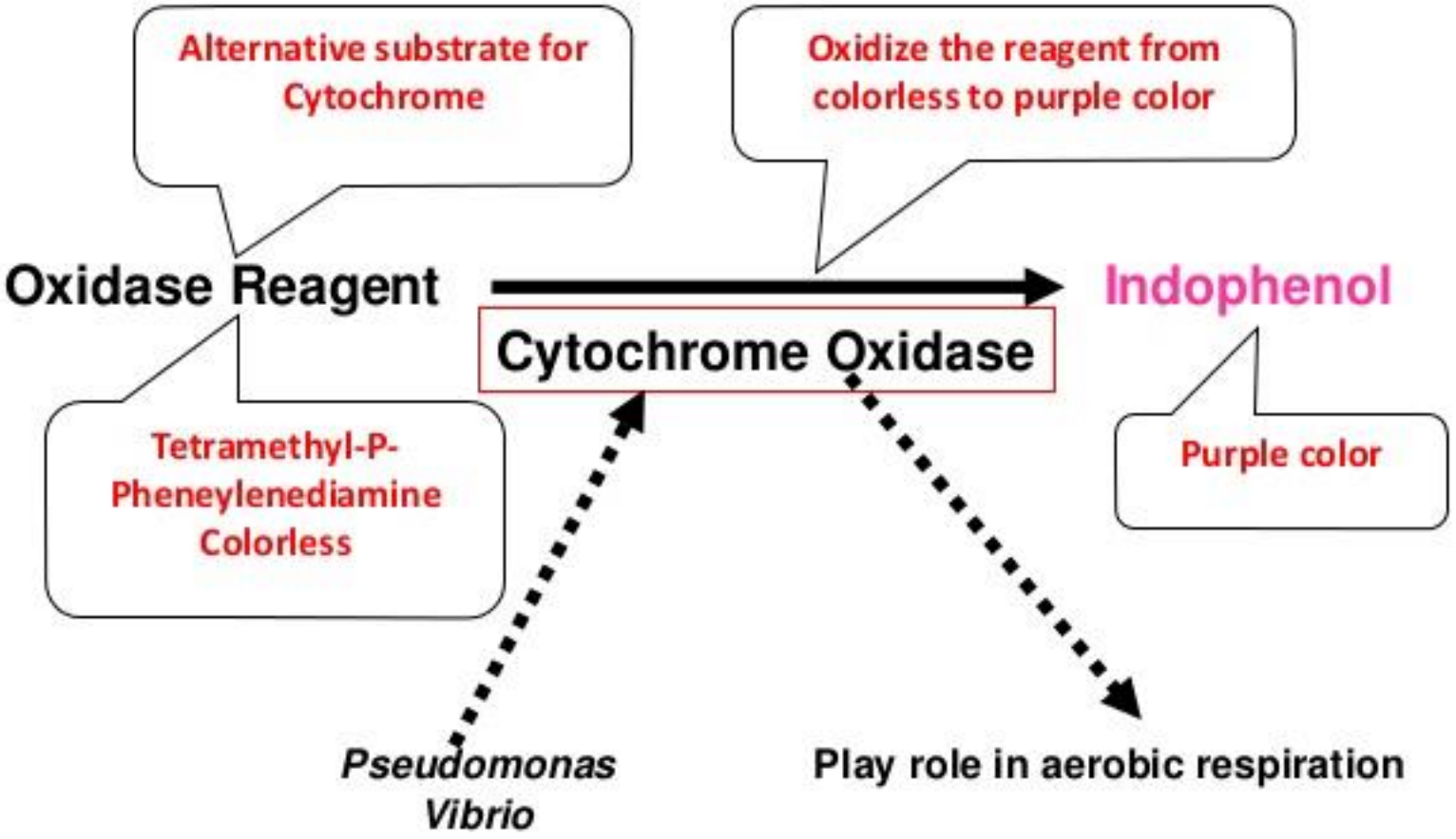
colonies turns to deep purple after adding a colourless oxidase reagent (drops)

Note: all Enterobacteriaceae: oxidase -
Pseudomonas & Neisseria : oxidase +

7- H_2S production test



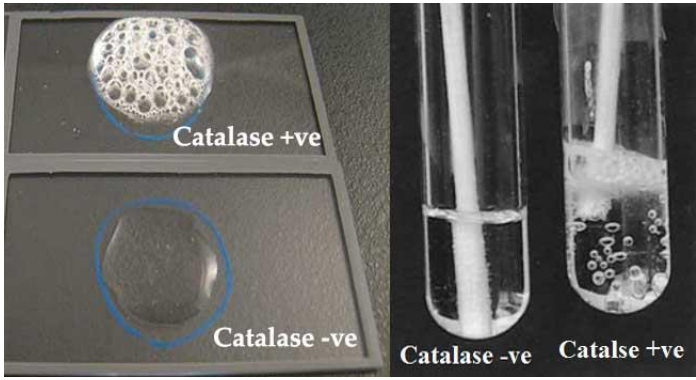
Oxidase Test: Principal



Biochemical reactions

8- Catalase test

- H_2O_2 —(Catalase enzyme)→ O_2 (gas bubbles) +H_2O



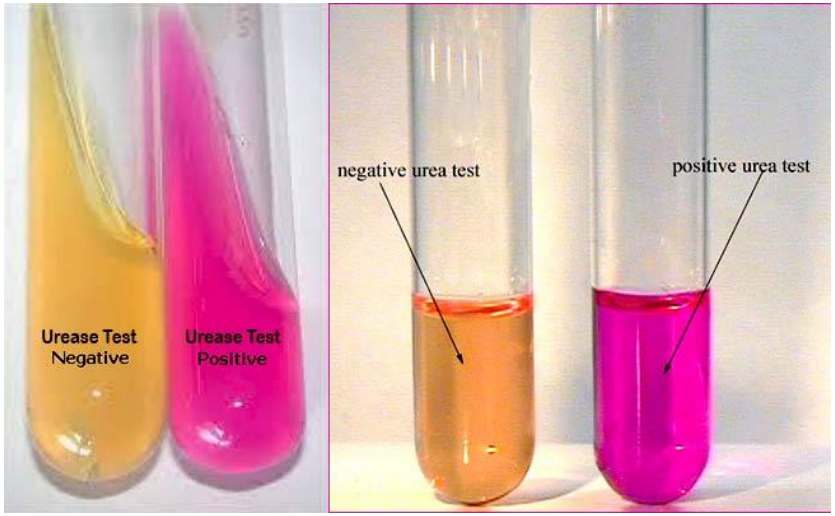
9- Coagulase test

- Fibrinogen —(Coagulase enzyme)→ fibrin (clot formation)

Biochemical reactions

10- Urease test

- Urea $\xrightarrow[\text{enzyme}]{\text{Urease}}$ NH3



Alkalinity of the media turns the colour of indicator from yellow to pink

Home work assignment

1. What do we mean by MIC and MBC, How to assess the antibacterial activity by disc diffusion test and tube dilution technique

2. What are the important and basic principles for each biochemical tests:

A. Bile esculin agar
B. Coagulase test
C. Optochin test
D. Manitol salt agar (MSA)

By the end you will be able to answer these questions

1. What are the important and basic principles for each biochemical tests?

2. Describe the antibacterial activity by two different methods?

- http://www.uwyo.edu/molb2210_lab/info/biochemical_tests.htm
- https://microbeonline.com/culture-media/
- https://microbeonline.com/bacterial-identification-methods/