BIOCHEMICAL TESTS

Nitrate reduction test

The presence of enzyme nitrate reductase which can convert nitrate into nitrite can be identified by using this test.

Steps:

Bacteria is grown in media containing 1% w/v potassium nitrate for 5-7 days at 37^{o}

ii. Test reagent is prepared by mixing solutions of sulphanilic acid and α-naphthylamine in acetic acid.

iii. 1 ml of test reagent is added to the culture.

iv. Presence of red coloration in few minutes indicates the positive results.

This test is mainly conducted for differentiation between members of *Enterobacteriaceae* that produce enzyme nitrate reductase from gram-negative bacteria which do not produce the nitrate reductase enzyme.

Urease test:

Urease is an enzyme produced by some microorganisms which can convert urea into ammonia. The presence of ammonia turns the pH of the medium alkaline which can be noted by the change of coloration of phenol-red indicator to a deep pink color.

i. Christensen's urease agar medium or Christensen urease medium is inoculated with test species of bacteria. Indicator phenol-red is also added to it.

ii. The culture is incubated for 5-7 days.

iii. Presence of deep pink color indicates the positive results.

This identification test is mainly used for differentiation of *Proteus vulgaris.*

Litmus milk test:

This test helps in differentiating among the microbes which can enzymatically transform different milk substrates into varied metabolic end products.

Steps:

i. Litmus milk is prepared by adding litmus to the milk.

ii. Microbial sample is carefully inoculated to the litmus milk.

iii. Any observable changes are noted.

Following reactions may take place in the litmus milk:

- Lactose fermentation
- Gas production
- Litmus reduction
- Curd formation
- Proteolysis
- Alkaline reactions

Hydrogen sulfide production test

Some microbes have the presence of enzymes such as Cystein desulfurase which can reduce the sulfur containing amino acids, or enzymes such as thiosulfate reductase which can reduce organic sulfate to produce hydrogen sulfide gas. This can be tested by reacting with lead acetate which on reaction of hydrogen sulfide produces lead acetic acid sulfide which is black in color.

i. Bacterial samples are inoculated carefully in the Trypticase soy broth culture.

ii. The culture is incubated for 25-48 hrs for short versions of samples.

iii. A filter paper strip soaked in lead acetate is dipped in the broth.

iv. Black coloration of filter-paper strip indicates the positive results.

Organisms such as *Citrobacter freundii, Salmonella, Proteus mirabilis, Proteus vulgaris, Edwardsiella tarda* are positive for this test.

Sugar Fermentation test

Sugar fermentation test is used to determine the capability of bacteria to use the carbohydrate. In this test, presence of gas produced due to fermentation of carbohydrate is determined.

Steps:

1. Trypticase soy broth culture is prepared.
2. Desired sugar (lactose/dextrose/sucrose) is added in the broth.
3. Phenol red is also added as an indicator.
4. Broth is transferred into the test tubes and inverted Durham tubes are inserted into the test tube ensuring that the Durham tube is fully filled with the broth.
5. Sterilization of the test tube in autoclave.
6. Inoculation of broth with the test organism.
7. Incubation of the test tube for 18-24 hours.

Result Interpretation:

- No changes will be seen in the test tube having bacteria which cannot use carbohydrate.
- Only change in color of media from red to yellow is observed when the organism is capable of utilizing carbohydrate but do not releases any gases.
- Change in color of media from red to yellow and also the formation of gas bubble in the Durham tube indicates that the organism is capable of reducing sugar along with release of gases.

Potassium cyanide test

The test is performed to know whether the bacteria capable of growth in medium containing KCN as the source of carbon or not.

Steps:

1. KCN broth is made by adding 0.75% KCN to the nutrient medium.
2. Test organism is transferred to the broth aseptically.
3. The tube is incubated for 1 day.

Result Interpretation

Positive result can be interpreted by the presence of turbidity in the broth.

Catalase test

Catalase is an enzyme responsible for degradation of hydrogen peroxide in microorganisms. The presence of this enzyme can be tested using catalase test.

Steps:

1. Soy broth culture is prepared and hydrogen peroxide is added to it.
2. The test microorganism is introduced to the culture and immediate formation of bubbles is noted.

Result Interpretation

Positive results can be interpreted by the formation of immediate bubbles in the broth.

Urease test

Urease is an enzyme present in many microorganisms which have the capability of cleaving the nitrogen and carbon bond in an amide such as urea to give ammonia. The test is helpful in identification of *Proteus vulgaris* then other species. Phenol red is taken as an indicator which turns into pink due to presence of alkaline ammonia.

Steps:

1. Trypticase soy broth culture is prepared and phenol red is added. Thereafter, it is sterilized using autoclave.
2. Test microbe is inoculated aseptically using loop method.
3. Test culture is incubated for 1-2 days.

Result interpretation:

Presence of pink color indicates the positive results.

Oxidase test

Cytochrome oxidase is an enzyme which catalyzes the oxidation of a reduced cytochrome by molecular oxygen to give hydrogen peroxide and water. This test is helpful in identification of *Neisseria* and *Pseudomonas* which are positive for this test from other microorganisms.

In this test, p-aminophenol is used as a reagent which provide electrons and become blackish due to oxidation.

Steps:

1. In a trypticase soy agar medium, p-aminophenol is added and it is then sterilized using autoclave.
2. Agar medium is transferred to the Petri dish and allowed to solidify.
3. A single line streaks of test organism are made on agar medium
4. Incubation of culture media for 1-2 days.

5. **Result interpretation:**

The presence of black color indicates the positive results, while no change in color or presence of slight pink color indicates negative test organism.

MCQs

1. The test reagent for nitrate reduction test consists of?

a. α-naphthylamine

b. Sulphanilic acid

c. Alpha-naphthol

d. Both a) and b)

2. Which test is mainly used for differentiation between *Enterobacteriaceae* from gram-negative bacteria?

a. Nitrate reduction test

b. Urease test

c. Litmus milk test

d. Hydrogen sulfide production test

3. Medium used for urease test is?

a. Kauser's citrate agar medium

b. Kauser's urease agar medium

c. Christensen's urease agar medium

d. Christensen's citrate agar medium

4. Hydrogen sulfide production test is used for identification of bacteria having presence of?

a. Cystein desflurase enzyme

b. Thiosulfate reductase enzyme

c. Urease enzyme

d. Both a) and b)

5. Reaction that may take place in the litmus milk is/are?

a. Lactose fermentation

b. Gas production

c. Litmus reduction

d. All of the above

6. For hydrogen sulfide production test, bacterial samples are inoculated in?

a. Kauser's citrate agar medium

b. Christensen's urease agar medium

c. Simple agar medium

d. Trypticase soy broth culture

7. Positive result for hydrogen sulfide production test is indicated by presence of which color?

a. Red

b. Yellow

c. Black

d. Colorless liquid