Microbiology

Biochemical tests

Biochemical tests:-complex chemical reactions of life the microorganism employs a large number of individual enzymes whose activities interlock. These enzymes worked utilize chemicals substrate in culture media as source of energy and as building blocks for growth and reproduction. These chemical reactions the pattern aids in the identification and differentiation of the microorganism from closely related species.

Types of Biochemical tests

1-Sugar fermentation test :-

- **Mechanism of action or Mode of action:-**

  This test used to distinguish between types of the bacterial which ability of ferment a wide groups from sugars and others ferment only a few.

The sugars fermentation end products are formed such as :-

1- **organic acids** :- lactic acid, acetic acid, prop ionic acid.
2- **Neutral products** :- acetone, butyl alcohol, ethyl alcohol.
3- **Various gases** :- methane, hydrogen and Co.

May be detected the formation of **organic acids by pH indicator** which add to the microbial growth medium.

A commonly used pH indicator is :-

1-Phenol red
2-Bromothymol blue
3-Bromocresol purple

May be detected the **Gas formation in broth** through the use of **Durham tube**, gas production in **agar media** is accompanying by the formation of gas pockets that appear as cracks in the agar.

Sugar fermentation medium(للاطلاع)

Peptone	1gm
Nacl	0.05gm
Phenol red	0.2% (add 5ml)
Distilled water	100ml

Autoclaving and distribution in sterile test tubes (10ml) then added 0.1gm from sugars (glucose ,fructose, maltose, rafinose, cellulose …etc). after dissolves 0.5ml D.W and sterilized by filtration (milipore) then to the sterilized medium. Inoculated tubes with bacteria and incubated at 37C° for 24 hours.

Result of this test:- **red color (-)** **Yellow color (+)**

2. Methyl red and Vogues proskaure (MR-VP):-those tests are important in diagnoses of G-ve rods (**enterobacteriace**) and some types of bacillus these tests measuring the end products Of to break down the sugar**.**

- **Mechanism of action or mode of action MR:-**

This test depend upon the ability of organism to break down the sugar (glucose) and give acid as end product which lead to decrease in pH to the less from 4.4. this can be detected by add **(methyl red indicator)**

MR medium:- (للاطلاع)

Peptone	0.5gm
Kepo4	0.5gm
D.W	100 ml

Autoclaving and distribution in sterile test tubes (5ml) then add 0.5ml from glucose sugar solution concentration 10% sterilized by filtration (milipore).

PH indicator :-

methyl red	0.1 ml
ethanol	300ml
distalled water	200 ml

Result of this test:- add 5drops from **MR indicator** to 5ml of cultured broth.

Red color (+) e,g E.coli **Yellow color (-)**

- **Mechanism of action or Mode of action VP :-** This test can detect the neutral products as final products break down the glucose such as pyrovic acid to acetone**.**

VP medium:- **(للاطلاع)**

Like the MR medium but different in pH indicator and incubator at 37C° for 24 hr. inoculated test tubes with bacteria for pH indicator (مطلوب)

VP indicators:-

1-Barritts A 5% alpha-naphthole

2-Barritts B 40% potassium hydroxide (KOH)

Result in this test :- Add 1ml from 40% KOH and 3ml of 5% alpha-naphthol to 5ml of culture broth incubate at 37C° for 5-20 minutes.

Red-brown (+) e.g. proteus Yellow (-)

3- Indole test:- Indole is a nitrogen-containing compound formed from the degradation of the amino acid tryptophan by various bacteria.

❖ **Mechanism of action or Mode of action :-**

$$\text{Peptone water (Tryptophan)} + H_2O \xrightarrow{\text{(Tryptophonase)}} \text{Indole ring + pyrovic acid} + NH_3$$

Indole medium:- (للاطلاع)

Peptone 2.3gm
Distilled water 100ml

Autoclaving and distribution in sterile test tubes (6ml) then inoculated with bacteria and incubate at 37C° for 24 hr.

pH indicator (Kovac's reagent):- للاطلاع

amyl alcohol 150ml
para-dimethylaminobenzaldehyde 10gm
HCL concentration 50ml

Result in this test :- Add 0.3 ml of **Kovac's reagent** to the 6ml of culture broth

Red ring (+) e.g. E. coli Yellow or green

4- Citrate utilization test :-

❖ **Mechanism of action or Mode of action :-** This test used to distinguish G-ve rods (**enterobacteriace**) that use citrate as carbon sole source of energy. This purpose used Simmon's citrate agar whose contain pH indicator **(bromothymol blue).**

Simmon's citrate agar medium (للاطلاع)

Nacl	5gm
MgSo4	2gm
NH4H2PO4	1gm
Na3C6H5O7 2H2O	5gm
Agar	26gm
Bromothymol blue	0.2%
Distilled water	1000 ml

Autoclaving and distribution in sterile test tubes slant then inoculated with bacteria and incubate at 37C° for 24 hours.

Result in this test :- Blue color (+) e.g. Klebsiella Green color (-)

5- Urea hydrolysis test (Urease test):

❖ **Mechanism of action or mode of action :-**

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} NH_3 + CO_2$$

Used to this purpose **(urea agar)** whose contain **(phenol red indicator).**

Urea medium :-(للاطلاع)

Peptone	1gm
KH2PO4	2gm
Nacl	5gm
Agar	20gm
Phenol red	0.2%
Distilled water	1000 ml

Autoclaving then add 10ml from glucose concentration 10% sterilized by milipore and 100 ml from urea supplement concentration 20%. distribution in sterile test tube slantly. Inoculated with bacteria and incubate at 37 C° for 24 hr.

Result in this test :- Pink color (+) e.g proteus Yellow color (-)