



IDENTIFICATION OF ISOLATE MICROORGANISM

Media and reagents:

1. Grams iodine
2. Grams crystal violet
3. Grams decolouriser
4. Safranin
5. MRVP media
6. Lactose broth
7. Simmons citrate agar
8. Sucrose
9. Lactose
10. Xylose
11. Mannitol
12. Rhamnose
13. Glucose
14. Urea broth
15. Nutrient broth
16. Gelatin
17. Oxidase disc
18. Peptone water
19. Cedar wood oil
20. Kovac's Reagent

Colony characteristics:

Observe the edge ,texture, Pigmentation ,Elevation of the colony.

Gram Staining:

Pick the colony from the plate with help of the nichrome wire loop and suspend in 1ml of sterile water tube. Put the one drop of suspension on the glass slide and prepare thin smear ,allow to air and fix by gentle heat . Flood with Gram's crystal violet for one minute. Wash with water and flood with Gram's iodine for one minute. Wash with water and decolorise with Gram's decoloriser until no further violet colour comes off. Wash with water and counterstain with 0.5% safranin for one minute. Wash with water, dry and observe under oil immersion objective. If Gm +ve bacteria shall be give violet colour image under microscope or If Gm -ve bacteria shall be give violet colour image under microscope.

Biochemical test:

1. Sugar fermentation:

Inoculate loopful culture in the 5 ml each sterile nutrient borth tube containing different 2.5% sugar such as Sucrose ,Xylose, mannitol, Rhamnose and incubate at 37° C for 24 hrs ,if give acid production Gm +ve Bacillus spp. Present



PHARMADEVILS

MICROBIOLOGY DEPARTMENT

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2. Indol test:

Inoculate loopful culture in the 5 ml of sterile peptone water and 5 ml macconkey broth. Incubate in a water bath at 43.5 to 44.5° C for 24 hours. To test for Indole, add 0.5 mL Kovac's Reagent, shake well, and allow to stand for 1 min. If a red colour is produced in the reagent layer Indole is present, confirming the Gm- Ve E Coli present

3. Methyl red voges proskaur test:

Inoculate loopful culture in the 5 ml in sterile MRVP media and incubate at 37° C for 24 hrs If give acid production turn colour change red to yellow Gm –ve pseudomonas spp. Present

4. Citrate utilization test:

Streak loopful culture on the Simmons citrate agar plate and incubate at 30-35°C for 24 hrs . If colour change greenish to blue Gm + ve Bacillus spp present

5. Gelatin hydrolysis:

Inoculate loopful culture in the 10 ml sterile nutrient broth containing 1.5% gelatin and incubate at 30-35°C for 24 hrs .If gel formation in the tube Gm + ve spp. Present

6. Urease test:

Inoculate loopful culture in the 10 ml sterile urea broth and incubate at 37° C for 24 hrs If give acid production turn colour change red to yellow Gm –ve spp. Present

7. Oxidase test:

Smear the suspected colonies on oxidase discs. If purple colour produce indicates the presence of Gm –Ve spp..