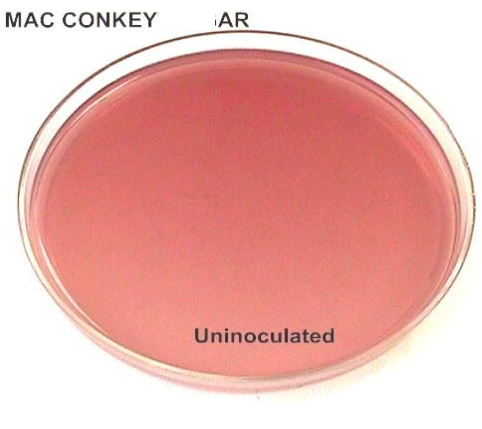
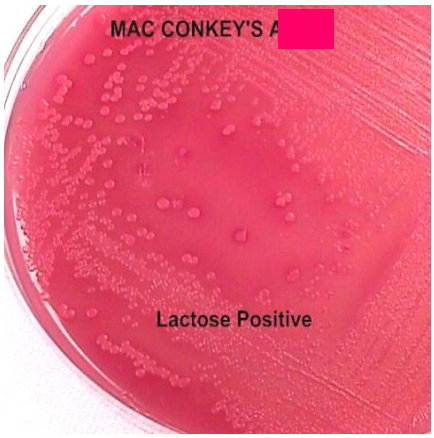
Enterobacteriaceae

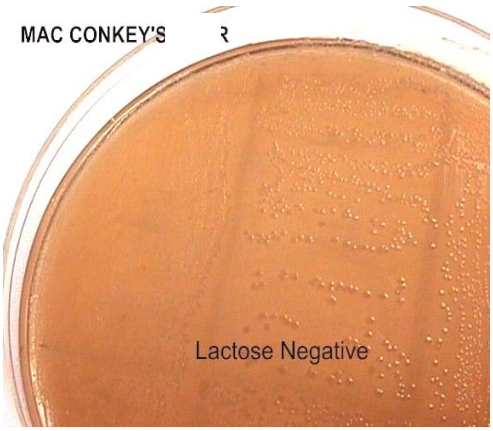
The enteric bacteria members of the family Enterobacteriaceae are the most commonly isolated normal or pathogenic microbes accounting for the most frequent cause of diarrhea and associate illnesses as well as over 50% of nosocomial infections(including gastroenteritis, respiratory and urinary tract infections).

They are gram negative bacilli group that are non-spore-forming) frequently motile (with the exception of Shigella and Klebsiella sp.). facultative anaerobes(oxidaes negative) that ferment glucose, reduce nitrates to nitrite, and break down peroxide(catalase positive). Although their normal habitat is the intestinal tract of humans and other animals, they may also be found in soil, water, and decaying material.

Based on their ability to ferment lactose, the enterics can be further subdivided into **Coli forms** (normal as well as opportunistic enterics that ferment lactose within 48 hours) for example: Escherichia coli, Klebsiella, Enterobacter, Citrobacter, **Non-Coli forms** (normal or pathogenic enterics that either fail to ferment lactose or do so with extremely slow kinetics) for example: opportunistic: Proteus, Providencia , Edwardsiella ; Hafnia and Serratia .







Pathogenic:

Salmonella , Shigella , Yesinia . The various genera of enteric bacilli can be differentiated by using selective and differential media and biochemical tests .

Specimen

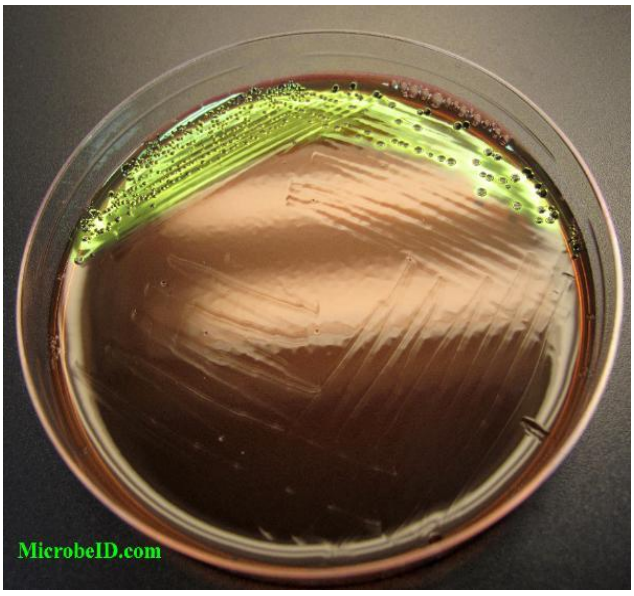
Urine , stools , blood, pus, spinal fluid, sputum, soil and water .

Lab diagnosis

1. Gram stain (gram negative bacilli)
2. Streaking on differential media

a) E.M.B (Eosine Methylene Blue)

Which gave excellent differentiation of **E. coli** and **Klebsiella aeroginosa** , The E-coli colonies usually show a dark center and have a greenish metallic sheen , colonies of Klebsiella aeroginosa are usually much larger than typical E. coli , pink , mucoid.



E.coli on EMB

E.coli on MacConkey agar

b) MacConkey agar

It gives amore clear – cut differential between the enteric pathogens lactose fermenter and non lactose fermenter . The colonies of coli form bacteria are red in color and may be surrounded by a zone of precipitated bile . This reaction is due to the action of the acids , produced by fermentation of lactose , The colonies of non- lactose fermenter bacilli do not greatly alter the appearance of the medium . these colonies give an alkaline reaction , are uncolored and transparent.

3- IMVC test :-

a) Indol Test

Certain bacteria such E .coli have the ability split the amino acid tryptophan into indol and pyruvic acid , indol which indicate by adding the **kovacs regent** after inoculating the tube contain peptone water (1%) for 18-24 at 37C° .

Indol production test

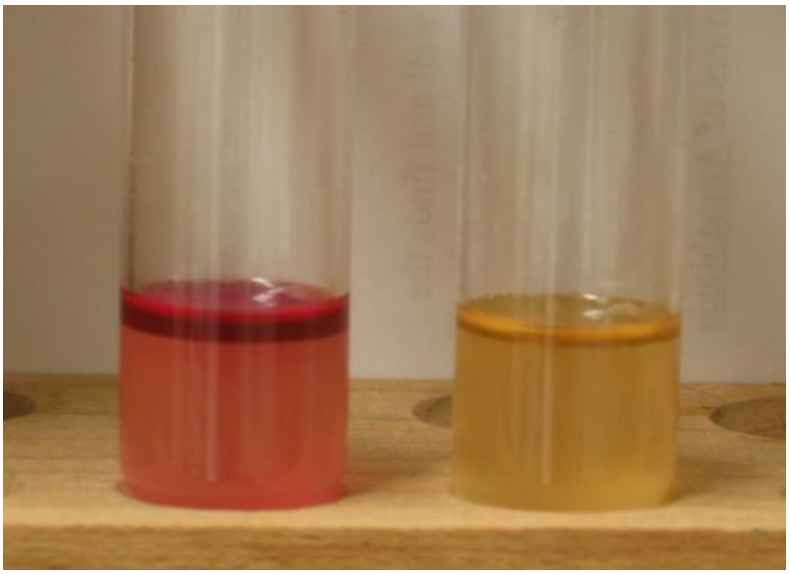
Aims : To determine the ability of microbe to degrade the amino acid tryptophan.

Principle:

Interpretation:

Development of cherry red colour at the interface of the reagent and the broth, within seconds after adding the Kovac's reagent indicates the presence of indole and the test is **positive. If no** colour change is observed, than the test is negative and organisms are not capable of producing tryptophanase.

Indole Production



When E.coli is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's reagent which contains 4 (p)-dimethylaminobenzaldehyde which reacts with the indole to produce a red coloured compound.

b) Methyl Red Test (MR Test)

Aims: to differential **E .coli** and **E. aerogen** and to demine the ability of microbes to oxidize glucose with production and stabilization of high content of acid end product.

Principle:

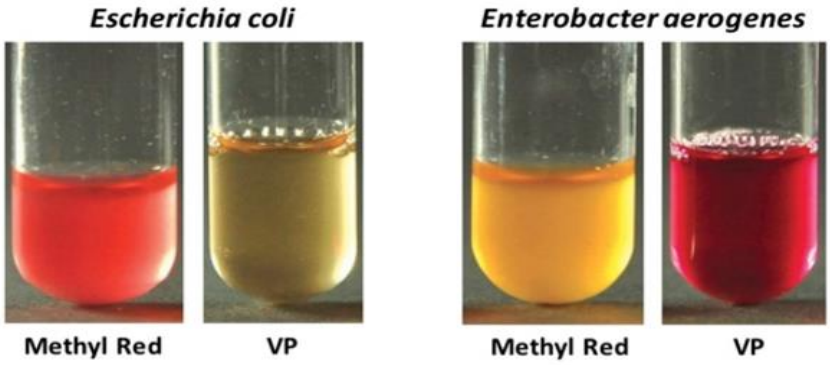
E .coli

Glucose+H_2O → **Lactic acid, Acetic acid, Formic acid** → **Methyl red** → **CO_2+H_2O, Red colour (positive test) (pH4)**

E . aerogen

Glucose+H_2O → **Acetic acid** → **2,3butanediol+ CO_2+H_2O + Methyl red** → **Yellow colour (negative test) (pH6)**

Although Methyl Red Test and Voger-Prauskuer Test uses the same medium (MR-VP broth) *E.coli* and E. *aerogenes* is giving different colors in each test (see the figure below). Please explain the reason. 5

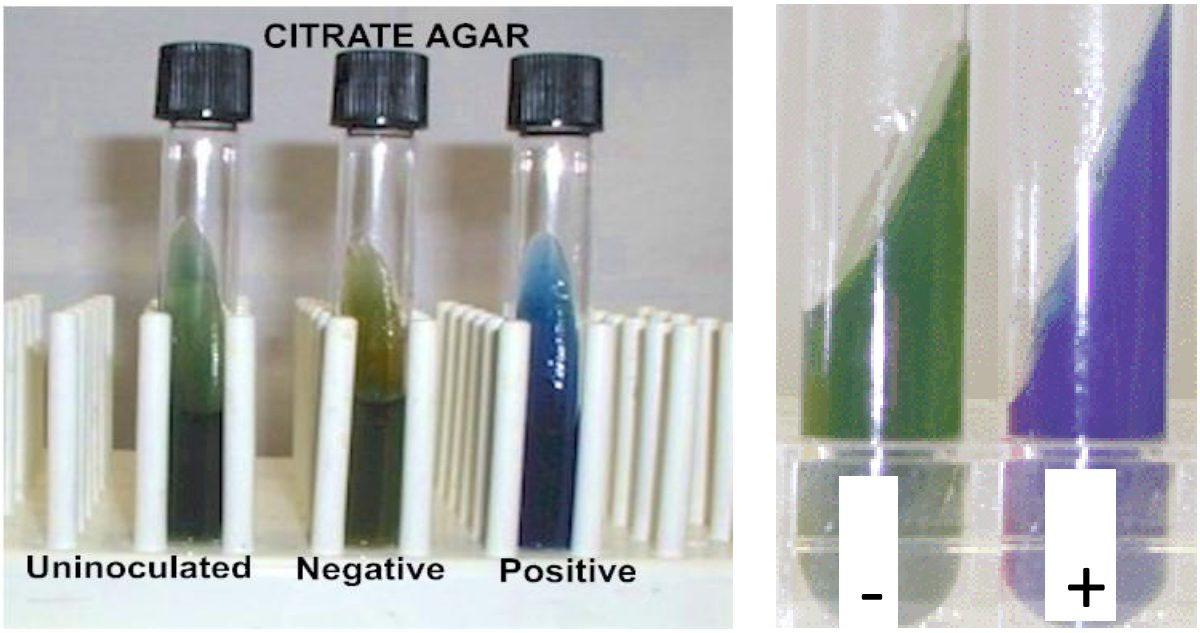


c) Voges – Proskaur test (Butanediol fermentation)

A negative methyl red test may indicate that these organisms being test produced a lot of(2,3 butanediol) and ethanol instead of acids . Indicator consist of alpha-naphthol + KOH 40% to detect acetyl methyl carbinol .

d) Citrate Utilization

The ability of some organisms to utilize citrate as a source of carbon . Simmons citrate agar is used to detect this ability . Indicator is bromothymol blue in this media will change from **green to blue** .



Citrate Utilization Test

4- Carbohydrate Fermentation Test

(Sugar fermentation tests)

Aims: To determine microbes to ferment carbohydrate with the production of an acid and / or gas

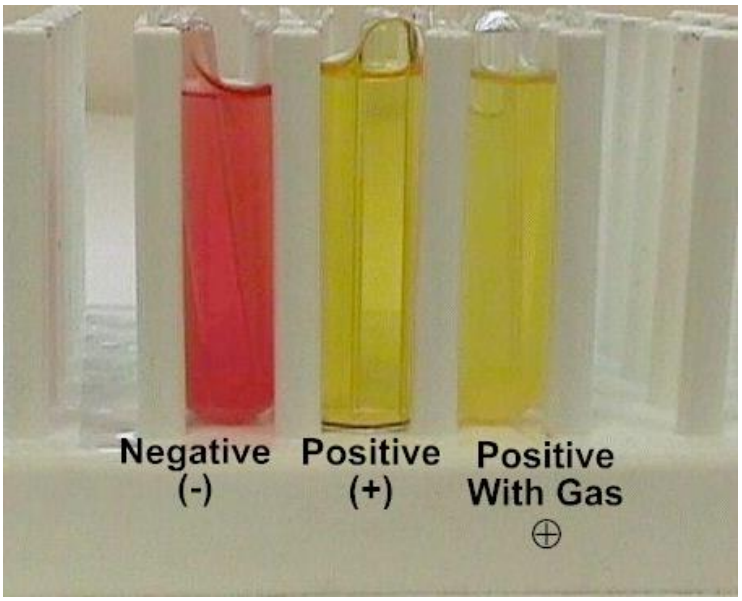
Principle:

Sugars are metabolized through different metabolic pathways depending on types of microbial species and aerobic or anaerobic environment. If fermenting bacteria are grown in a liquid culture medium containing the carbohydrate ,they may produce organic acid as by - products of fermentation. These acids are released into the medium and so lower pH of medium. If a pH indicator such as Phenol red or bromocresol blue is included in the medium, the acid production will change the medium from its original color to yellow.

Gases production during the fermentation process can be detected by using a small, Inverted tubes, called a **Durham tubes**, within the liquid culture medium. If gas is produced, the liquid culture medium inside the Durham tubes will be replaced ; by the **gas in the form of a bubble**.

Interpretation:

If the medium changes from colorless to yellow and **gas bubble**. Is found in Durham's tubes than it indicates acid and gas production. In some cases gas may not be evolved during the process. If no change Observed colour of medium then suger is not degraded by the organism.



Carbohydrate Fermentation Tests (Durham Fermentation Tube)

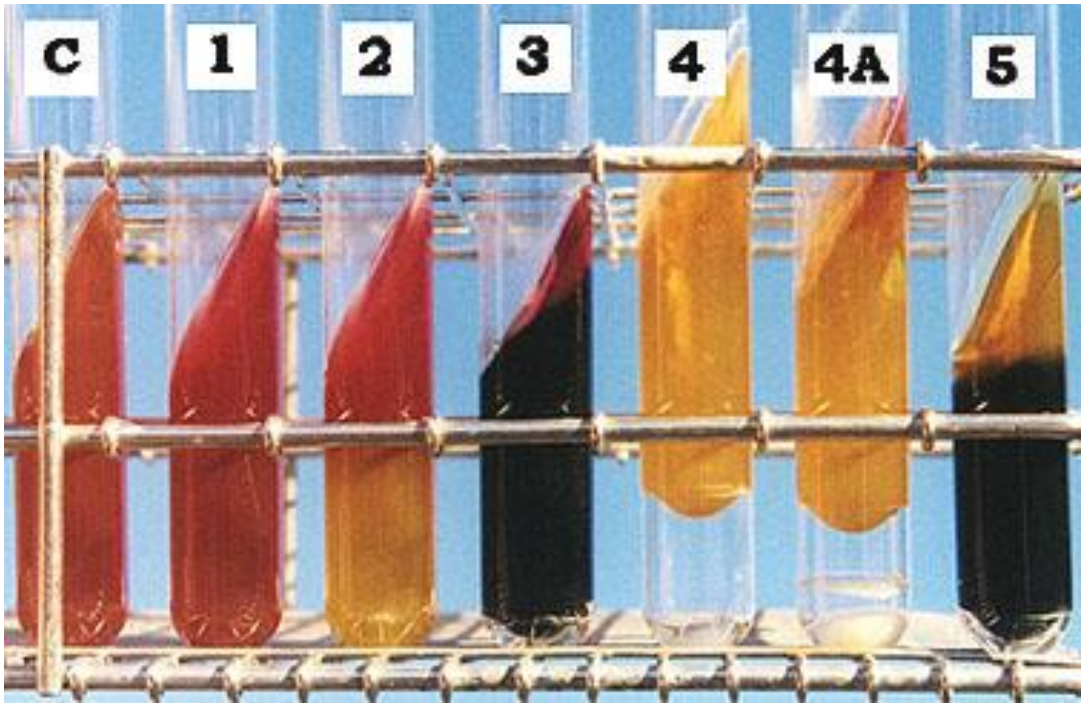
5- Triple Sugar Iron (TSI) agar:

Aims: to differential among and between the members of Enterobacteriaceae family.

Principle:

- Triple sugar iron (TSI) agar is a tubed differential medium used in determining carbohydrate fermentation and H_2S production. Gas from carbohydrate metabolism can also be detected. Bacteria can metabolize carbohydrates aerobically (with oxygen) or fementatively (without oxygen). TSI differentiates bacteria based on their fermentation of lactose, glucose and sucrose and on the production of hydrogen sulfide. TSI is most frequently used in the identification of the Enterobacteriaceae, although it is useful for other gram-negative bacteria.
- TSI agar slant contain a 1% lactose and sucrose ,and 0.1% glucose.
- The pH indicator (phenol red)
- from is red in colour due to presence of phenol red dye.
- sodium thiosulfate and ferrous sulfate make H_2S indicator system.

- thiosulfate reduced to by several species of bacteria and H_2S combines withand form insoluble black precipitates.$FeSO_4$ present in the medium.
- Blackening usually occurs in butt of tube.
- Incubation is for 18-24 hours in order to detect the presence of sugar fermentation, gas production, and H_2S production.

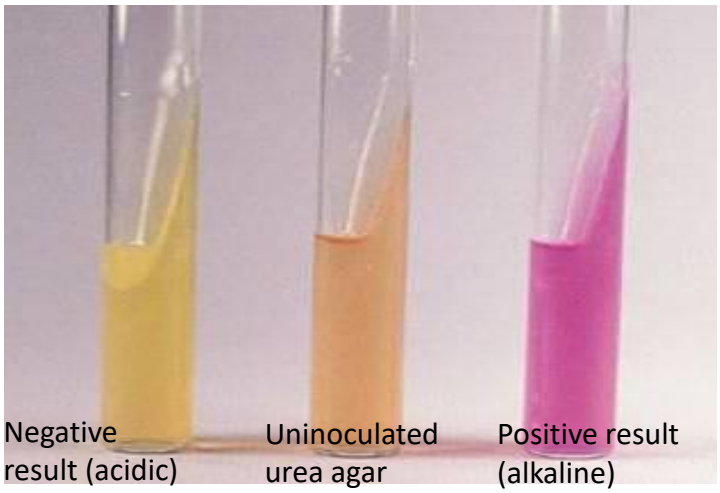


6- Urase test :-

The test used for detection of Proteus and other Gram negative intestinal bacteria which are capable of splitting urea by producing urase enzyme .

The test organism is cultured in a medium which contains urea and the indicator phenol red.

When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.



7- Motility test :-

The test differentiate between motile bacteria from non motile bacteria by using the sterile medium is inoculated by stabbing through the center of the medium and is incubated at the proper temp for 24-48 hours .

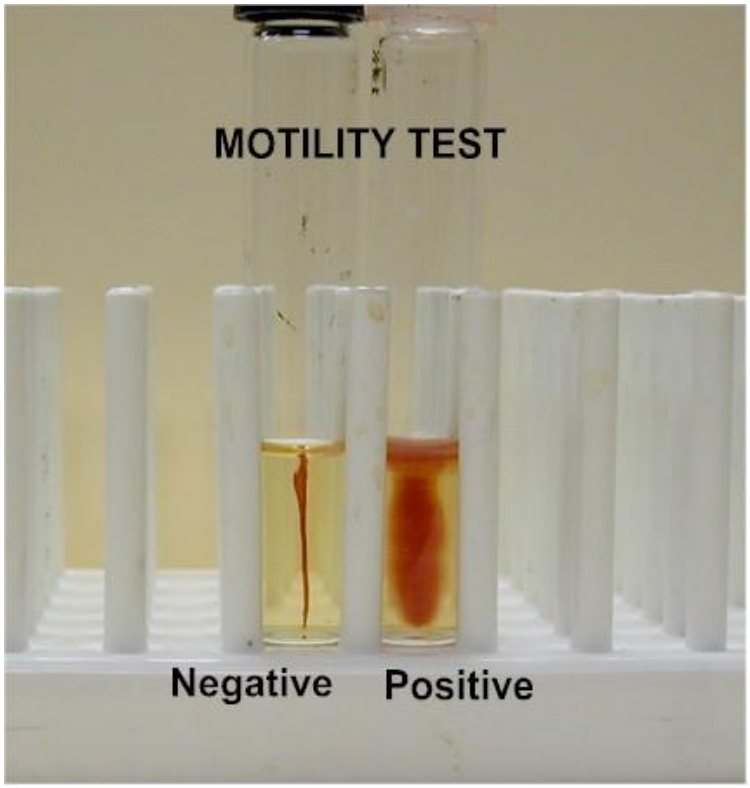


Table: Differentiation of Coli form Bacilli

Organism	Indol	MR	VP	SC	Urease	motility	TSI	GAS	H2S
E.coli	+	+	-	-	-	+	A/A	+	-
Klebsiella									
K.pnumoniae	-	-	+	+	+	-	A/A	+	-
K. oxytoca	+	-	+	+	+	-	A/A	+	-
Enterobacter									
E.aeroginosa	-	-	+	+	-	+	A/A	+	-
E.cloacae	-	-	+	+	d	+	A/A	+	-