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Genus : Clostridium

General character : G+ve bacilli , spore formation , obligated anaerobe , vary in their requirement for reduce O₂ .

Classification upon biochemical reaction :

1-**Saccharolytic** :CHO ferment Clostridium perfringens

2-**Proteolytic** : protein decompose Clostridium botulinum

3-**non Saccharolytic** :weak proteolytic Clostridium tetani

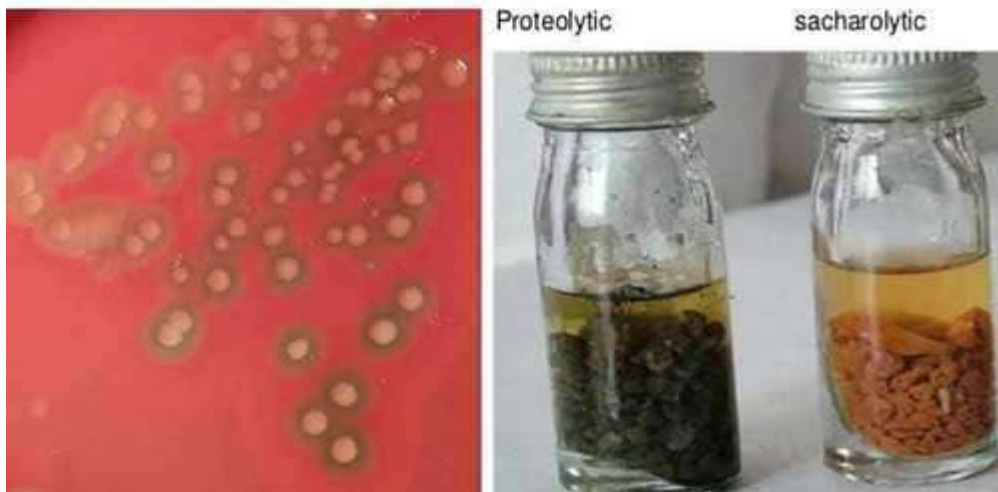
***Reactions on cooked meat medium:**

1. Saccharolytic reaction :

Causes fermentation of the muscle glycogen with production of turbidity, acid, gas bubbles and the meat particles remain intact.

2. Proteolytic reaction :

Causes digestion of meat particles leading to formation of black, foul smelling sulfur compounds.



Clostridium perfringens (welchii)

G+ve bacilli , non motile , central spore , Microaerophilic .

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Lab diagnosis :

- 1- G+ve bacilli , non motile , central spore
- 2-lactose fermentation
- 3-on B.A colony appear low convex, semi opaque , entire edge
- 4-selective media :B.A + Neomycin sulphate
- 5-litmus milk stormy fermentation
- 6-Double zone on B.A(β -hemolysis ...hemolysin)
(α -hemolysis.. α -toxin-lecithinase) ---detect by Nagler test +ve .

Litmus Milk Medium :

** It is a multi – purposed medium distinguish between different species of bacteria, contains the lactose (milk sugar), casein (milk protein) and litmus (pH indicator).

** Sugar users will make (acid reaction) causing a change to (pink), whereas casein protein users will produce (alkaline reaction) causing a change towards (blue), and if the casein is completely hydrolyzed, NH_3 is released and the medium turns a clear brown, in addition sometimes the litmus indicator get reduced and becomes white , some organisms produce enzyme renin (curd formed) and gas in the curd.

Reaction on Litmus Milk



Diagnostics: Litmus Milk Reaction showing Saccharolytic and proteolytic properties of *Cl. perfringens*

1- Acidic Reaction

Lactose in Milk $\xrightarrow{\text{Fermented to}}$ Acid $\xrightarrow{\text{Litmus Indicator}}$ Pink Color



2- Basic Reaction

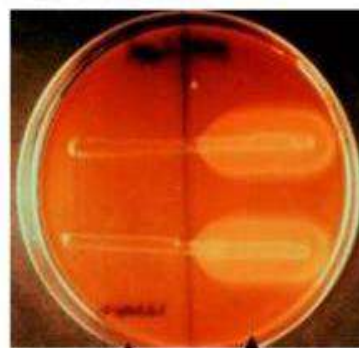
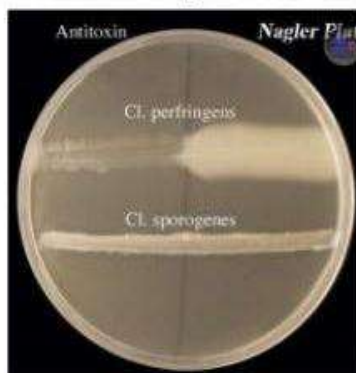
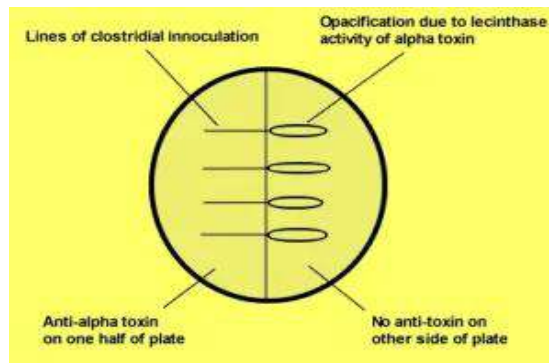
Casein in Milk $\xrightarrow{\text{Digestion}}$ Alkaline amines $\xrightarrow{\text{Litmus Indicator}}$ Blue Color



2- Stormy fermentation Too much acid and gas



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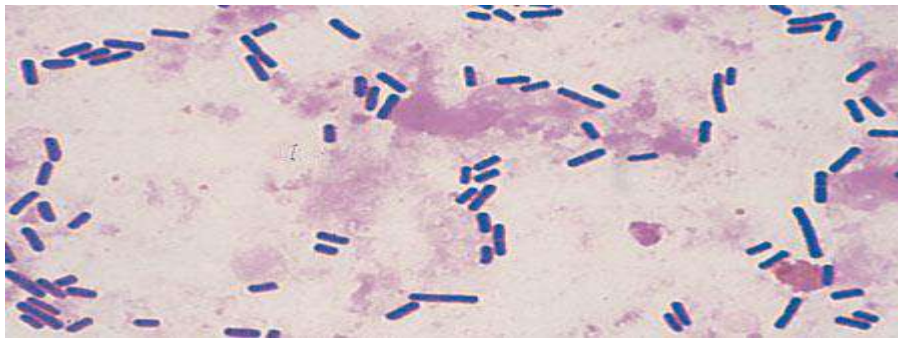
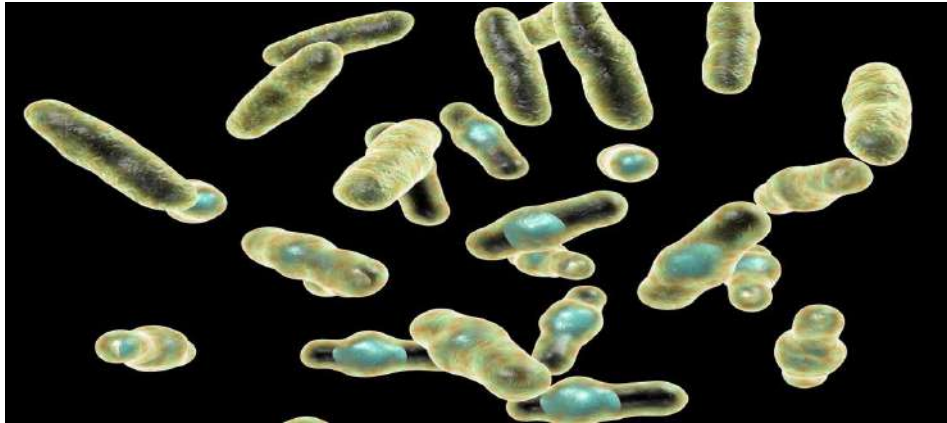


+ Antitoxin No antitoxin

20

Nagler test

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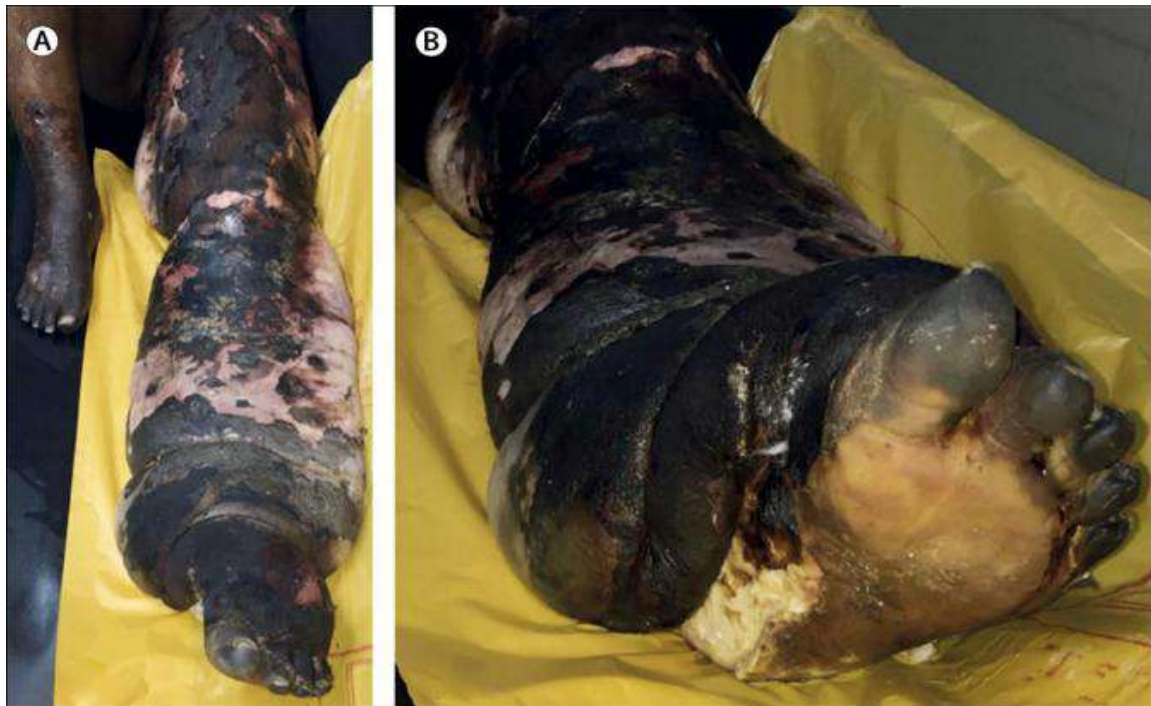


cl. perfringens



gas gangrene

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Gas gangrene

Pathogenicity : gas gangrene & mild gastroenteritis

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Clostridium botulinum

G+ve bacilli , sub terminal spore, motile.

Lab diagnosis :

- 1- G+ve bacilli , sub terminal spore, colony irregular or circular and translucent with granular surface.
- 2- selective media :B.A + Neomycin sulphate
- 3- milk litmus show proteolytic activity with violet color
- 4- on B.A β -hemolysis
- 5- glucose fermenter.
- 6- Indol -ve.

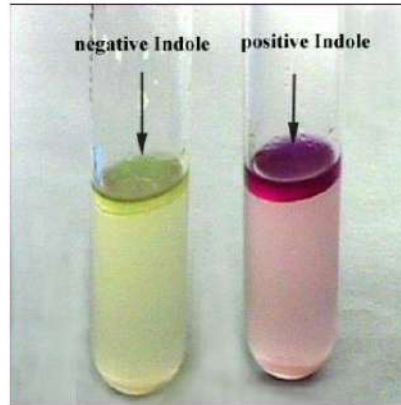


Cl. botulinum

Pathogenicity : food poisoning in canned food special in fish .

Indole Test

- Determine if the organism can produce the enzyme tryptophanase which cleaves tryptophan, generating indole
- Add 4-5 drops of Kovacs reagent



Clostridium tetani

G+ve bacilli , terminal spore , motile.

Pathogenesis:

Tetanus and locked jaw , rigidity in muscle lead to paralysis and produce toxins so called tetano spasma.

Lab diagnosis :

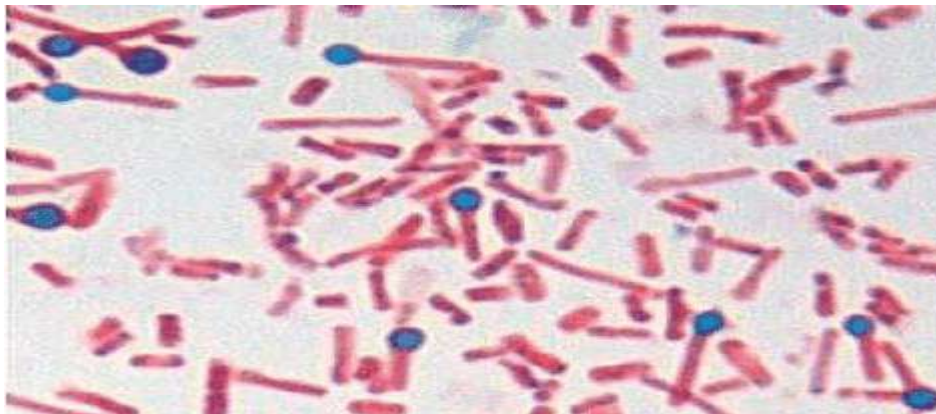
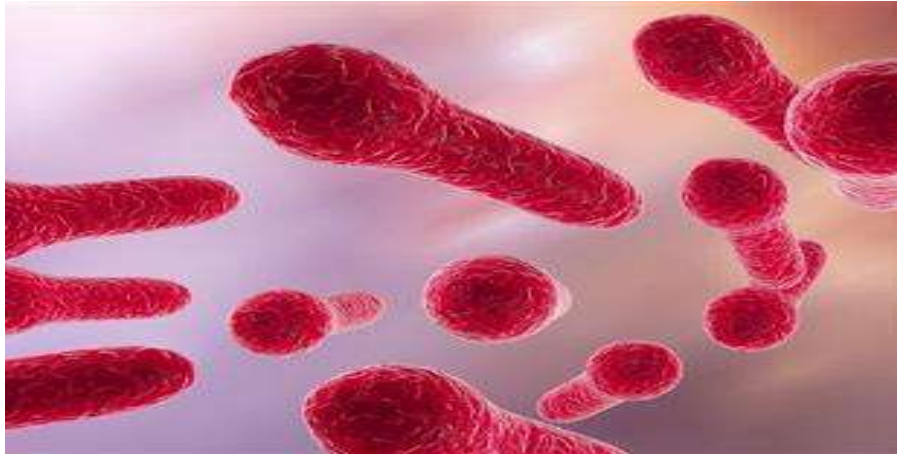
- 1- G+ve bacilli , terminal spore , motile and in smear G+ bacilli , spherical and terminal have drumstick appearance
- 2- on B.A β -hemolysis
- 3-strictly anaerobic , grow fine rhizoid
- 4-gelatinase +ve
- 5-litmus milk change to grey color

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6- Renin like enzyme producer

7- Glucose -ve.

8-Indol +ve.



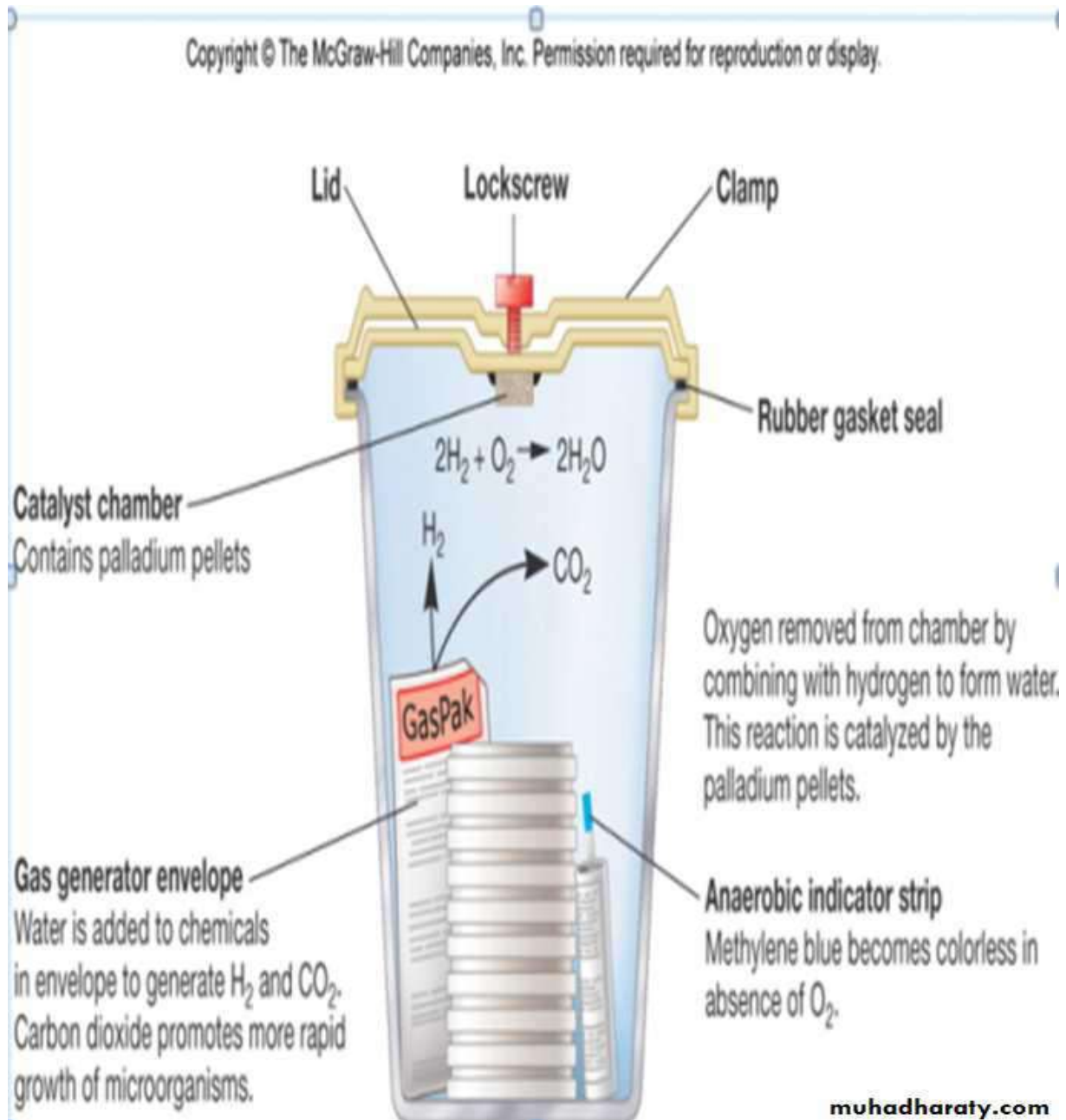
Cl. tetani

Selected Techniques for the Cultivation of Anaerobes:

1. Using media containing reducing agents, that reacts with oxygen and reduces it to water, e.g. sodium thioglycollate, ascorbic acid, strips of iron.
2. Anaerobic Jar : like McIntosh and Filde's anaerobic jar with a disposable envelope containing chemicals generate H₂ and CO₂ when water is added (Gas Pak system).

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3. Shake culture technique : nutrient agar is melted, cooled to approximately 45°C, inoculated with microorganisms and shaking to distribute contents evenly, incubation of the re solidified culture allows the development of separated colonies especially obligate anaerobes.



Genus Haemophilus (blood loving)

Lab diagnosis :

General character:

G- ve coccobacilli , non motile, non sporing, un able to grow on ordinary media without addition of whole blood or promoting factors:

X factor \longrightarrow heat stable iron protoporphyrin of hemoglobin =heme

V factor \longrightarrow NAD (nicotin amide adenin dinucleotide) co enzyme

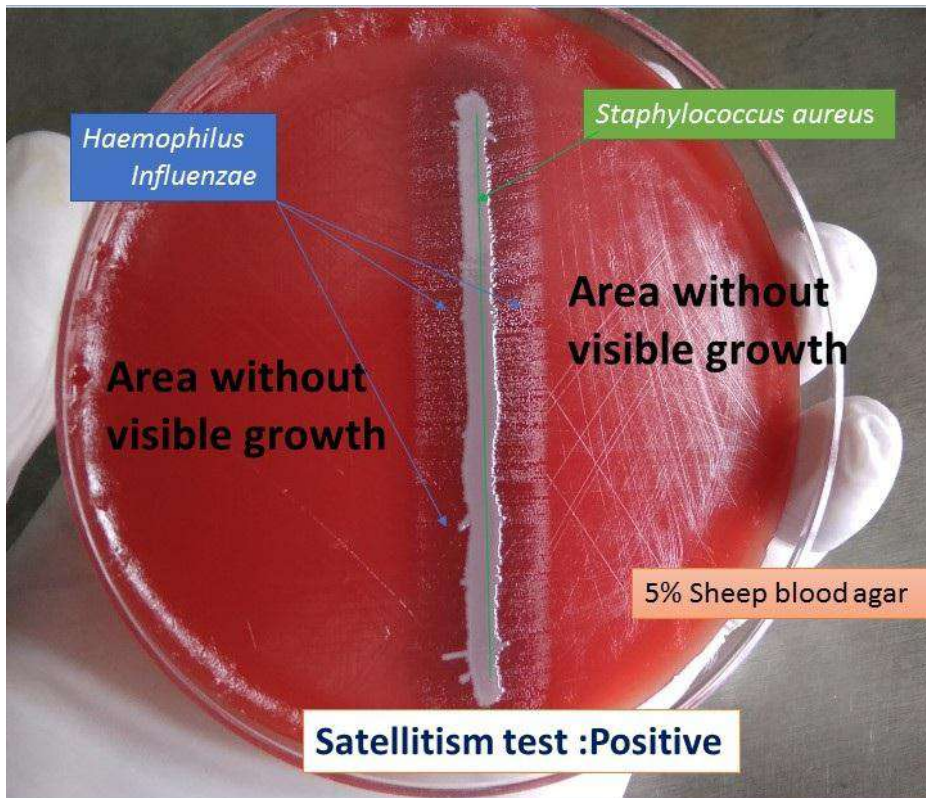
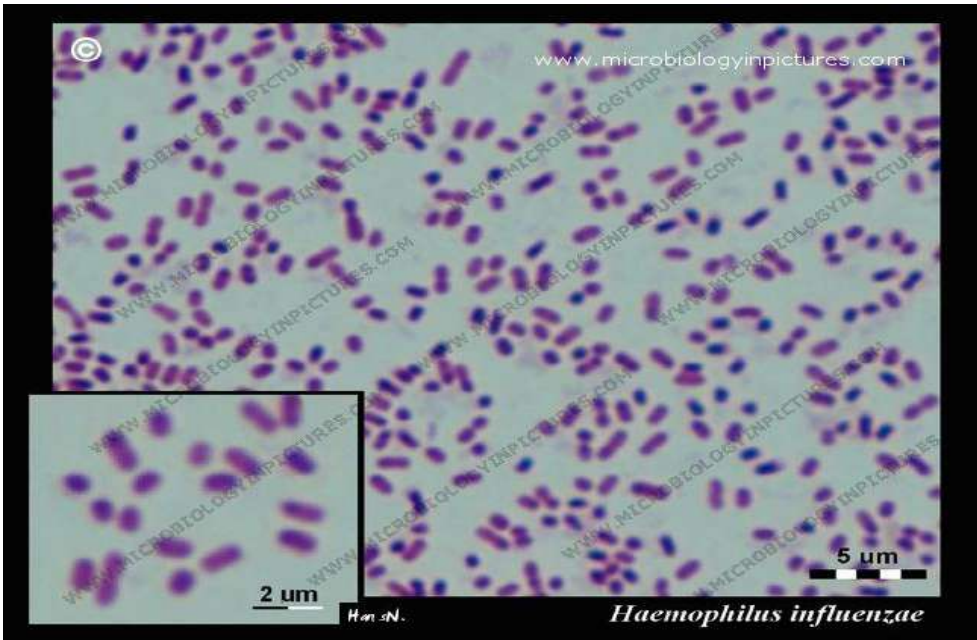
Chocolate agar contain X,V factors

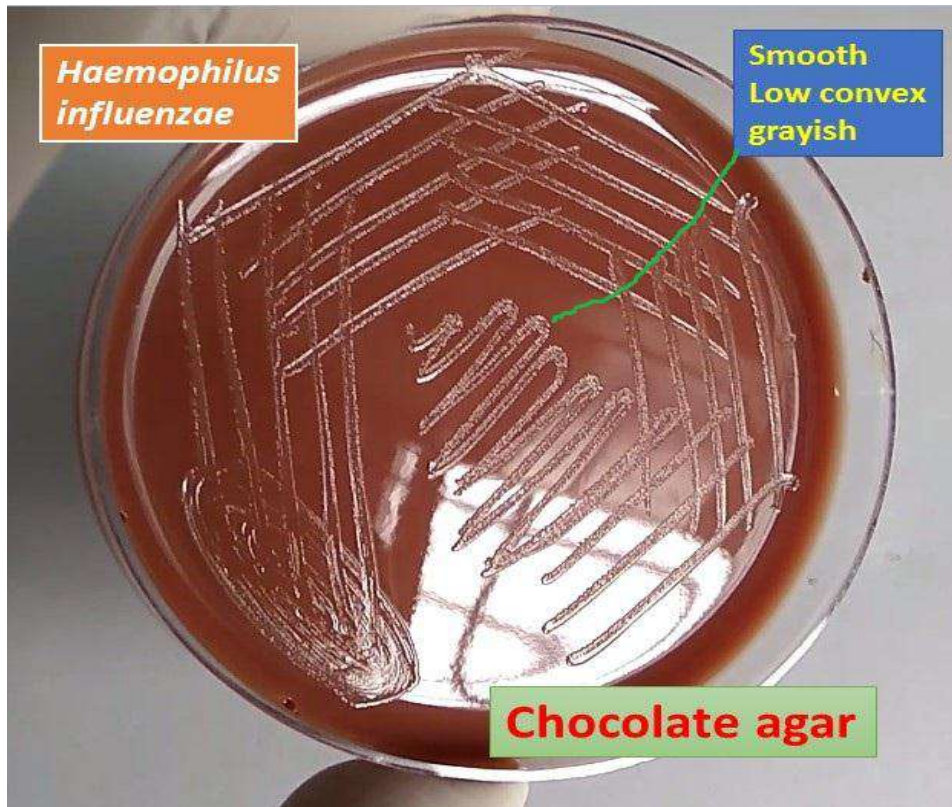
Blood agar contain X factor only

Colony appear semi opaque, mucoid, gray white in color and a virulent strain small , transparent and bluish

Satellitism phenomenon:

Many organism including staph, Neisseria, certain species of yeast, can synthesis NAD (V factor)when these organism are present in mixed culture , species of haemophilus need V factor growth around the microorganism as dew drop colonies with in the zone of NAD around the colonies of other bacteria. This phenomena occur on blood agar only.





Clinical samples:

CSF, sputum, nasopharyngeal swab

Pathogenesis:

Meningitis, laryngo epiglottitis, otitis media, pneumonia, arthrititis, endocarditis, chronic bronchitis.

Species	X factor	V factor	location	hemolysis	pathogenicity
H. influenzae	+	+	Respiratory tract	–	Meningitis in 5 month -5years ,septic arithritis, epiglottitis, otitis media, sinusitis, conjunctivitis
H. para influenzae	–	+	Normal in URT	±	Normal in URT , endocarditis, urethritis
H. aegypticus	+	+	Eye	–	Conjunctivitis (red eye)
H. vaginalis	±	-	vagina	±	Vaginitis
H. ducreyi	+	–	Sex organs	–	Soft sore in sex organs
H. haemolyticus	+	+	Non pathogenic URT	+	Normal in URT adult, pathogenic in children
H. suis	+	+	RT in swine	–	Pathogenic in swine
H. aphrophilus	+	–	Norml in URT	–	Norml in URT, endocarditis.

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Neisseria Genus:

General characters : G-ve cocci arrange in pair , oval or spherical Kidney in shape, aerobic, non motile , polymorpho nuclear, oxidase +ve, catalase +ve.

Oxidase :transport of electrons from the bacteria (electron doner) to the reagent by oxidase enzyme which reduced it to deep purple color (+ve).

Oxidase reagent :is a Tetramethyl paraplenylene diamine dihydrochloride .

Growth factors (**fastidious bacteria**):

1-enriched media (chocolate agar)

2- selective media(thayer martin media) contain **vancomycin** (inhibition G+ve)+**colistin** (inhibition G-ve)+**Nystatin** (inhibition fungi)

3-CO₂ (5-10%) **4**-humidity **5**-temp 37c & pH 7.4

Colony morphology :moist, elevate ,smooth ,round convex ,entire edge ,no hemolysis.

Species : **1-Neisseria gonorrhoeae**

2 -Neisseria meningitidis

Neisseria gonorrhoeae

Pathogenicity : GC (GonoCocci)-urogenital tract infections , cutaneous lesion , septicemia &anal canal infection ,rarely conjunctivitis ,endocarditis, gonorrhea, hepatitis.

Clinical sample Urine, seminal fluid ,rectal swab, vagain swab

Lab. Diagnosis :

1-G-ve diplococci (been shape) and not have capsule , extra &intra cellular if smear take from purulent discharge

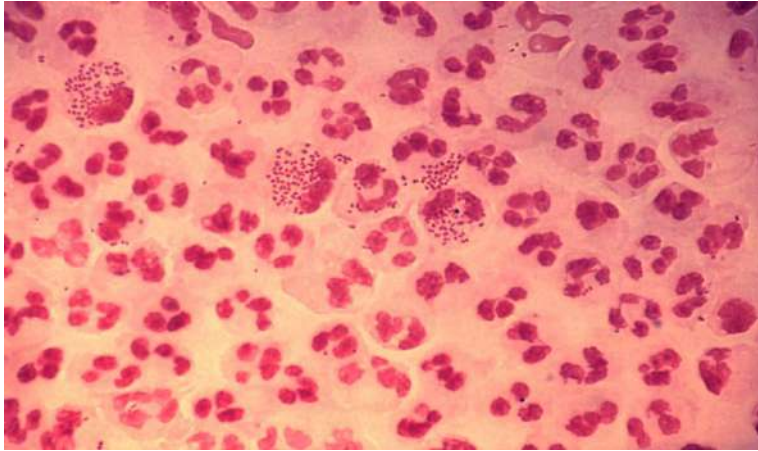
2-culture on B.agar ,chocolate agar ,thayer martin media (selective).

3-oxidase +ve

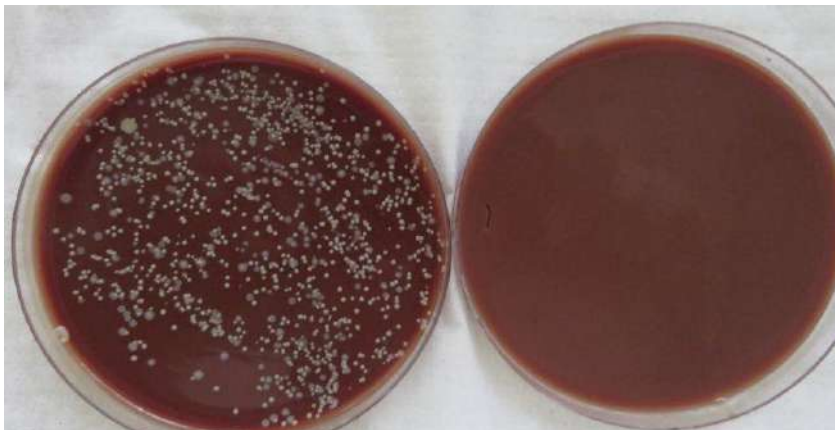
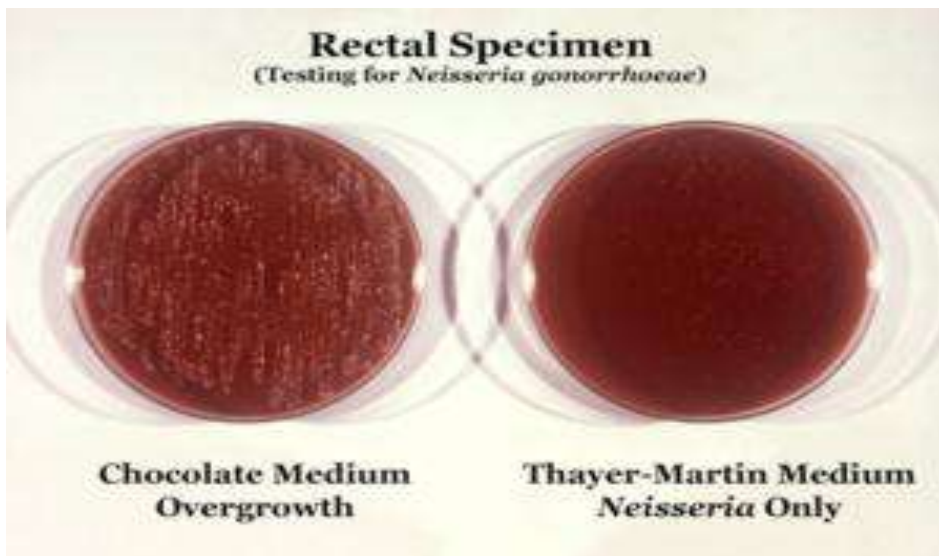
4-Ferment glucose only

5- Latex agglutination test (serological tests).

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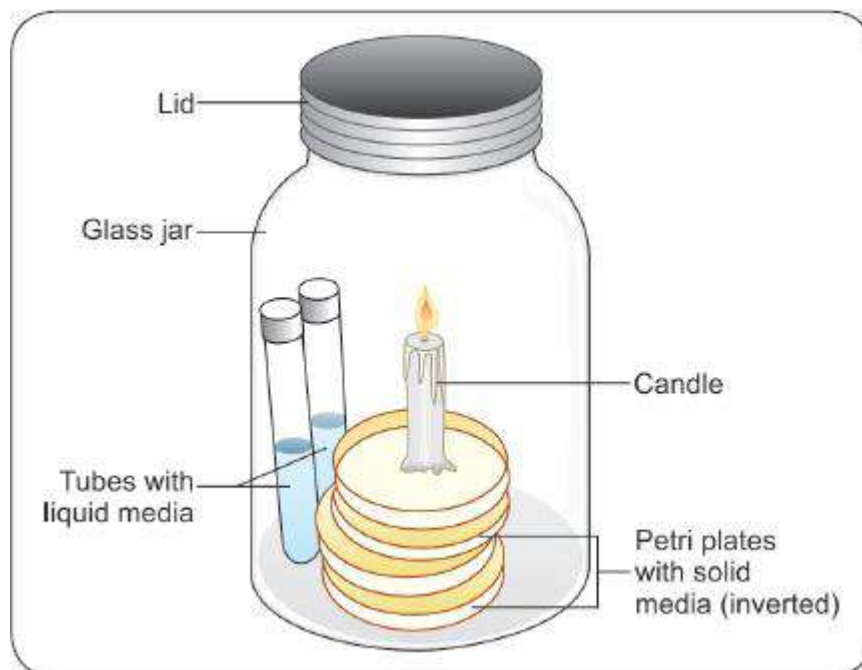
Neisseria gonorrhoeae



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Oxidase Test



Candle jar



Glucose +ve

Maltose -ve

Sucrose -ve

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Neisseria meningitidis

Pathogenicity : meningitis, septicemia.

Clinical sample : C.S.F, blood, nasopharyngeal swab.

Lab. Diagnosis

1-Microscopically : direct smear from c.s.f after centrifugation & Gram stain will show G-ve diplococci flattened intra&extracellular

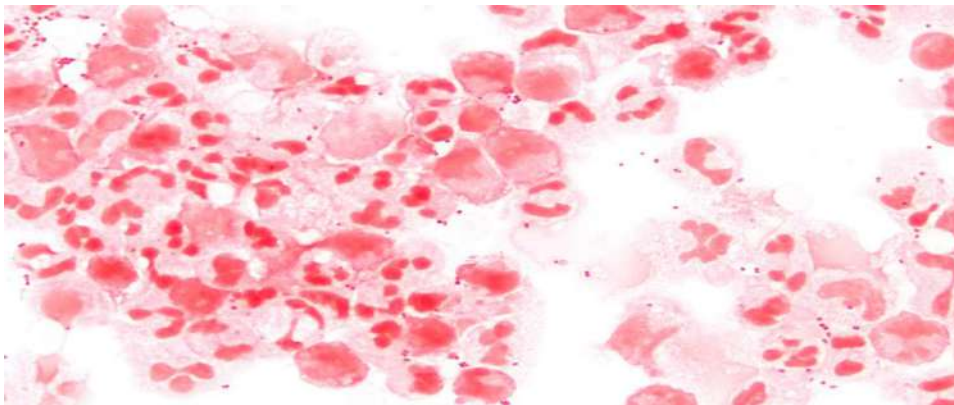
2-culture on B.agar, chocolate agar, thayer martin media

3-oxidase +ve

4-Ferment glucose & maltose

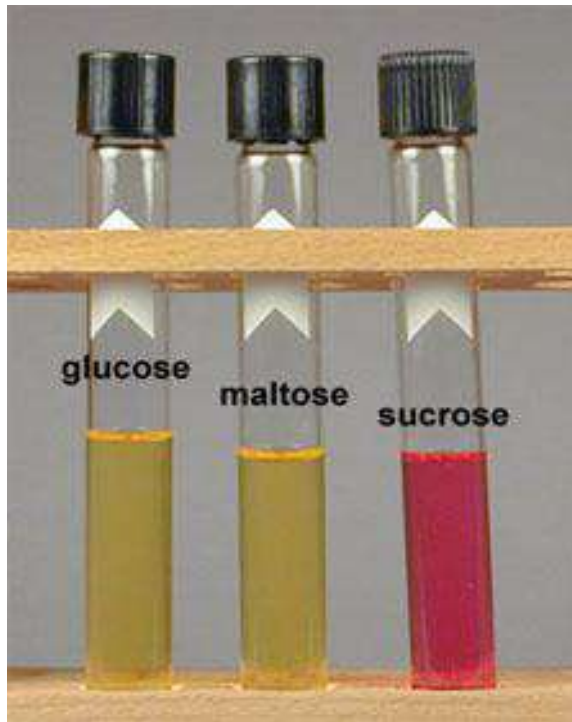
5- Quelling test for capsule (**have capsule**).

6- serological tests.



Neisseria meningitidis

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Glucose +ve

Maltose +ve

Sucrose -ve

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API for *Neisseria* species (Analytical Profile Index)

Glucose
+ ve

Maltose
- ve



Neisseria gonorrhoeae: Glucose +ve,
Maltose -ve

Neisseria catarrhalis

Non pathogenic, grow on ordinary media at 22 c , oxidase +ve, catalase +ve,
do not fermenter glucose and maltose.

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Enterobacteriaceae is a family of gram negative, facultative anaerobic non spore forming rods, motile, catalase positive and oxidase negative, reduction of nitrate to nitrite $\text{NO}_3 \rightarrow \text{NO}_2$, glucose fermentation resulting acid, grow on ordinary media or selective media.

Classification on the basis of lactose or non lactose fermentation to:

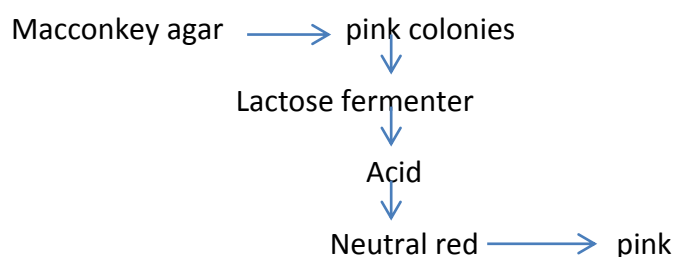
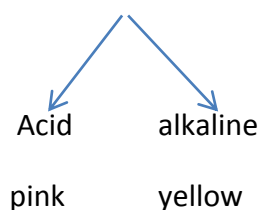
- 1- Lactose fermenter \rightarrow pink colonies after 18 hrs e.g E.coli
- 2- weak lactose fermenter \rightarrow pink colonies(light pink) after 18 hrs then lose the color (yellow colonies after 2-3 days e.g Klebsiella)
- 3- late lactose fermenter \rightarrow pink colonies after 48 hrs e.g Shigella sonnei
- 4- non lactose fermenter \rightarrow yellow colonies e.g Salmonella, Shigella, Proteus.

Media:

1) Macconkey agar (selective and differential) used for primary isolation

Composition:

- 1- lactose
- 2- bile salt (inhibit G+ ve)
- 3- indicator (neutral red)



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2) **The triple sugar iron test** : is a microbiological test named for its ability to fermenter sugar and to produce hydrogen sulfide, it is often used to differential enteric bacteria including Salmonella and Shigella.

Triple sugar iron agar (TSI) (**diagnostic media**)

Composition:

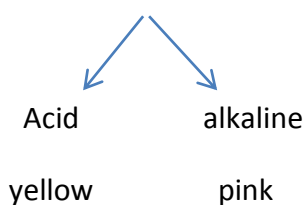
1- glucose 1 gm

2- lactose 10 gm

3- sucrose 10 gm

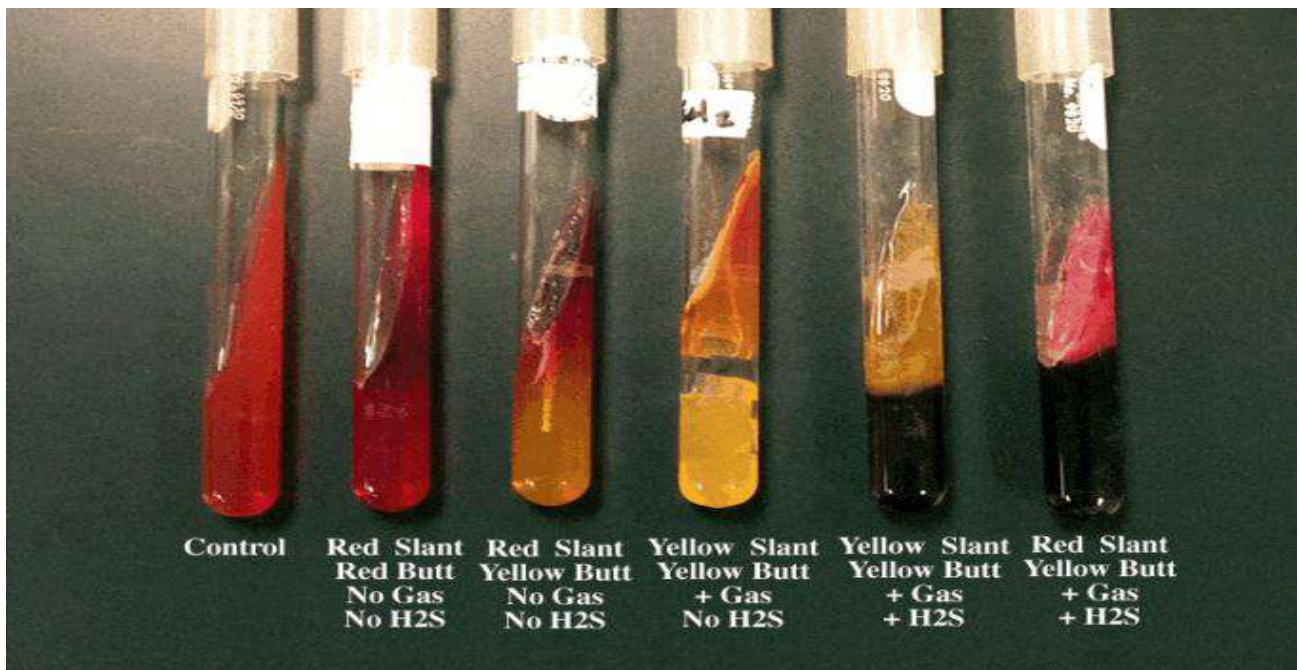
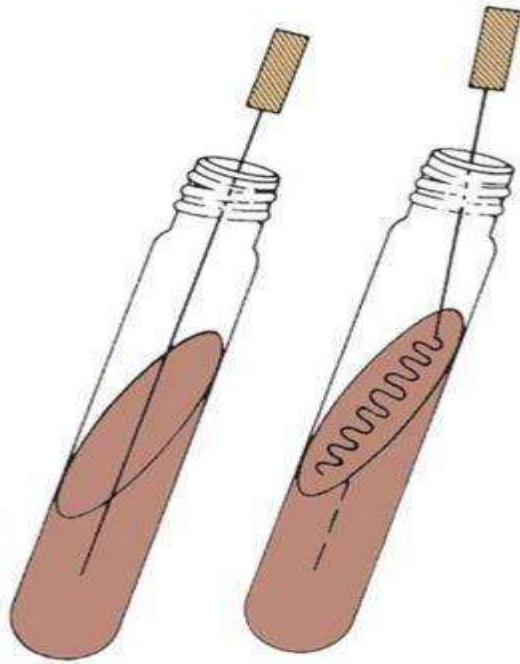
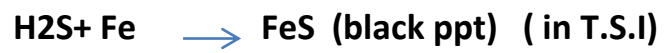
4- Fe ++

5- indicator phenol red



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Mechanism:



TSI agar

Triple Sugar Iron Agar

0.1%
dextrose

1.0%
sucrose

1.0%
lactose

(a) **Red/red** (no sugar fermentation)

(b) **Control**

(c) **Red/yellow** (Glucose fermented but lactose and sucrose not fermented)

(d) **Yellow/yellow** (Glucose fermented. Lactose and/or sucrose fermented)

(e) **Red/yellow with H₂S**

A B C D E



Figure 5-68

3) **DCA (deoxy cholate citrate agar)** inhibit E.coli

4) **S.S agar (Salmonella Shigella agar)** permit salmonella , shigella to growth

5- **Eosine methylene blue** (E.coli greenish metallic sheen)

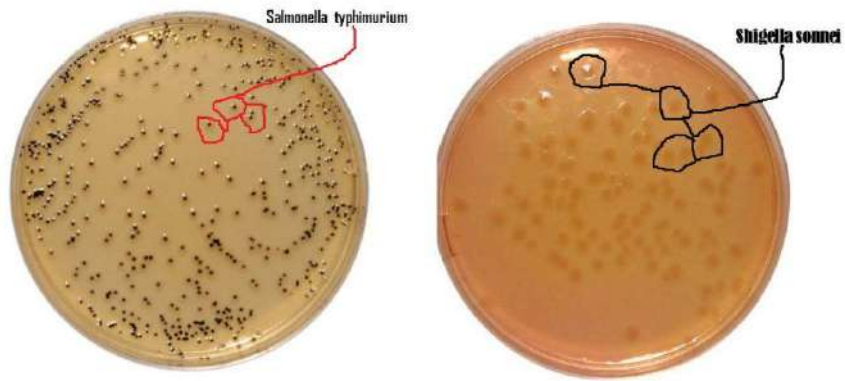
Other lactose fermenter appear purple

6- **Bismuth sulfate** for Salmonella typhi (black colonies)

7- **tetrathionate** (enrichment media, increase number of bacteria from small to plenty.

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Clinical samples → macconkey → T.S.I → IMViC tests



Deoxycholate Citrate Agar (DCA)

Source: Medical-Labs

Deoxycholate citrate Agar

- Suitable for isolation of dysentery bacilli, food poisoning Salmonella and S.paratyphi B, and less so, but superior to MacConkey agar for S. typhi.
- It is a heat sensitive medium It should not be autoclaved or remelted
- When prepared from commercial medium it should be dissolved and sterilized at 100^oc for a short period



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Salmonella-Shigella (SS) Agar

Salmonella Shigella (SS) Agar

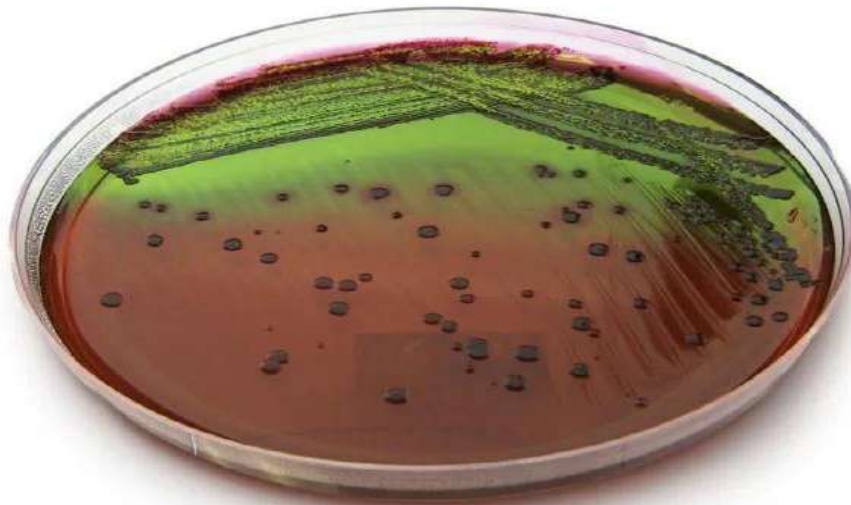
Principle, composition, uses & result interpretation.

Salmonella on SS Aga

Shigella on SS Agar

Escherichia coli

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**Escherichia coli colonies in Eosin Methylene Blue Agar
(Note: Greenish Metallic Sheen)**

Two petri dishes showing bacterial cultures on Salmonella Shigella (SS) Agar. The left dish shows a dark, metallic sheen, which is characteristic of Salmonella spp. The right dish shows a brownish, metallic sheen, which is characteristic of Shigella spp. A small white circular object is visible on the right dish. The MBcell logo is present in the bottom right corner of the image.

MB-S1394 Salmonella Shigella (SS) Agar
Cultivated Shigella flexneri (L)
/ Salmonella typhimurium (R)

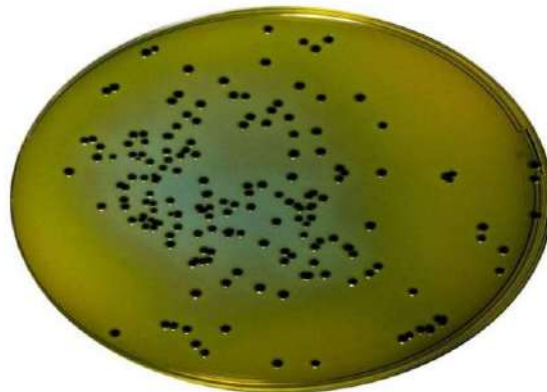
Bismuth sulfate agar is selective
for *Salmonella* spp.

Bismuth Sulfite Agar

- When plated on Bismuth Sulfite agar, *Salmonella enteritidis* produces black (hydrogen sulfide producing) colonies surrounded by black zones.



Bismuth Sulfite agar



IMViC tests: series in a group of four individual tests that are commonly used to identify bacterial species . The capital letters in MIViC each stand for one of the four tests

I= Indol test

M=Methyl red test

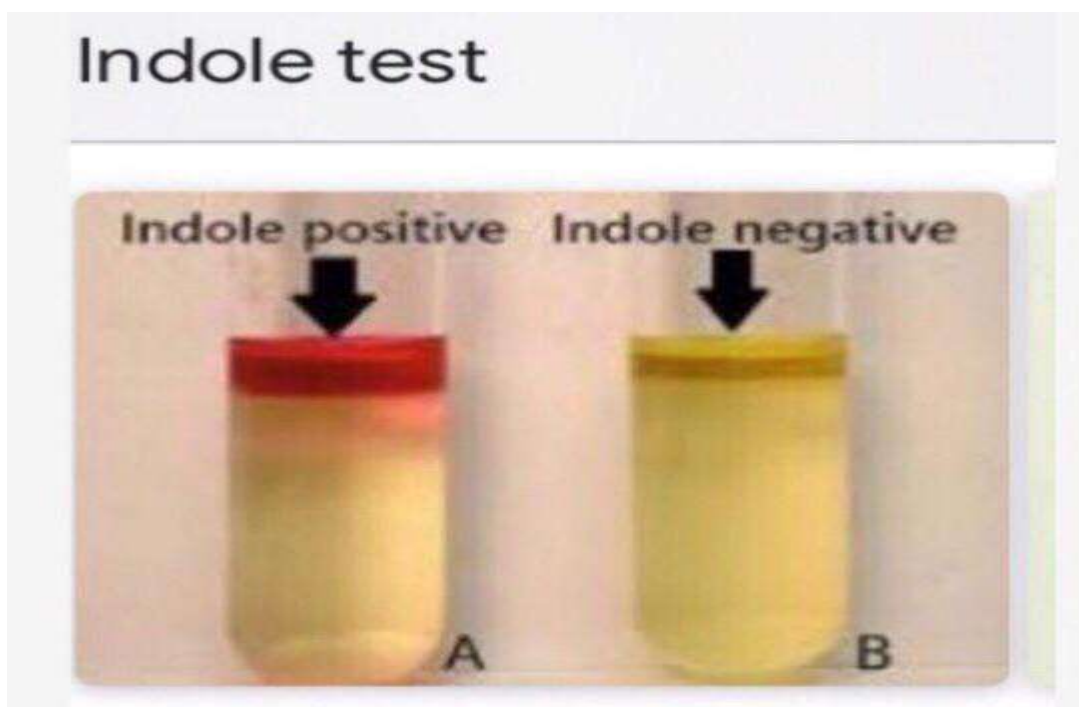
V=Vogas Proskaur test

C=Citrate utilization tests

1)Indol test:

The Indol test is a biochemical test for bacteria species to determine the ability of the organism to convert tryptophan in peptone water to indol by tryptophanase by adding Kovacs reagent and the +ve result is red ring.

Bacteria = peptone water $\xrightarrow{\text{tryptophanase}}$ Indol+ Kovacs reagent (red ring).



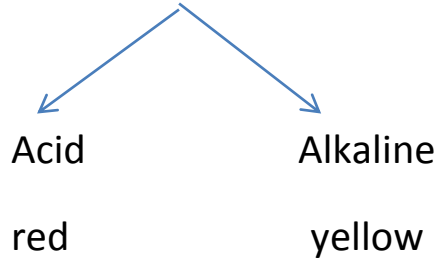
2) Methyl red :

Ability of bacteria to ferment sugar (dextrose)(complete fermentation) till acid

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Bacteria+ dextrose — incubation 37 C for 24 hrs → Acid+few drop of methyl red (complete fermentation) in PH= 4.5 → red color

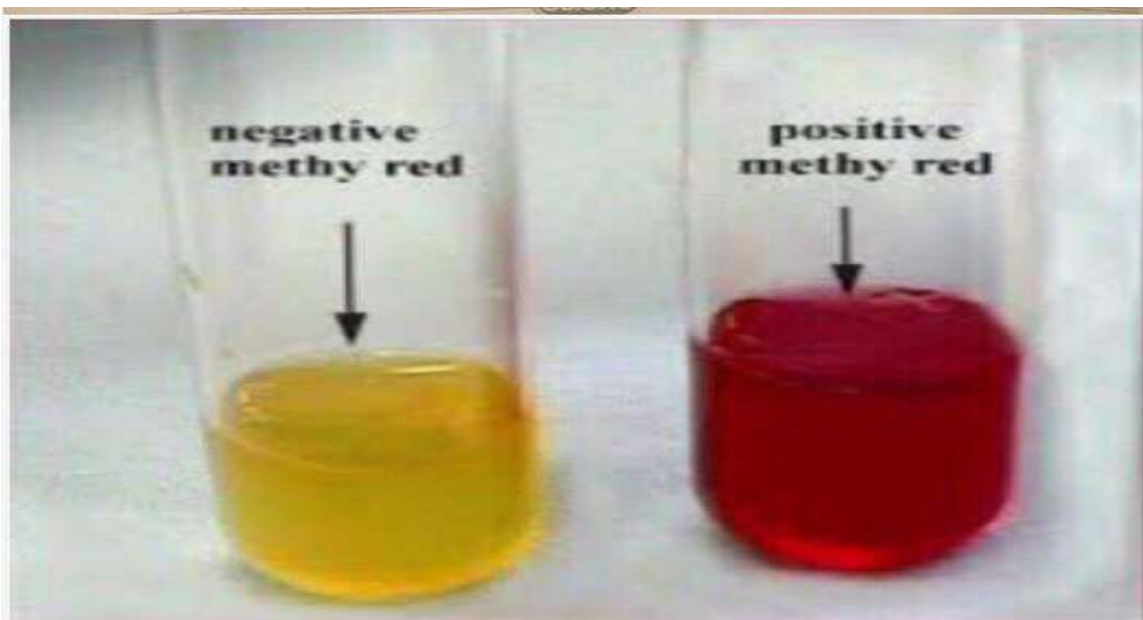
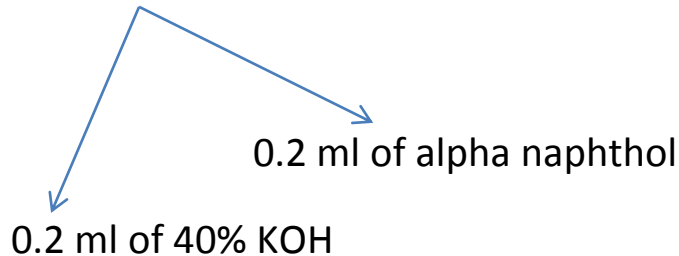
Methyl red indicator



3) Vogas Proskaur test:

Ability of bacteria to fermenter sugar dextrose (partial fermentation) to intermediate compound (acetyl methyl carbinol).

Bacteria+ dextrose incubation 37C for 24 hrs → acetyl methyl carbinol → Crimson color



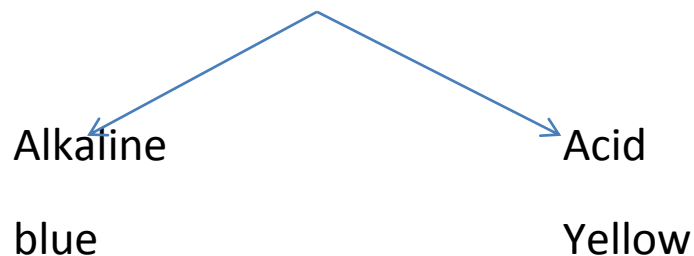


4) Citrate utilization test:

Ability of bacteria to utilize citrate salt as source of carbon and ammonium salt as source of nitrogen, media used to this test is Simmon citrate

Composition of simmon citrate

- 1- citrate salts
- 2- Ammonium salts
- 3-indicator (Bromo thymol blue)



bacteria+ Simmon citrate incubation 37C for 24 hrs → blue +ve (alkaline).

Citrate Utilization Test

