Biochemical test

Production of ammonia from urea (Urease test(

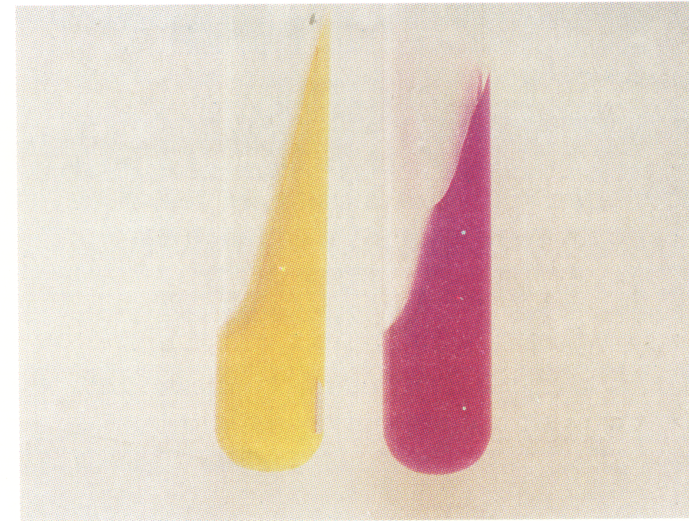
 Medium: urea broth or urea agar containing phenol red indicator

 Inoculation: as for broth media, incubation for 24 - 48 hr. at 37c ْ.

Result: change of color yellow to pink in +ve cases due to the hydrolysis of urea to ammonia by the action of urease enzyme produced by the bacteria and the medium because alkaline affecting PH of the indicator urea urease → 2NH3(Ammonia) + CO2+ H2O

In – ve cases the color of the medium not change.

e.g. E. coli is ureases –ve , proteous spp + ve



Indole production from tryptophan:

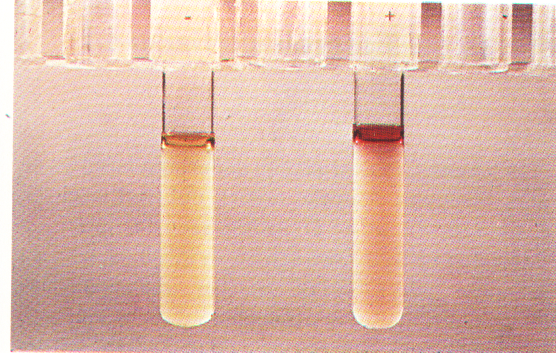
 Medium: nutrient broth containing peptone which is rich in tryptophan.

 Inoculation: as for broth cultures.

 Indicator: add kovac's reagent.

 Result: a deep red colored ring develops in the presence of indole and this is a positive result.Tryptophan→ indole + pyruvic acid + ammonia

In – ve result the ring stays yellow.E.g. E. coli is indole +ve , Salmonella spp – ve

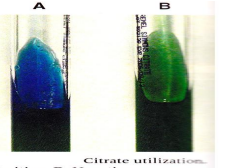


Citrate utilization test:

 Medium: Simon's citrate agar containing Bromothymol blue indicator

 Inoculation: streaking of the slant after stabbing of the bottomincubation at 37c ْ for 24 - 48 hr.

 Result: utilization of citrate as the source of carbon for energy and growth of the organism.Growth of organism on the citrate agar result in an alkaline reaction which makes the bromothymol blue change from green to blue color in positive cases, in negative cases there is no change in color of the medium and no growth of the organism. e.g E. coli is – ve Salmonella spp + ve .



oxidation / fermentation (O/F) test.

Medium: Hugh and Leifson's medium containing 1% glucose and bromothymol blue indicator.

Inoculation: by stabbing 2 tubes for the same organism and then adding paraffin to one of them only.

Result: Acid production is shown by a change in the color of the medium from green to yellow due to fermentation of glucose and change in color of the indicator (bromothymol blue) in acidic pH.

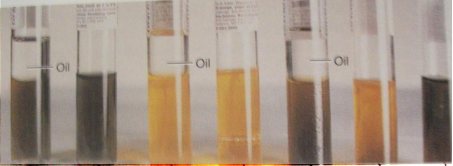
Fermentative organism (bacteria that ferment glucose in the absence of oxygen) will produce acid in the paraffin coated tube only while oxidative organisms (bacteria that ferment glucose in presence of O2) will produce acid in the open tube only.So the results are read as follows:

O/F = oxidative / fermentative organism.

O/-= oxidative organism.

-/F= fermentative organism.

-/- = Inert (not active) organism



Catalase test:

Aim: to detect the production of catalase enzyme by the microorganism

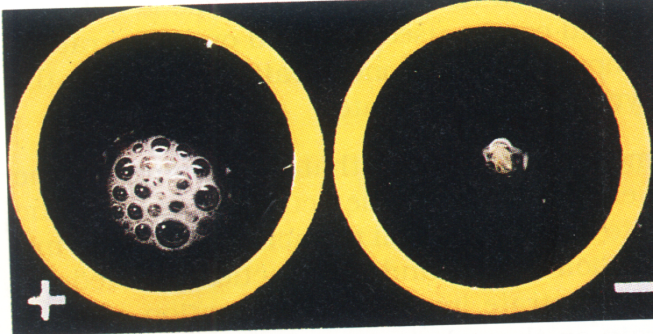
growing aerobicall).

Reagent (indicator): 3-6% hydrogen peroxide (H2O2).Result: a loopful of bacteria growth is emulsifieal with a loopful of H2O2 on clean slide, the production of Effervescence or foam causal by liberation of free O2 as gas bubbles indicates the presence of catalase.

2H2O2 → catalase → 2H2O + O2

E.g. Staphylococcus spp + ve .

Streptococcus spp – ve .



oxidase test:

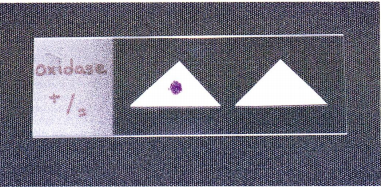
Aim: to detect production of oxidase enzyme by bacteria.

Reagent: 1% a queous solution of tetramethyl – p- phenylene diamine –HCL.

Result: few drops of the reagent are added to a filter paper and using wooden stick or glass rod, some of the bacterial growth is transferred to the impregnated filter paper; a purple coloration is produced within 5 -10 sec indicating the presence of oxidase enzyme. Delayed + ve result may appear with in 10-60 sec. but more than that (or a colorless result) is – ve .

E.g. Staphylococcus spp. And Enterobacteriacae spp. – ve .

Streptococcus spp and pseudomonas spp + ve .



methyl red / voges proskauer (MR/ VP) test.

Medium: glucose phosphate broth.

Inoculation: as for broth media incubation at 37c ْ for 2-7 days.

Reagent (indicator): methyl red added for MR test.

α-naphthol and 40% KOH or NaOH added for VP test .

Result: the inoculated broth is divided info 2 portions, in the MR part, a

red color indicates acidic pH (4.5 or less) and positive result, while yellow color is negative.

In + ve cases, glucose is fermented and end products of fermentation are acids.

E.g. lactic acid and butyric acid.

In the VP part development of a red colour within 5 minute, constitutes a + ve reaction due to formation of Acetyl methyl carbinol (Acetone) from glucose fermentation. Yellow color appearance is – ve.

e.g. E. coli is MR+ / VP –

Klebsiella spp is MR -/VP +

Note: some organisms may have both tests – ve, but very rare and not

find organisms with both tests + ve.

