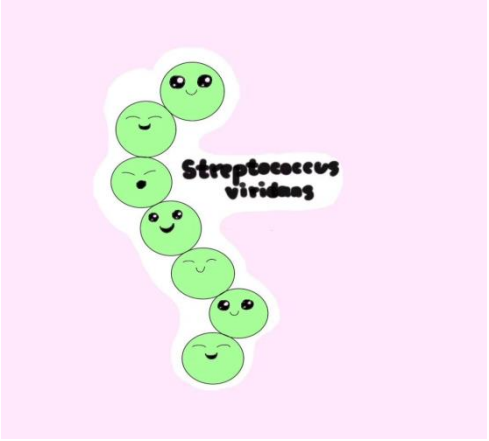
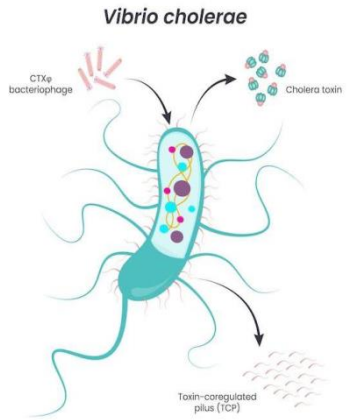
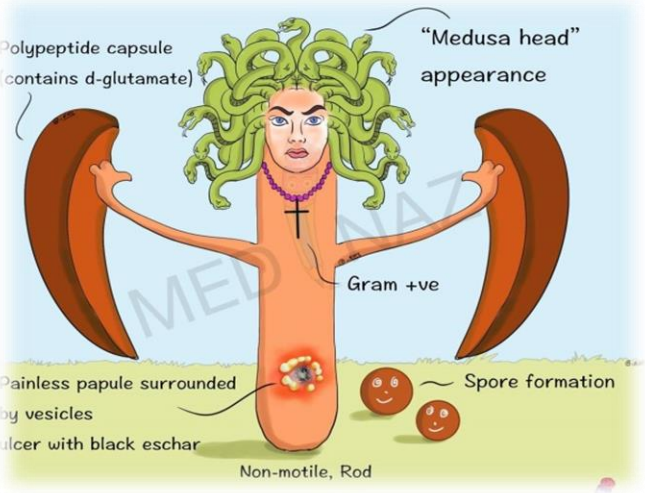


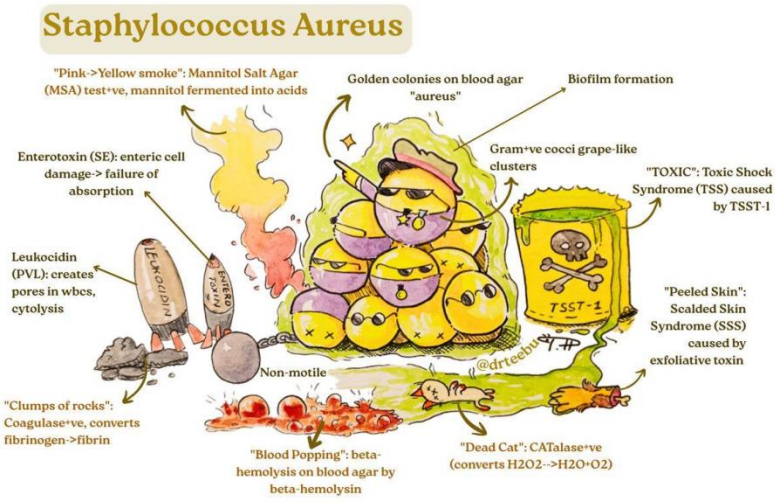












UNIVERSITY OF DIYALA _ COLLEGE OF SCIENCE _ DEPARTMENT OF BIOTECHNOLOGY

3RD GRADE / FIRST SEMESTER _ PATHOGENIC BACTERIA (PRACTICAL)

TEACHERS:

LECTURER HIBA ALI HILAL _ LECTURER ASST. MUSTAFA THAER HAMEED

University of Diyala
College of Science
Department of biotechnology

Lab1 Introduction in practical bacteriology

►**Bacteria:** Bacteria are microscopic living organisms that have only one cell. The word for just one is "bacterium." Millions (if not billions) of different types of bacteria can be found all over the world, including in your body. They're on your skin and in your airways and mouth. They're also in your digestive system , reproductive system and urinary tract. Scientists estimate you have 10 times more bacterial cells than human cells in your body. Bacteria divided into two types **pathogenic** and **nonpathogenic** bacteria .

► **bacteriology :** A branch of microbiology dealing with the identification, study, and cultivation of bacteria and with their applications in medicine, agriculture, industry, and biotechnology.

► **Pathogenic bacteria :** are bacteria that can cause disease. They can reproduce quickly in your body and give off poisons (toxins) that can cause infection. **For examples *: Escherichia coli* , *Staphylococcus aureus* , *streptococcus pyogens* .**

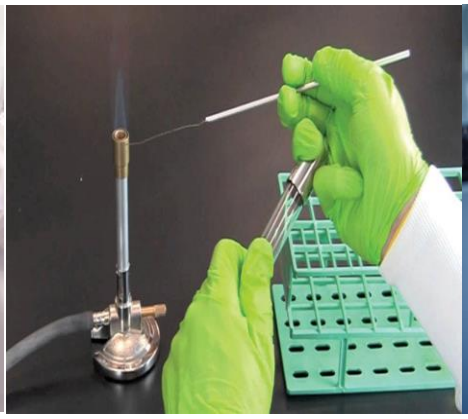
General Guidelines about Medical Bacteriology Lab

Lab Safety

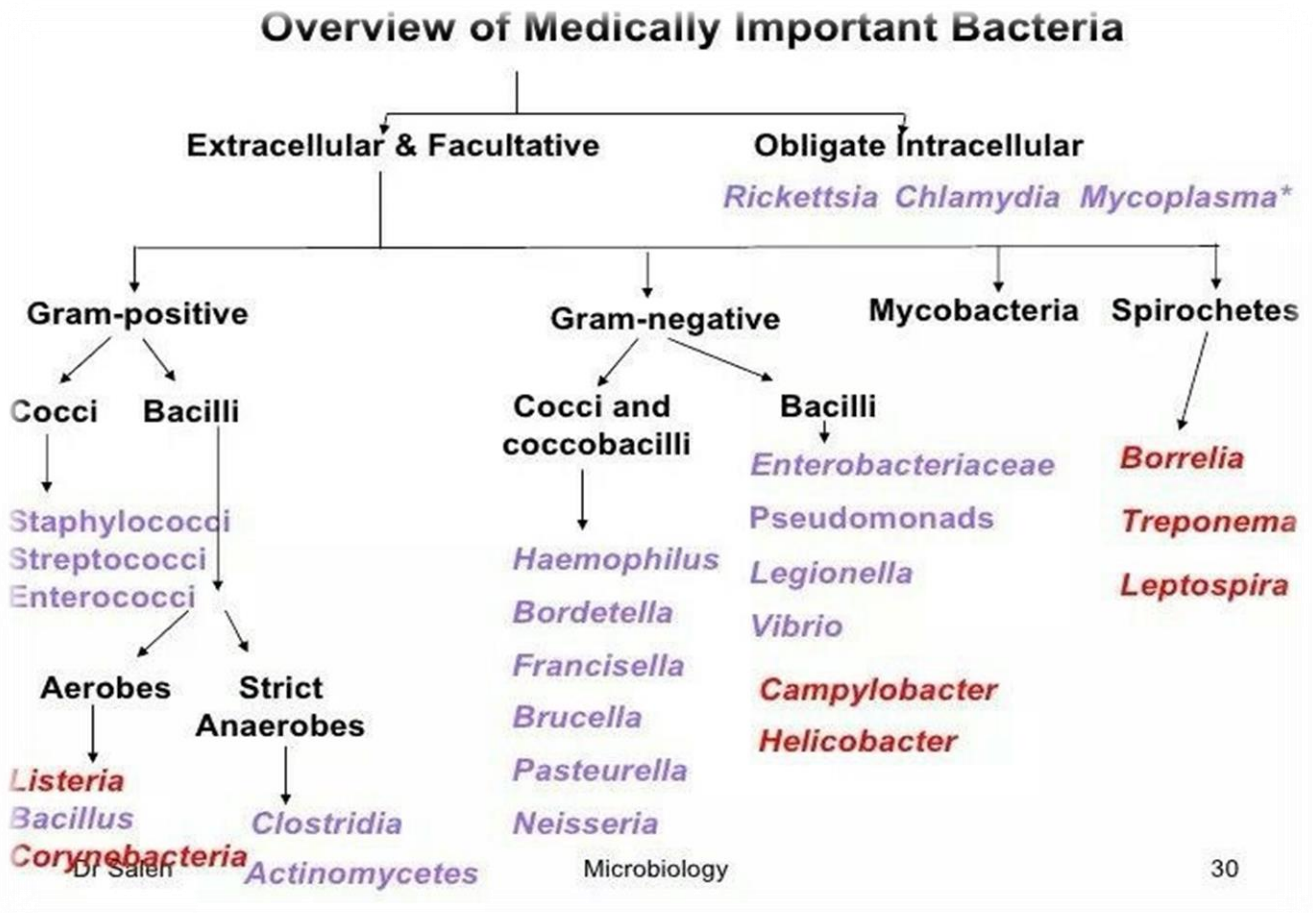
1. **All materials and clothes other than those needed for the laboratory are to be kept away from the work area.**
2. **A lab coat or other protective clothing must be worn during lab. The lab clothing is not to be worn outside of the laboratory.**
3. **Clean the lab table before and after lab with the disinfectant solution provided.**
4. **Wash hands before leaving lab.**
5. **Any item contaminated with bacteria or body fluids must be disposed of properly. Disposable items are to be placed in the BIOHAZARD container. Reusable items are to be placed in the designated area for autoclaving prior to cleaning. Sharps are to be disposed of in the appropriate container.**
6. **Reusable items should have all tape and marks removed by the student before being autoclaved.**
7. **Because organisms used in this class are potentially pathogenic, aseptic technique must be observed at all times. NO eating, drinking, application of cosmetics or smoking is allowed. Mouth pipetting is not allowed.**
8. **Cuts and scratches must be covered with Band-Aids. Disposable gloves will be provided on request.**
9. **Long hair should be tied back while in lab.**
10. **All accidents, cuts, and any damaged glassware or equipment should be reported to the lab instructor immediately.**
11. **Sterilization techniques will involve the use of Bacticinerators that are fire and burn hazards.**
12. **Bacticinerators reach an internal temperature of 850oC or 1500oF. Keep all combustibles (capable of catching fire) away from the Bacticinerators. Do not leave inoculating loops or needles propped in the Bacticinerator.**
13. **Microscopes and other instruments are to be cared for as directed by the instructor.**
14. **It is the responsibility of the student to know the location and use of all safety equipment in the lab (eyewash, fire extinguisher, etc).**
15. **Cultures may not be removed from the lab. Visitors are not allowed in the lab.**

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16. **Doors and windows are to be kept closed at all times.**
17. **For the best lab experience, read labs before coming to class. Make notes as necessary. Wait for a laboratory Background by the instructor before starting work.**

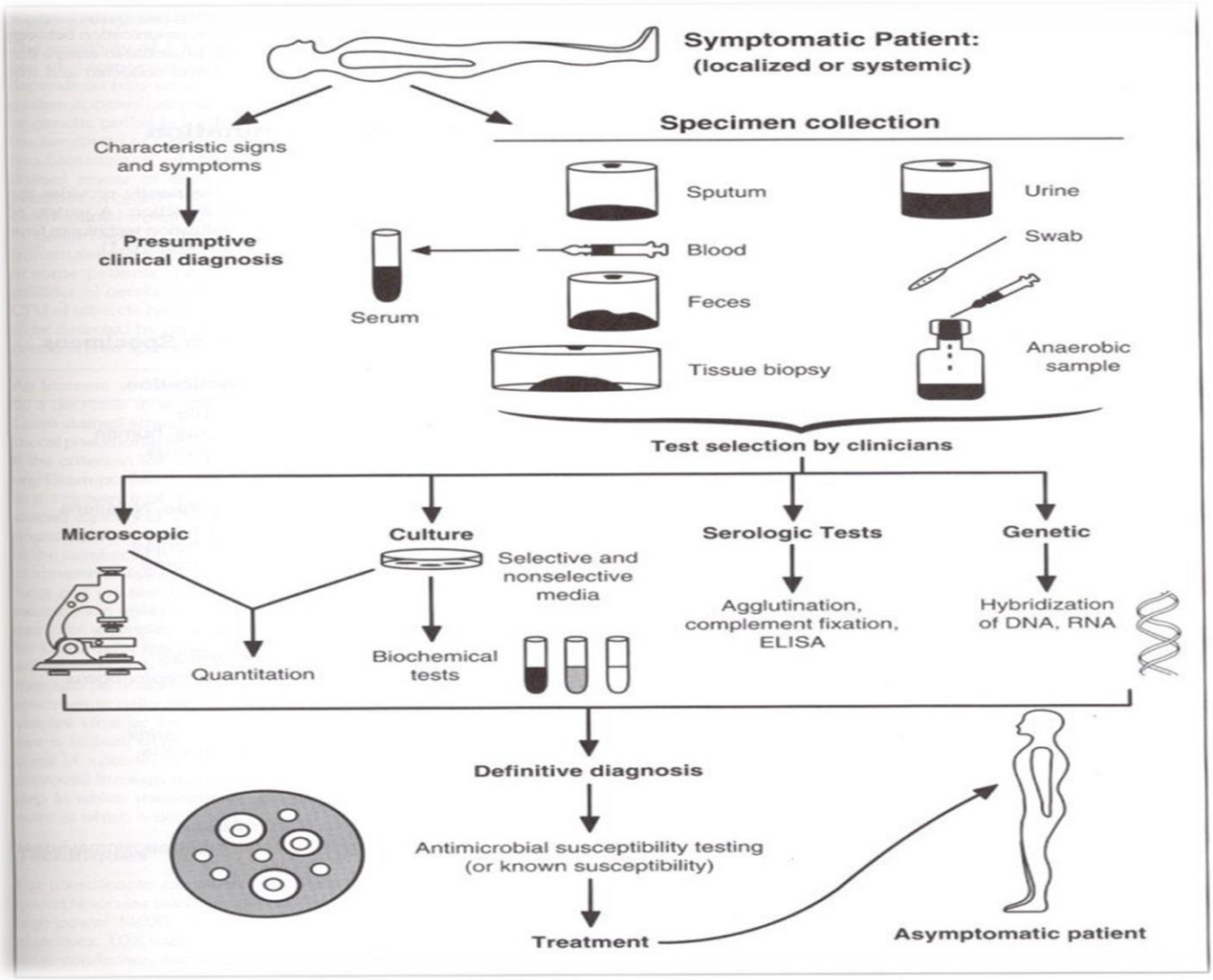




Medically important bacteria According to Gram's stain:



Principles of pathogenic bacteria identification : Identification of the organism causing an infectious process is frequently essential for effective antimicrobial and supportive therapy.

1. **Specimens collection .**
2. **Direct microscopic visualization of the organism.**
3. **Cultivation and identification of the organism.**
4. **Detection of microbial antigens [immunohematology]**
5. **Detection of an inflammatory or host immune response to the microorganism [immunohematology]**
6. **Detection of microbial DNA or RNA [molecular biology] and All laboratory studies must first be directed by the patient's clinical information (that is, their history and physical examination), and then evaluated, taking into consideration the sensitivity and specificity of the test.**



Basic steps for infectious diseases diagnosis

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Routine microbiology specimen collection procedures:

1. **Specimens collection :**

NOTE\Samples should be collected before the start of any treatment .

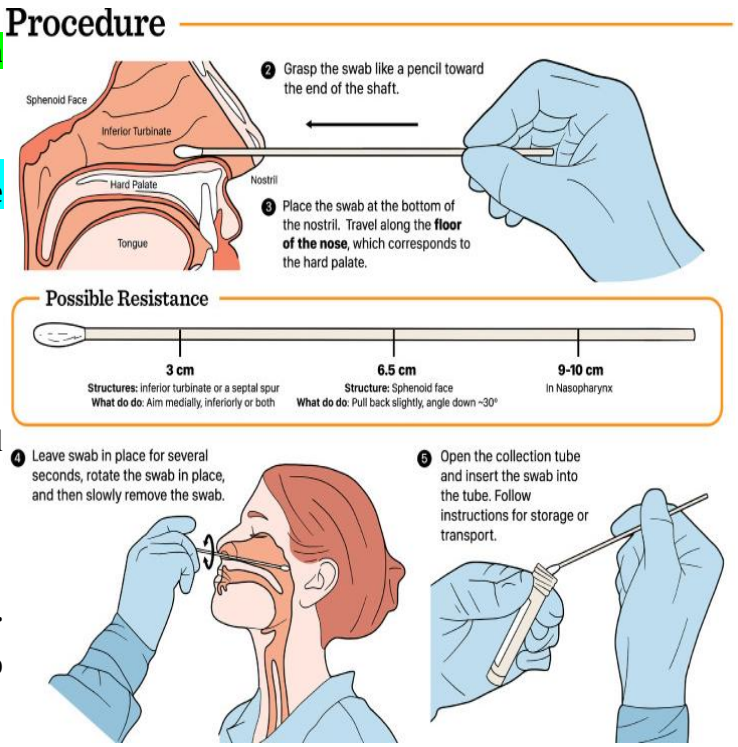
A: -Respiratory tract specimens

A-Nasopharyngeal swab :

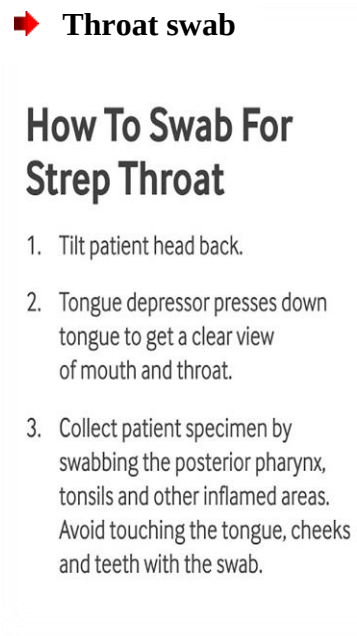
• Insert a sterile swab into the nose until resistance is met at the level of the turbinate.

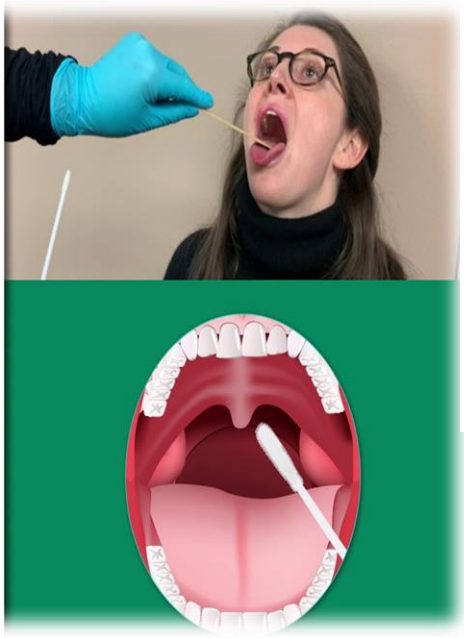
• Rotate the swab against the nasal mucosa.

• Place into swab transport system, and deliver promptly to the lab. Rayon / Dacron Swab to collect samples



Throat swab





Sputum **Have patient rinse mouth and throat with fresh water, cough deeply and expectorate into Sputum Collector. An early morning specimen is usually more productive. 5-10 cc of specimen is desired. Label specimen and send to laboratory immediately. If there is a delay in transport to the lab, specimen should be refrigerated.**

B. Eye specimens

- Where possible ask the patient to look upwards and gently pull the lower lid down or gently part the eyelids. Use a sterile cotton wool swab and gently role the swab.

- over the conjunctival sac inside the lower lid or using gauze. Hold the swab parallel to the cornea to avoid injury if the patient moves, Place the s swab in the transport medium.



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C. Ear swabs:

►If there is purulent discharge this should be sampled.

►Place a sterile swab into the outer ear and gently rotate to collect the secretions.

► Place swab in transport medium.

► For deeper ear swabbing a speculum may be used

D. wound and miscellaneous specimens:

►Use a sterile swab and gently rotate on the area to collect exudate from the wound and place into transport medium.

► Where there is pus collect as much as possible in a sterile syringe or sterile container (do not use a swab) and send to the laboratory.





E. Urinary tract specimens (Midstream or 'clean catch' specimen)

- **Ask the patient to void a small amount of urine into the toilet first.**
- **Then ask the patient to urinate 10-20 ml directly into the specimen container.**
- **Instruct the patient that the remaining urine can passed into the toilet.**
- **Place the lid securely on the specimen container**



Containers for urine & feces sample collection

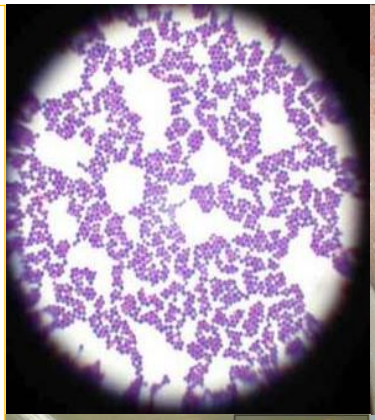
F. Fecal specimens: Collect feces from patients as soon after onset of illness as possible, and before the start of treatment. For liquid stool specimens, no more than 10 ml should be added to the Cary Blair medium and mixed.

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Third Grade / First Semester
Pathogenic Bacteria / Practical

lab 2: Staphylococcus aureus :

► ***Staphylococcus aureus:*** is a gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as "grape-like." that cause a wide variety of clinical diseases. Infections caused by this pathogen are common both in community-acquired and hospital-acquired settings. The treatment remains challenging due to the emergence of multi-drug resistant strains such as **MRSA** (Methicillin-Resistant ***Staphylococcus aureus***).













► **laboratory diagnosis :**

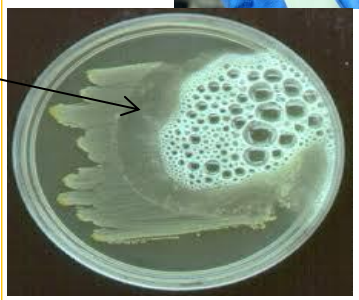
1. **Gram staining:** Gram-positive cocci in clusters, cocci may appear singly in pairs or in short chains.

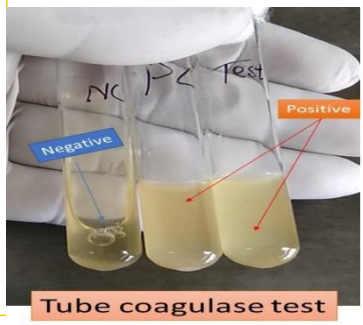
2. Culture media

- **Blood Agar:** growth occurs abundantly within 18 to 24 hours, yellow or golden yellow colonies with ***S.aureus*** on Blood Agar. large, smooth, hemolytic colonies. The lysis of the RBCs is due to alpha toxin are seen.
- **Mannitol Salt Agar (MSA)** is a selective media commonly used for the isolation of *S. aureus.* After inoculation, MSA plates were incubated at 35°C for 24 to 48 hours. *S. aureus* is mannitol fermenting bacteria and gives yellow or gold colonies

3. biochemical tests:

- **Catalase test :** The catalase test is important in distinguishing ***streptococci*** (catalase-negative) ***staphylococci*** which are catalase positive. The test is performed by flooding an agar slant or broth culture with several drops of 3% hydrogen peroxide. Catalase-positive cultures bubble at once. The test should not be done on blood agar because blood itself will produce bubbles.





4-Coagulase Test (tube coagulase test):

Coagulase producing strains of ***S. aureus*** form a clot (solid fibrin gel) when grown in plasma whereas coagulase negative do not form a clot

- **Principle of Tube coagulase test:**

The free coagulase secreted by ***S. aureus*** reacts with coagulase reacting factor (CRF) present in plasma to form a complex, thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma.

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Procedure for Tube coagulase test:

1. Take three test tubes and label them as “test”, “negative control” and “positive control”.

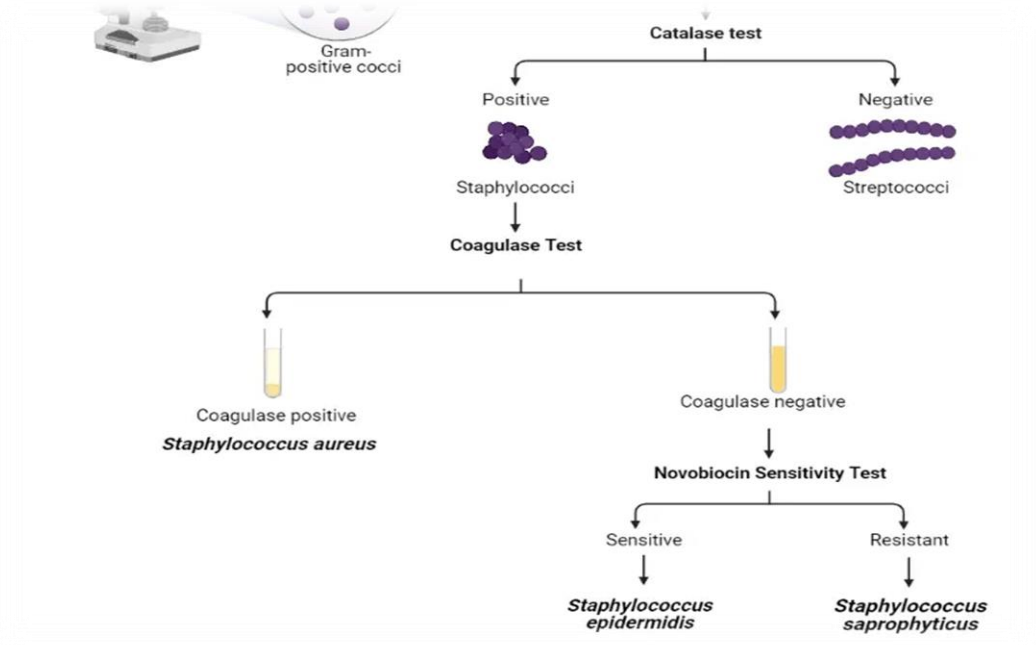
2. Fill each test tube with 1 ml of 1:6 dilution of rabbit or human plasma in normal saline.

3. Add 0.1 ml of overnight broth culture to the tube labeled test. Also add 0.1 ml of overnight broth culture of known S. aureus to the tube labeled positive control and 0.1 ml of sterile broth to the tube labeled negative control.

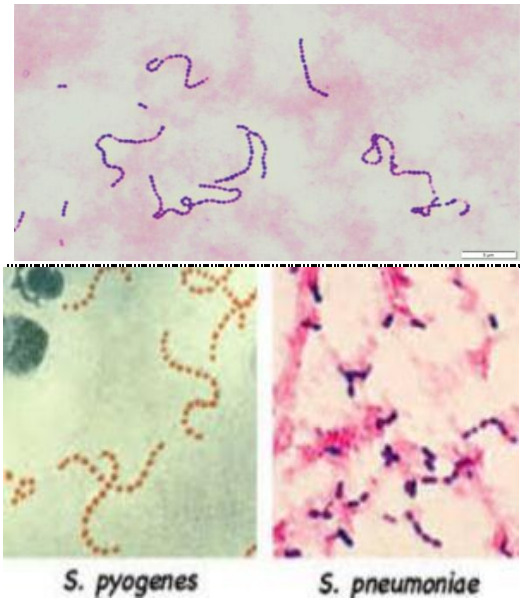
4. Incubate all the tubes at 37oC and observe up to four hours.

Most important test for S. aureus identification

No.	Test	result
1	Gram staining	+
2	Catalase	+
3	Oxidase	-
4	Co agulase	+
5	DNAase	+
6	Mannitol fermintation	+
7	Hemolysis	Beta
8	Growth on N.A	Production golden pegment on agar



Lab 3: Gram positive cocci (*Streptococci spp*).



- ***Streptococci*** **:** are Gram-positive cocci arranged in chains or pairs. They are part of the normal flora of humans and animals. Some of them are human pathogens. The most important of them is Streptococcus pyogenes causing pyogenic infections, with a characteristic tendency to spread, as opposed to staphylococcal lesions, which are typically localized. It is also responsible for the nonsuppurative lesions, acute rheumatic fever and glomerulonephritis which occur as sequelae to infection.
- **Common characteristics shared by these gram-positive cocci are:**

1. Gram-positive cocci, arranged in chains or pairs.
2. Non motile, non spore forming.
3. Some strains are capsulated, which are important in pathogenicity.
4. Catalase-negative.
5. Majority are facultative anaerobes, few are obligate anaerobes.
6. They are fastidious microorganisms grow on enriched media such as blood agar, have small, pin head, opaque, circular colonies.
7. Sensitive to drying, heat, and disinfectant.

- **Classification of Streptococci based on :**

-reactions on blood agar) (Brown in 1903) The type of hemolytic reaction on blood agar has long been used to classify the streptococci.

1. **Alpha (α)** hemolytic streptococci Alpha (α) hemolytic streptococci produce a greenish discolouration with partial hemolysis around the colonies known as viridans streptococci" or ***Streptococcus viridians*** (from „viridis" meaning green). The alpha streptococci are normal commensals in the throat, but may cause opportunist infections rarely. Pneumococcus (***Streptococcus pneumonia***) is also an alpha hemolytic streptococcus.

2.Beta (β) hemolytic **streptococci Beta:** (β) hemolytic streptococci produce a sharply defined, clear, colourless zone of hemolysis, 2-4mm wide, within which red cells are completely lysed. Most pathogenic streptococci belong to this group e.g. Streptococcus pyogenes.

3. Gamma (γ) or nonhemolytic streptococci Gamma (γ) or nonhemolytic streptococci produce no change in the medium. They include the fecal streptococci (***enterococci, Str faecalis***) and related species (enterococcus group).

Blood Agar:

Shows three types of hemolysis

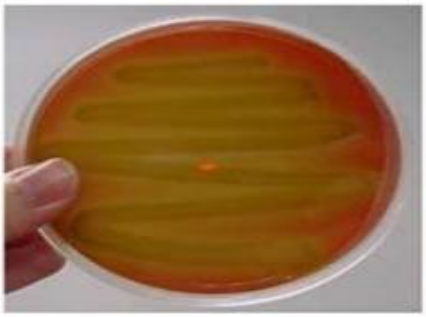
α Hemolysis

β Hemolysis

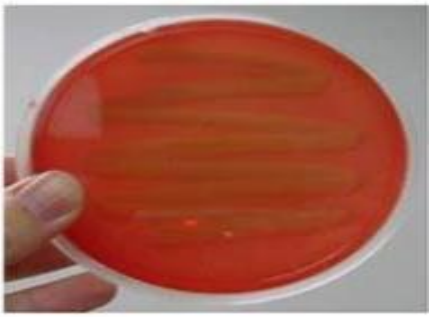
γ Hemolysis



Beta Hemolysis



Alpha Hemolysis



Gamma Hemolysis

Classification Based on O_2 requirement

- **Aerobes** → Growth on BA
  - **α hemolysis** → Incomplete hemolysis (green color) → Strep. viridans Strep. pneumoniae
  - **β hemolysis** → Complete hemolysis → Lancefield grouping (C carbohydrate Ag on cell wall) → Group A – U (21 groups) → Griffith typing of Group A on MTR proteins into > 100 types
  - **γ hemolysis** → α / β / no hemolysis → specific → *Enterococcus fecalis*
- **Anaerobes** → Peptostreptococci

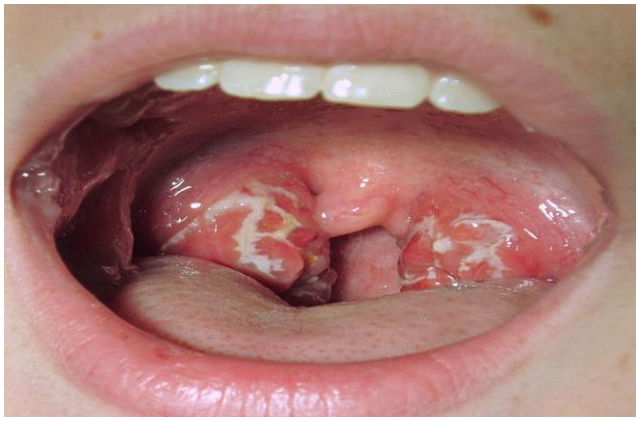
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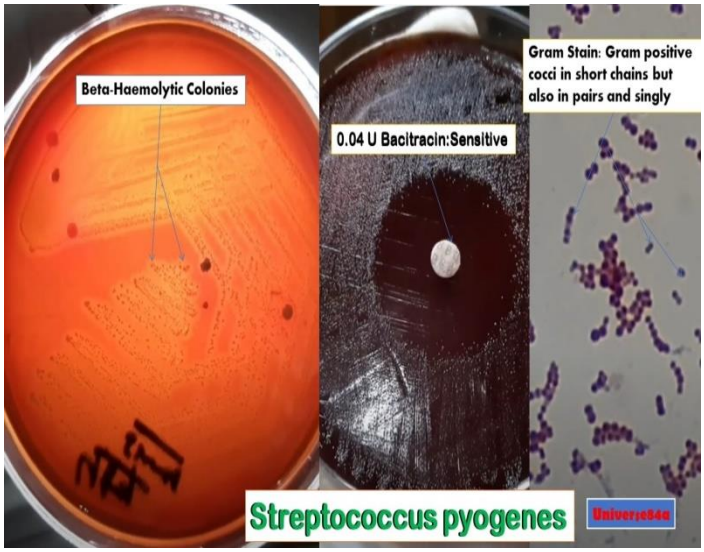
HUMAN STREPTOCOCCAL PATHOGENS

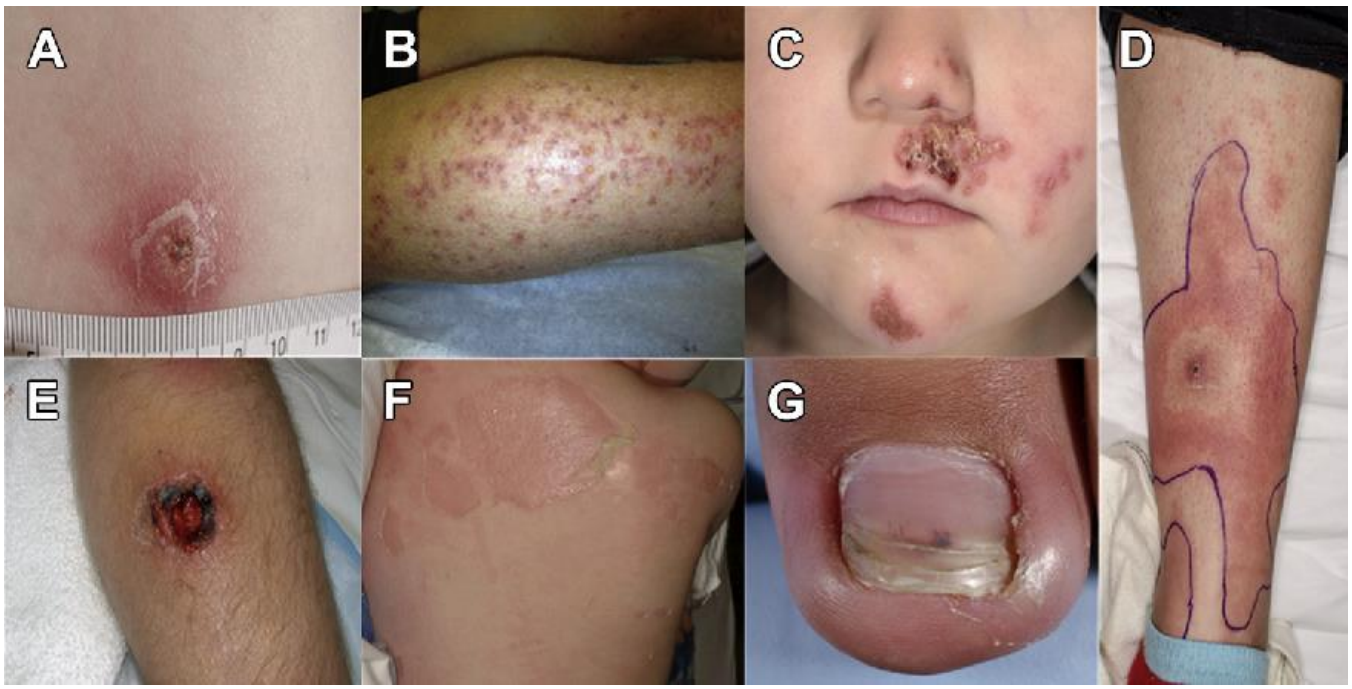
- ***S. pyogenes***
- ***S. agalactiae***
- ***S. Viridans streptococci***
- ***S. pneumonia***
- ***Enterococcus faecalis***

Human streptococcal pathogens (β-hemolytic)

Group A streptococci - *S. pyogenes:* Most serious streptococcal pathogen, Inhabits throat, nasopharynx and occasionally skin.







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Laboratory diagnosis of *S. pyogenes*

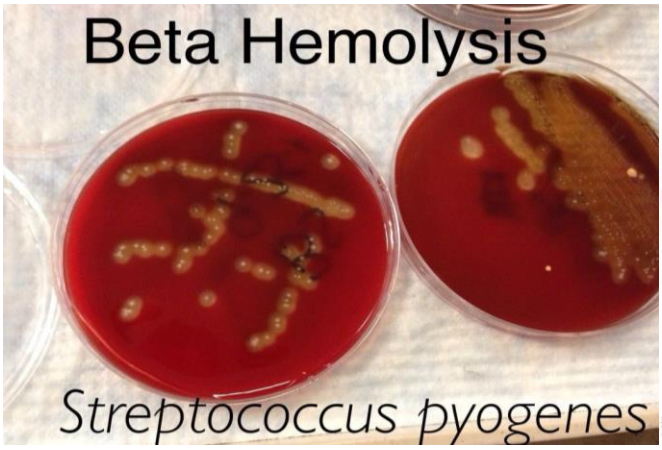
►*Specimens:* Throat swab, pus, blood

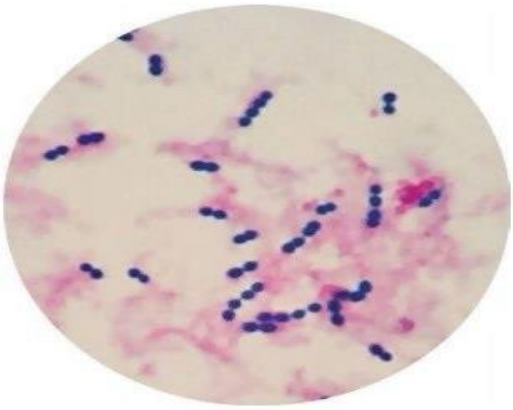
►Microscopy :Gram stain: G+VE in short chains arrangement

►Culture: Blood Agar : beta hemolytic colonies

►Identification tests:

- **Catalase : Negative**
- **Bacitracin: sensitive**
- **ASO titer /Ab. titer: normal < 200< positive result.**





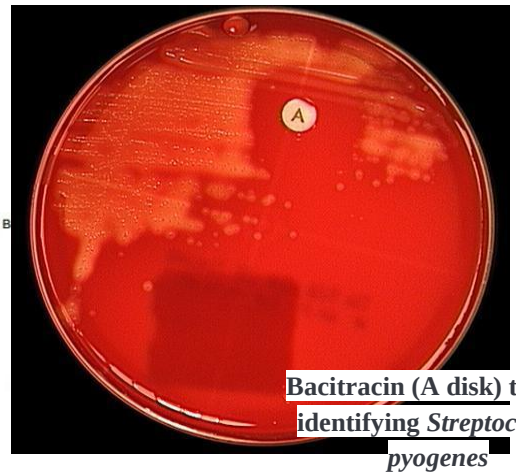
Gram staining of *Streptococcus pyogenes*

Sensitivity to Bacitracin and Optochin:-

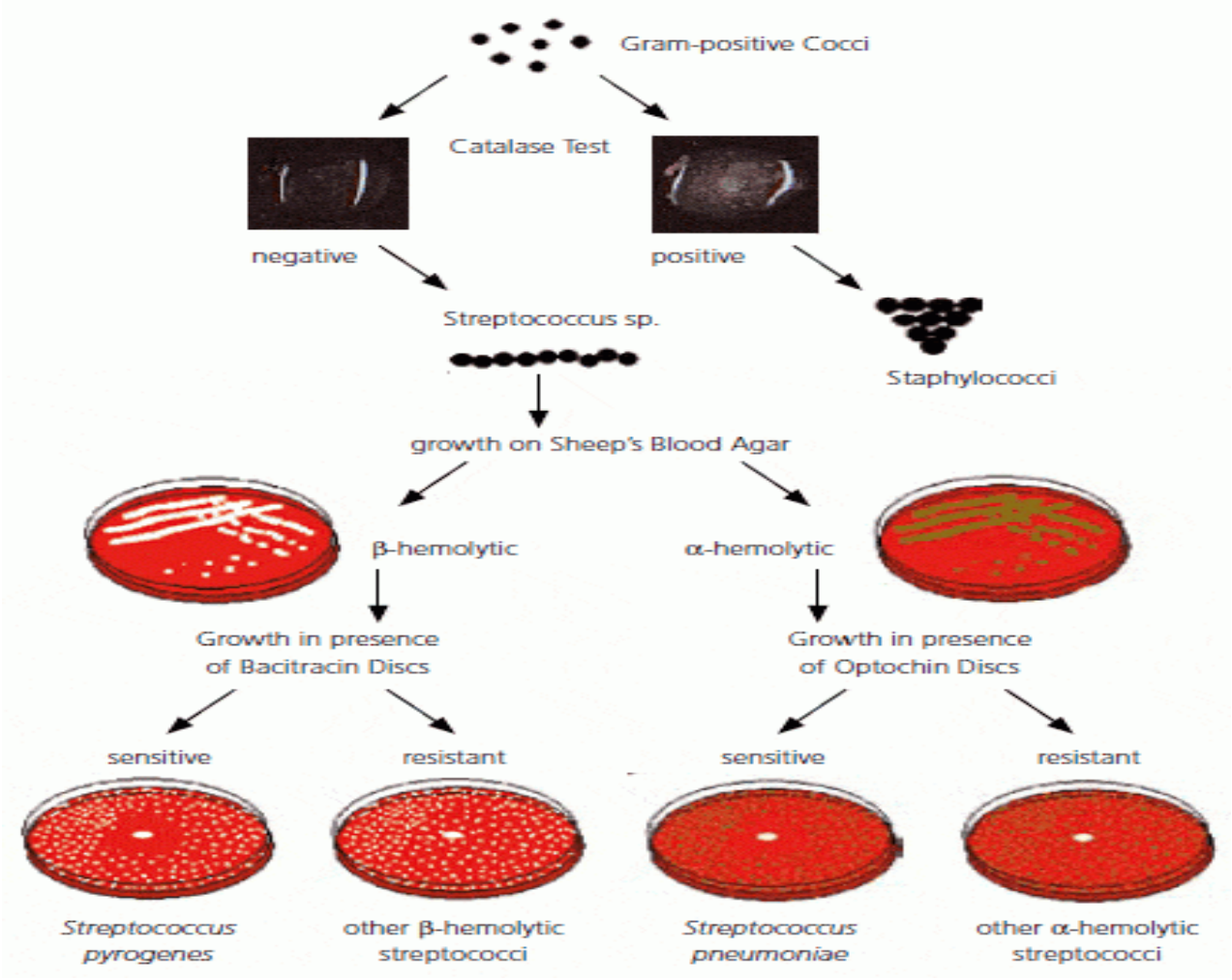
Each disk of drug is placed on blood agar or nutrient agar and incubate with Streptococcus after incubation, an inhibition surrounding the growth indicate positive result.



Fig. Optochin Test using optochin disk
A. S.viridians, Resistance to optochin
B. *S. pneumoniae*, Susceptible to optochin



Bacitracin (A disk) test for identifying *Streptococcus pyogenes*



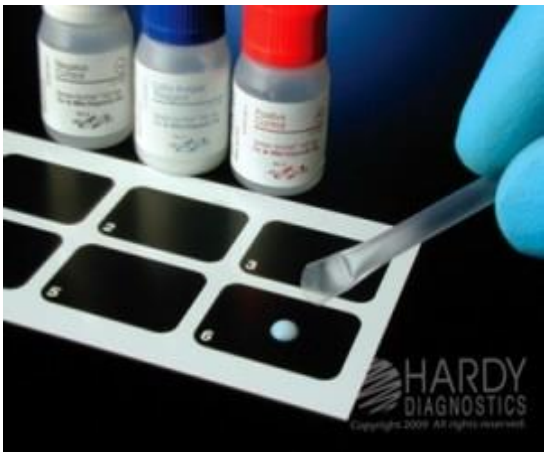
Anti-StreptolysinO Test (ASOT):

►Principle: ASOT this test is serological test used in post streptococcal infection complications depending on the presence of anti streptolysin –O anti body in the blood of patient's previously infected with ***S. pyogenes.***

►Anti bodies .titer: normal <200< positive result.

►Materials used in the ASO Test

1. **ASO antigen:** A stabilized buffered suspension of polystyrene latex particles coated with Streptolysin O and 0.1% sodium azide as a preservative. Shake well prior to use.
2. **ASO positive control**: Human serum-containing more than 200 IU/ml ASO and 0.1% sodium azide as a preservative.
3. **ASO negative control**: Human serum containing 0.1% sodium azide as a preservative.
4. Sufficient disposable pipettes.
5. Glass test slide.



►Sample Collection and Handling:

- Only fresh serum specimens should be used.
- Plasma must not be used since fibrinogen may cause non-specific agglutination of the latex.
- It is preferable to test samples on the same day as collected. Serum samples may be stored at 2-8°C for up to 48 hours prior to testing.
- If longer storage is necessary, sera should be stored frozen at -20°C.

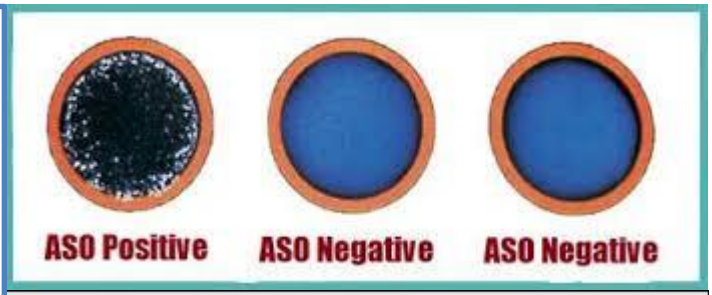
►Procedure for Antistreptolysin O Test:

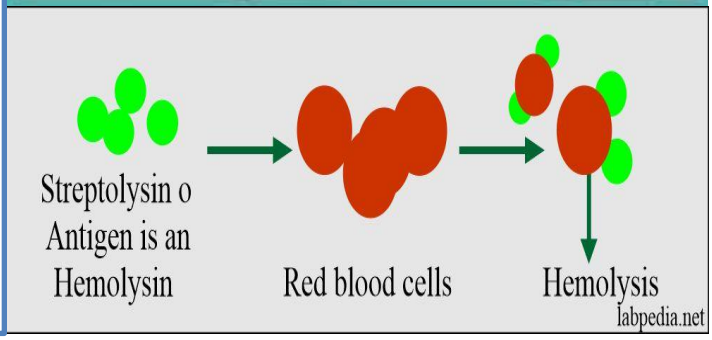
1. **Fresh blood sample collect from vein .**
2. **Centrifuge and separate serum from blood cell .**
3. **Mix ASOT antigen solution full drop with serum (1ml) .**
4. **Mix gently with wooden stick**
5. **Observe the result through 3_5 mints.**

►Test Result:

-A test sample is considered to contain ASO antibodies in excess of 200 IU/ml when agglutination (clumping) is observed when compared to the result of the negative control.







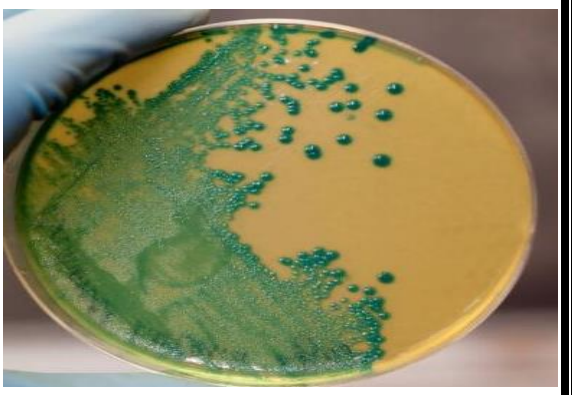
Differentiation between β-hemolytic streptococci:

Streptococcus spp.	Hemolysis	Bacitracin	Bile solubility	6.5% NaCl	Optochin
S. pyogenes	Beta	+	-	-	-
S. agalactiae	Beta	-	-	-	-
E. faecalis	Gamma	-	-	+	-
S. viridans	Alpha	-	-	-	-
S.pneumoniae	alpha	-	+	-	+

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LAB 4 Gram positive rods :

Listeria monocytogenes :



Listeria monocytogenes : is a Gram-positive, catalase-positive, non-spore forming, aerobic or anaerobic intracellular rod bacterium in the genus *Listeria* and family *Listeriaceae*. *L. monocytogenes* can also exist as coccobacilli or short chains (resembling *Streptococcus* and *Corynebacteria* species) and as saprophytes in the environment. Its motility varies with temperature. *L. monocytogenes* is motile at 18-20°C with a tumbling motility in liquid media but at 37°C, the bacterium is weakly motile or non-motile.

Pathogenicity : *L. monocytogenes* I s a commonly encountered bacterial pathogen in food products especially vegetables, meat and milk products which they ubiquitously inhabit. The bacterium is a **Psychrotolerant organism** i.e. it can grow at very low temperature such as the refrigerator temperature. Growth at low temperatures allows *L. monocytogenes* to thrive in refrigerated stored-food products via which likely human infection may occur after consumption. It is noteworthy that low temperatures slow down the growth and multiplication of some pathogenic bacteria and this underlies the reason for preserving food products in the refrigerator.

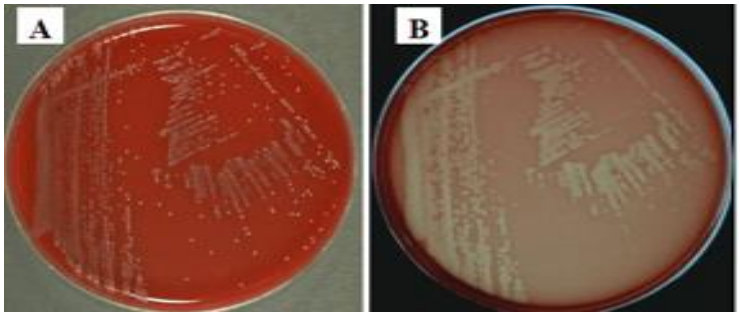
Laboratory diagnosis :

1. Specimens collection :

- Blood, cerebrospinal fluid (CSF) and focal lesions are the primary specimens obtained from infected patients .
- Food samples (meat , dairy products , poultry prducts) .

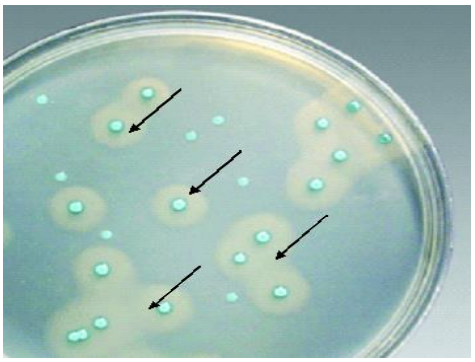
2. Culture media :

- ***Blood agar :*** are preferably used for the cultivation of ***L. monocytogenes*** in the laboratory. *L monocytogenes* produces small, grayish, haemolytic and translucent colonies on BA.



colonies of *L. monocytogenes* on blood agar.
A: is showing Pinpoint to small, semi-transparent colonies
B: is showing same plate examined with transmitted light.
The colonies are surrounded by a narrow haemolytic zone.

- **Selective media :** Culture plates are incubated for **48hr** at temperature range of 3-40°C.Selective media (e.g. *Listeria* oxford agar base) also exist for the isolation of *L. monocytogenes* from clinical and environmental samples.
- **Oxoid Brilliance *Listeria* agar (OBLA) .**

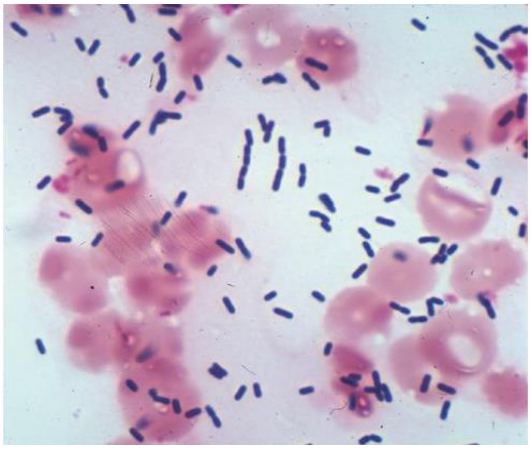


***L. monocytogenes* growing on Oxoid Brilliance *Listeria* agar (OBLA) plate**



***Listeria monocytogenes* on Oxford Agar.**

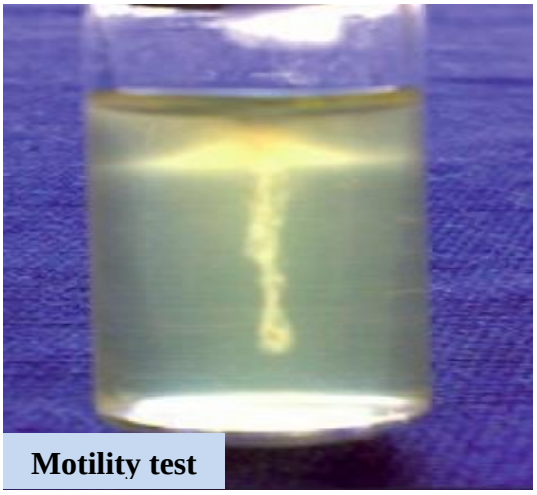
3. Gram staining: gram positive rods take blue purple color, regular short rod with a diameter of approximately 0.5 µm and a length of 0.5–2.0 µm **.**



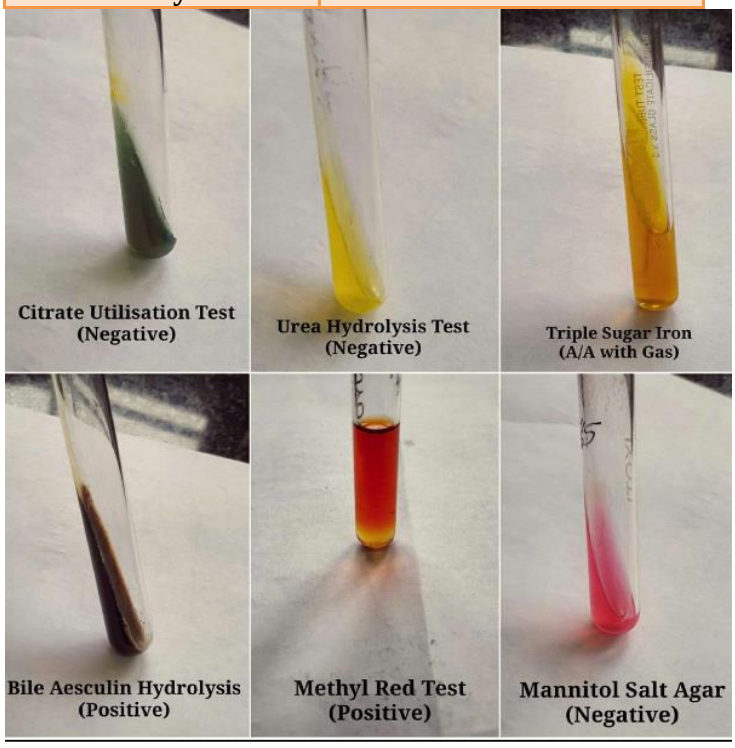
4. Biochemical test:

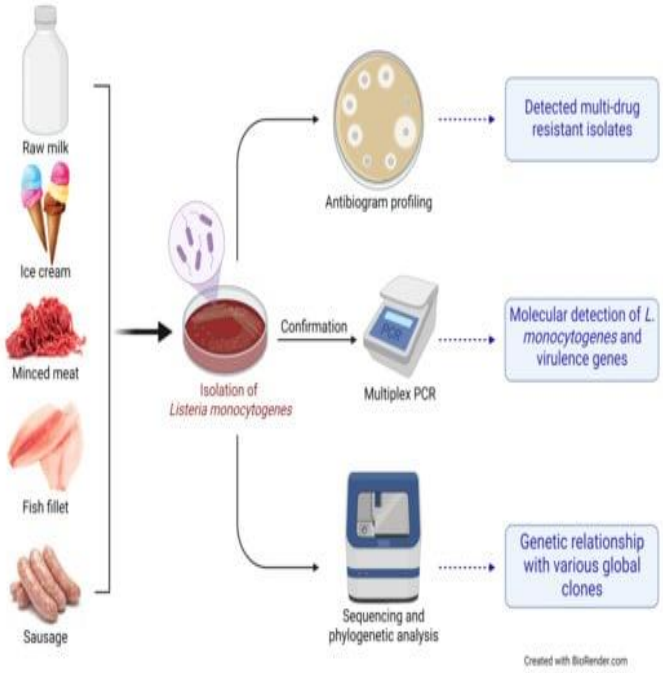
- **Catalase test : positive**
- **Oxidase test : negative**
- **Mannitol fermentation : negative**
- **Citrate utilization test : negative**
- **Urea hydrolysis test : negative**
- **Motility test : positive**
- **Haemolytic test : Beta hemolysis**

Most important biochemical test for *L. monocytogenes* identification	
Test	**Result**
Gram stain	**+ ve**
catalase	**+ ve**
Oxidase	**- ve**
Mannitol fermentation	**- ve**
Hemolysis	**β**
Citrate utilization	**- ve**
Urea hydrolysis	**- ve**
Motility test	**+ ve**



Motility test

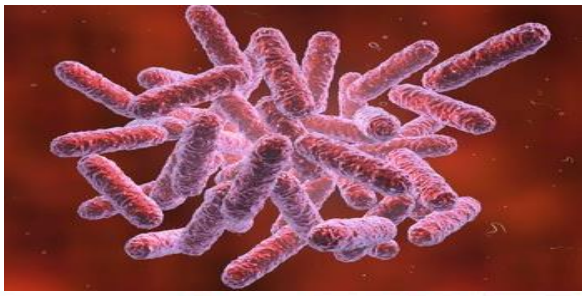




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Third Grade / First Semester
Pathogenic Bacteria / Practical

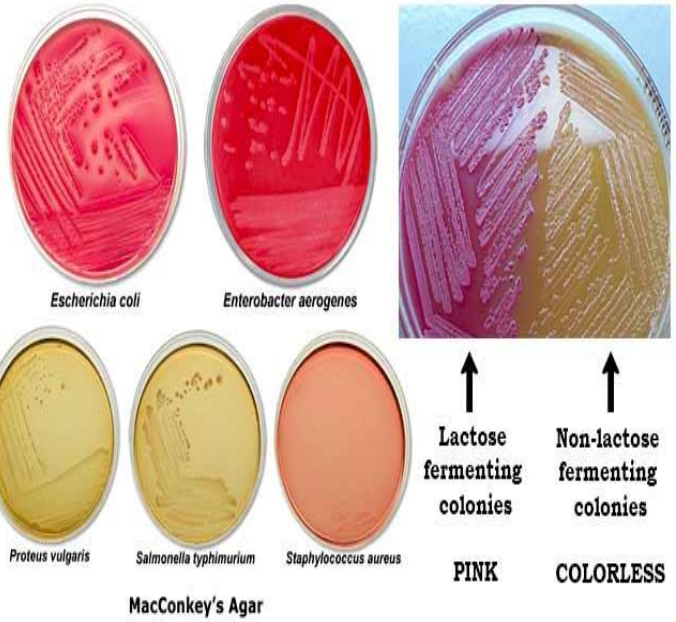
Lab5: *Enterobacteriaceae* family (*Escherichia coli*)

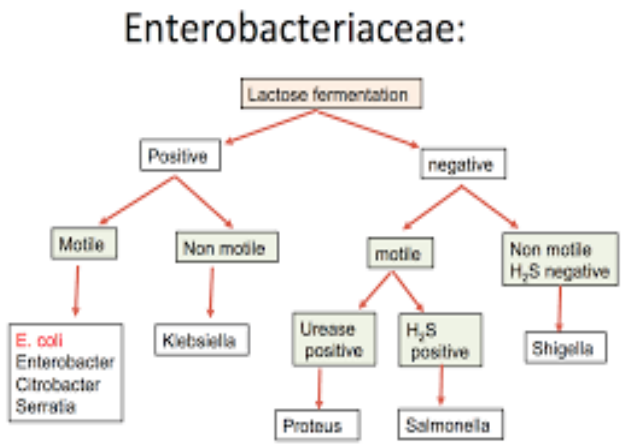


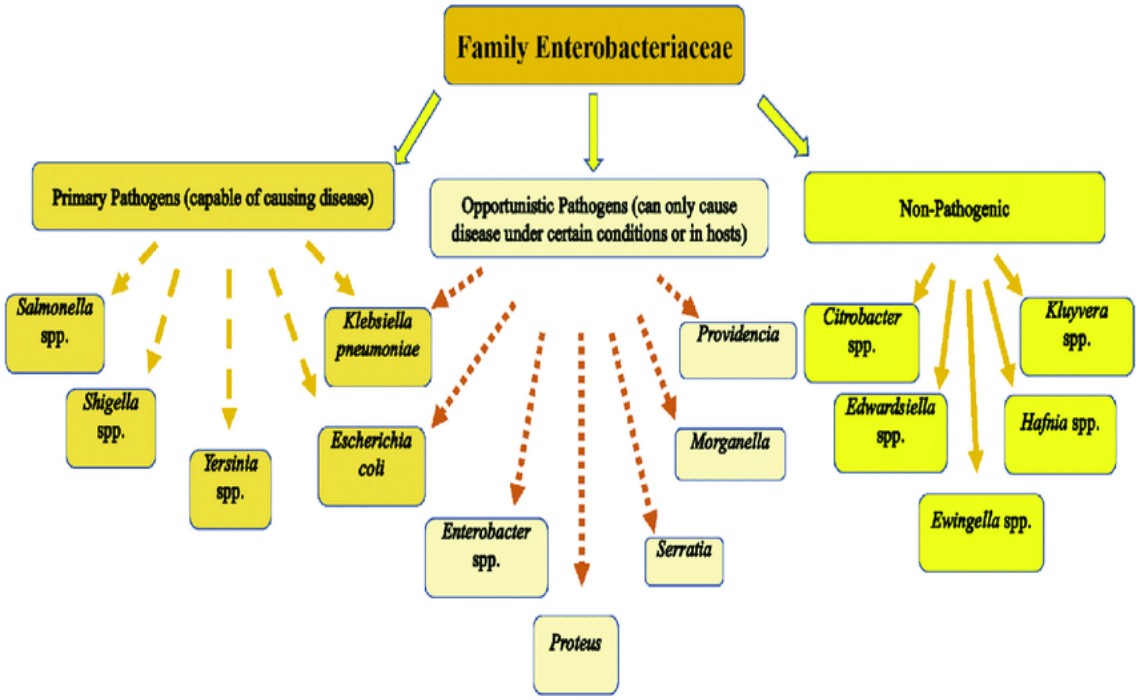
- ***Enterobacteriaceae* family :** a large group of microorganisms that most of them live as a normal microbiota in the intestine (colon) of human and may be other parts of the body, and cause several enteric diseases as well as other diseases that involve the respiratory, genitourinary, the meninges and the skin.

- **General characters:**

1. **Gram negative**
2. **Non spore-forming rods.**
3. **Some are motile, others are capsule producing.**
4. **Facultative anaerobes**
5. **Ferment glucose with or without gas**
6. **Grow on ordinary media**
7. **Oxidase negative .**



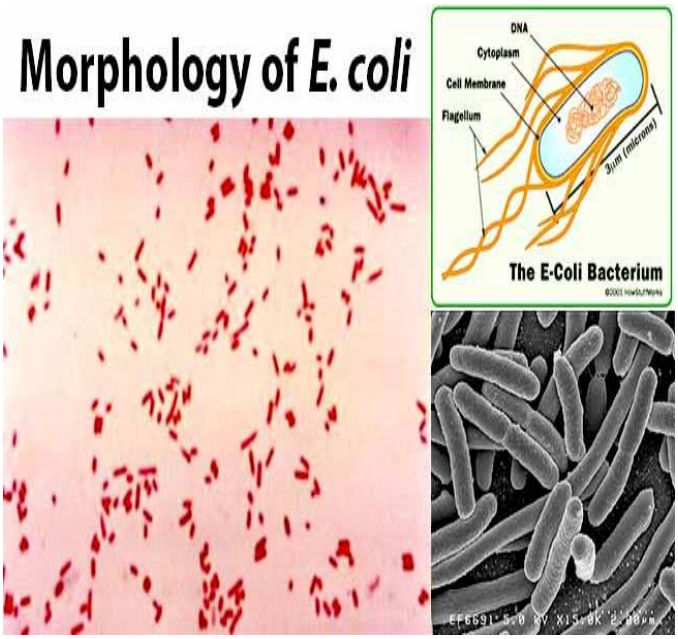




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Lactose fermenter *Enterobacteraceae* (*Escherichia coli*): a gram-negative bacillus :



►***E. coli*** was discovered by Theodor Escherich in 1885 after isolating it from the feces of newborns.

►***E. coli*** is the normal flora of the human body.

►The niche of *E. coli* depends upon the availability of the nutrients within the intestine of host organisms.

►Most commonly found in the colon of the large intestine. Most of them are harmless and opportunistic.

►***E. coli*** helps with the absorption of vitamin K and other vitamins in the colon.

►*E. coli* constitute about 0.1% to 1% of GI tract bacteria, It is a facultative anaerobe.

►*E. coli* is also found in human feces. When excreted from the intestinal tract, the bacteria are able to

►***E. coli*** is found outside the body in fecally contaminated environments such as water or mud or sediments.

►***E. coli*** can also be found in environments at a higher temperature, such as on the edge of hot springs.

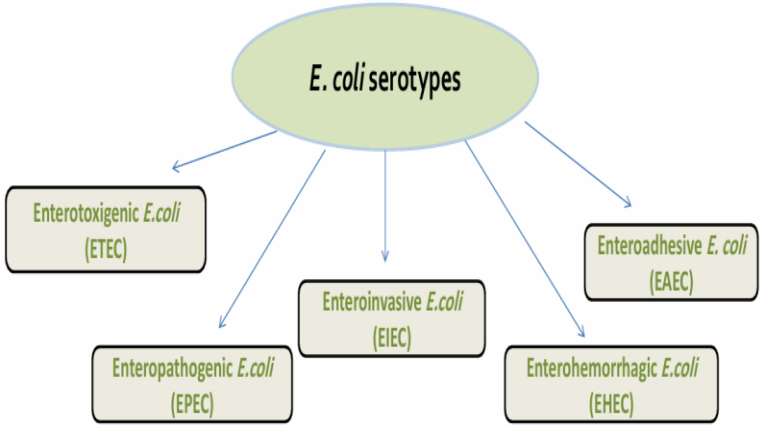
►Humans are most likely to be infected with *E. coli* **O157: H7** found in cattle.

E. coli (Escherichia coli) infections

1) Intestinal infections: (caused by some strains) (e.g. Traveler's diarrhea, dysentery, hemorrhagic infection).

2) Extra intestinal infections:

UTI, Wound infections, Pneumonia, Bacteremia, Septicemia. It is the common cause of G-ve nosocomial infection.



Laboratory diagnosis :

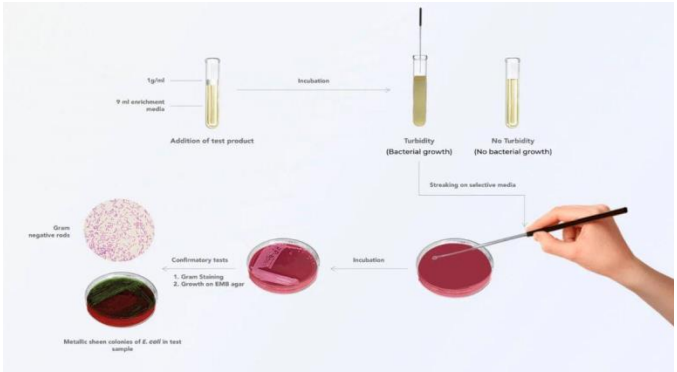
A. Samples collection

1. Clinical specimens include :

- **Urine**
- **Stool**
- **Wound swabs**
- **Blood samples**

2. Non clinical samples includes:

- **Water samples**
- **Food samples**
- **dairy products**
- **fresh and industrial juices.**



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B. cultivation on culture media :

1. selective & deferential media

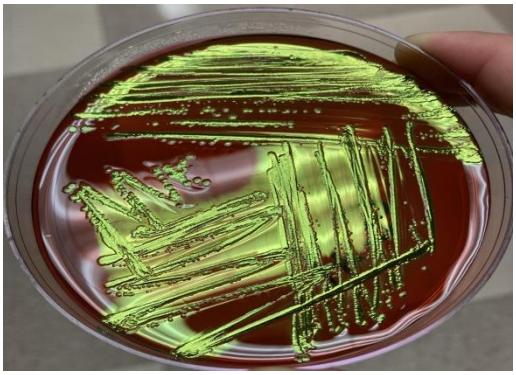
►MacConkey agar :

1. Colonies are circular, moist, smooth, and of entire margin.
2. Colonies appear flat and pink.
3. They are lactose fermenting colonies.



►Eosin Methylene Blue (EMB agar):

E. coli colonies typically appear with a dark-centered, metallic green sheen due to their strong fermentation of lactose and subsequent production of acid. This specific appearance is caused by a combination of factors: the acid lowers the pH, causing the dyes to be absorbed and precipitate, which creates a dark center and metallic sheen effect when light reflects off the surface



►*E. coli* on Blood agar (B.A)

1. Colonies are big, circular, gray, and moist.
2. Non-hemolytic colonies (gamma-hemolysis) (A) or Beta (β) hemolytic (B) colonies are formed.



C- Gram staining :

Gram staining of ***E. coli*** results in **gram-negative**, **pink**, **rod-shaped** bacteria. This is because ***E. coli*** has a thin peptidoglycan layer and an outer membrane, which allows it to lose the crystal violet primary stain during the decolorization step and take up the pink counterstain, safranin.

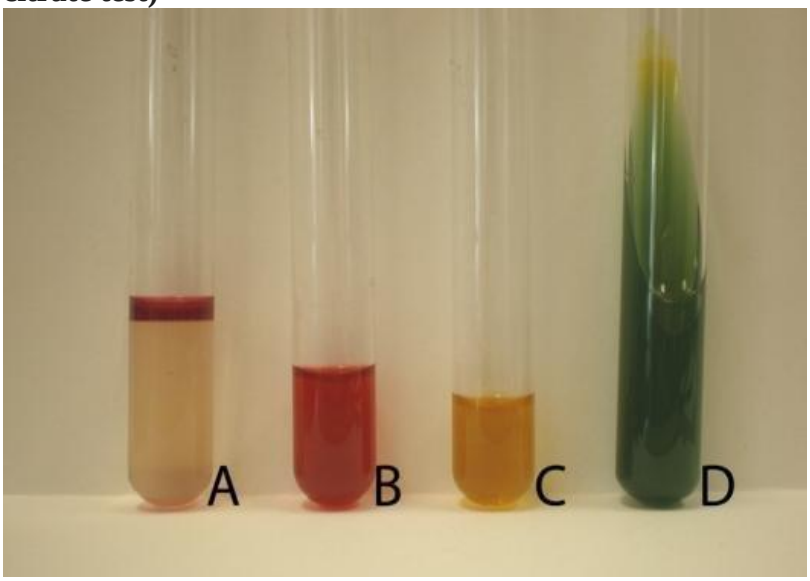


E. coli in Gram stain showing gram negative bacilli

D. Biochemical tests:

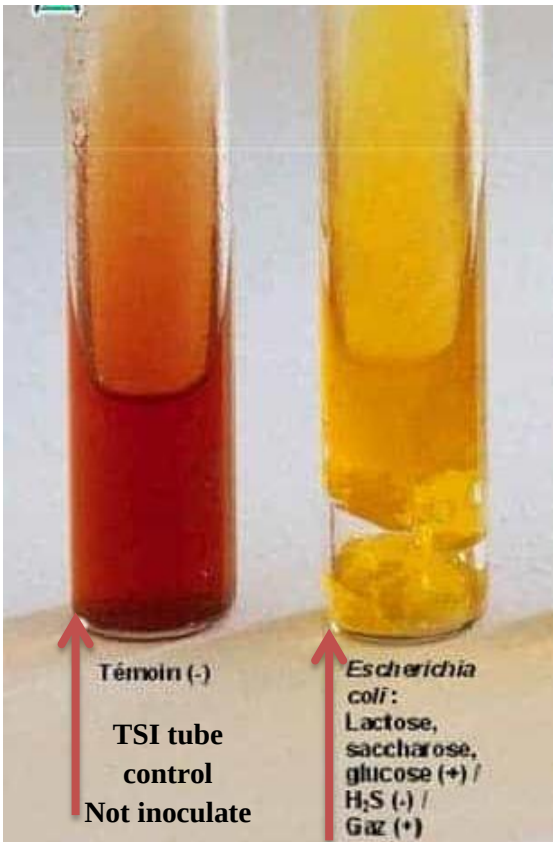
Biochemical test	Result
Catalase	+ ve
Oxidase	- ve
TSI	A/A+ gas production
Indole	+ ve
Methyl red	+ ve
Voges – Porskaur	- ve
Citrate utilization	- ve
Urease	- ve
Nitrate reduction	+ ve

1. **IMViC test : (indol, methyl red , voges- Proskaur ,Simon citrate test)**



Results of an IMViC series done on Escherichia coli after 24-hour incubation at 37°C. Tube A shows a positive indole test in tryptone broth. The positive result is indicated by the red layer at the top of the tube after the addition of Kovács reagent. Tube B shows a positive methyl red test as indicated by the red color after the addition of methyl red reagent

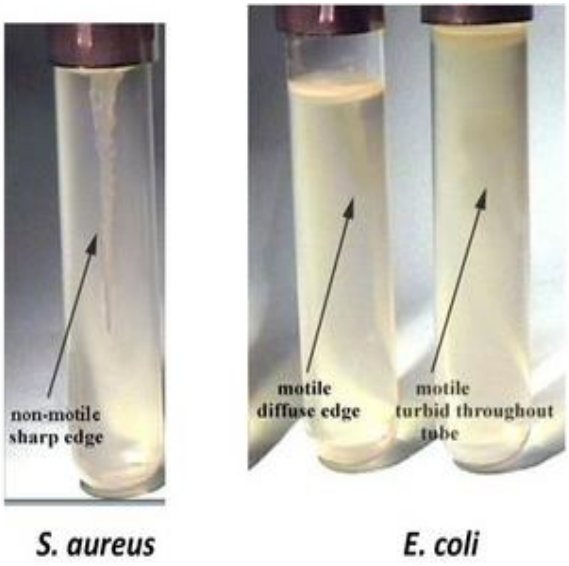
Tube C shows a negative Voges-Proskauer test as indicated by the lack of color change after the addition of Barritt's A and Barritt's B reagents. Tube D shows a negative citrate result as indicated by the lack of growth and color change in the tube.

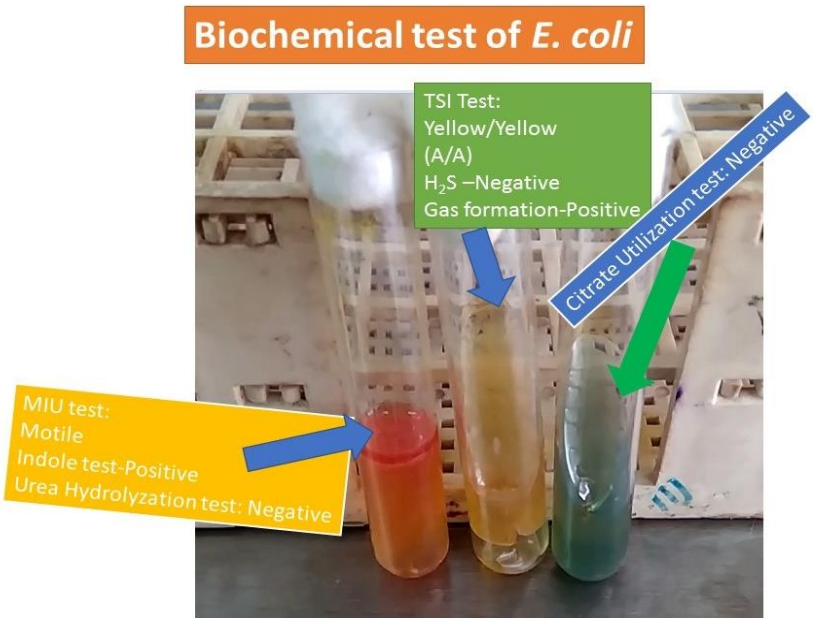


2. **TSI :**

- **Acid slant/ acid but**
- **No H2S production**
- **Gas formation shown by most strains**

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- **Motility test in SIM media**



Enterobacteriaceae

- *Escherichia*
- *Shigella*
- *Salmonella*
- *Edwardsiella*
- *Citrobacter*
- *Yersinia*
- *Klebsiella*
- *Enterobacter*
- *Serratia*
- *Proteus*
- *Morganella*
- *Providencia*

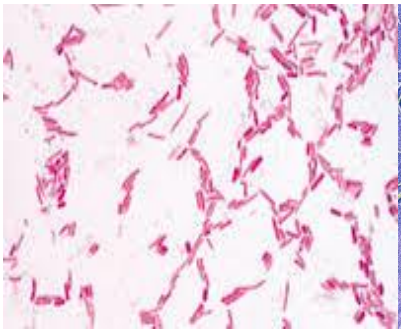


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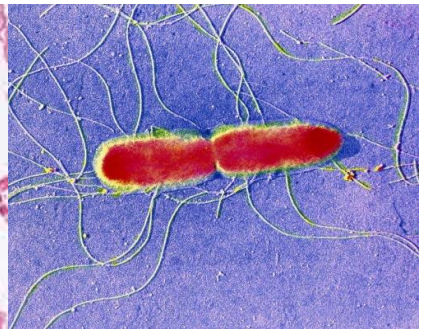
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Lab6: *Salmonella* and *Shegilla:*

- ***Salmonella spp.*** The genus ***Salmonella*** belongs to family ***Enterobacteraceae*** Gram negative bacilli , they possess interesting phenotypic/genotypic characteristics, history and peculiar nomenclature compared to other bacteria within and outside the family**.**



Salmonella **under light microscope**

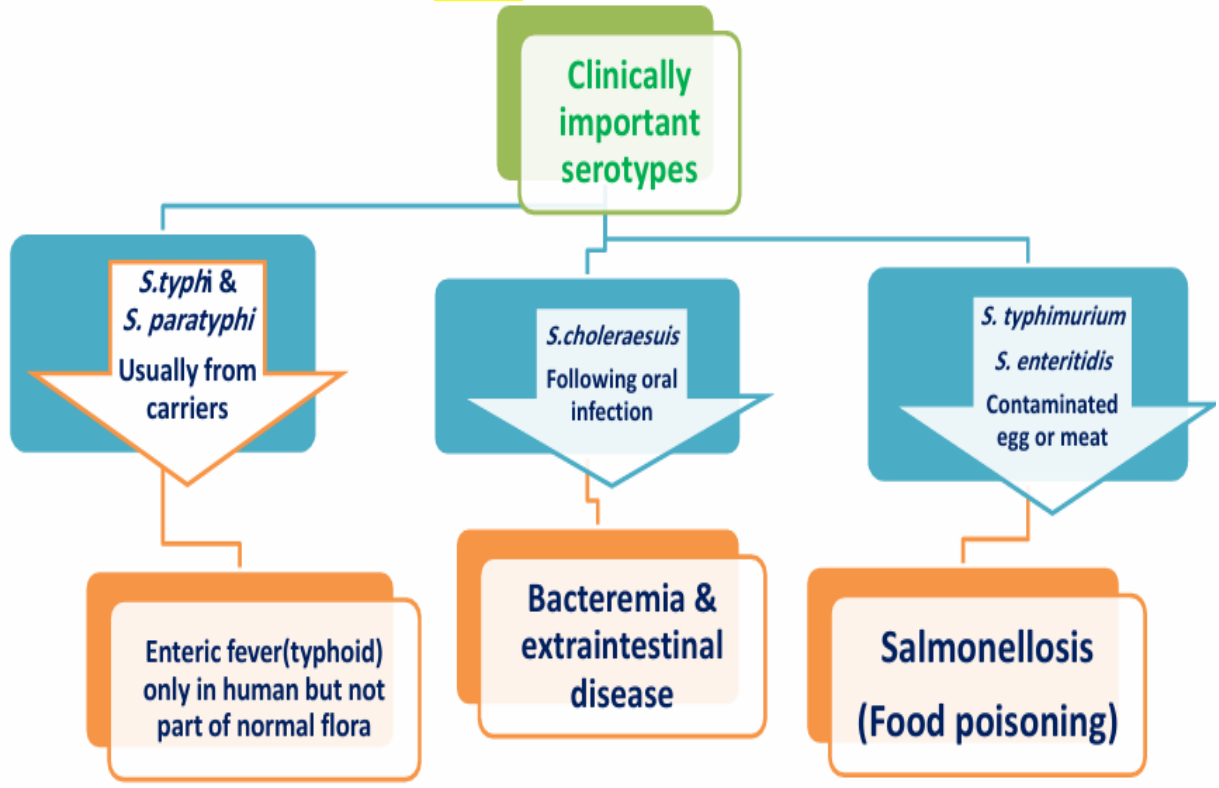


Salmonella **under electronic microscope**

- **General characteristics:**

1-Grams negative, bacilli or coccobacilli.
2- Flagellated **Motile**
3- Facultative anaerobic.
4-Grow on **MacConkey's agar**, **non lactose fermenter**.
5-Ferment glucose, produce acid and gas
6-Catalase **+ve** & oxidase **–ve**.
7-Most strains produces hydrogen sulfide **(H2S).**

Parameters	Survival (extreme values)	Growth	
		optimum	Extreme
Temperature (°C)	-23 (butter)	35-37	5-50
pH	/	7-75	3.8-9.5



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pathogenicity *of Salmonella spp.:*

- Enteric fever, one of the foremost bacterial infections worldwide, is a common infection in regions with poor economic development and with a limited public health infrastructure.
- **Typhoid fever**
- The World Health Organization **(WHO)** describes *Salmonella* as one of the four most important causes of diarrhea worldwide.

Salmonella Sources, Reservoirs, and Transmission

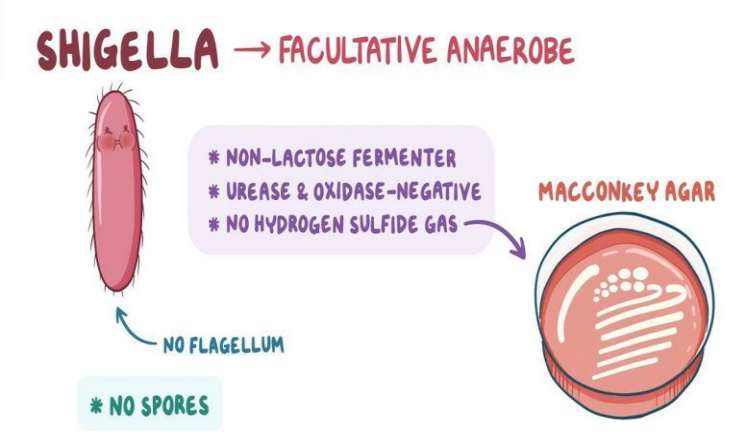
The sources of *Salmonella* infection include: Poultry and poultry products, which are considered the primary source of *Salmonella* infection in humans.

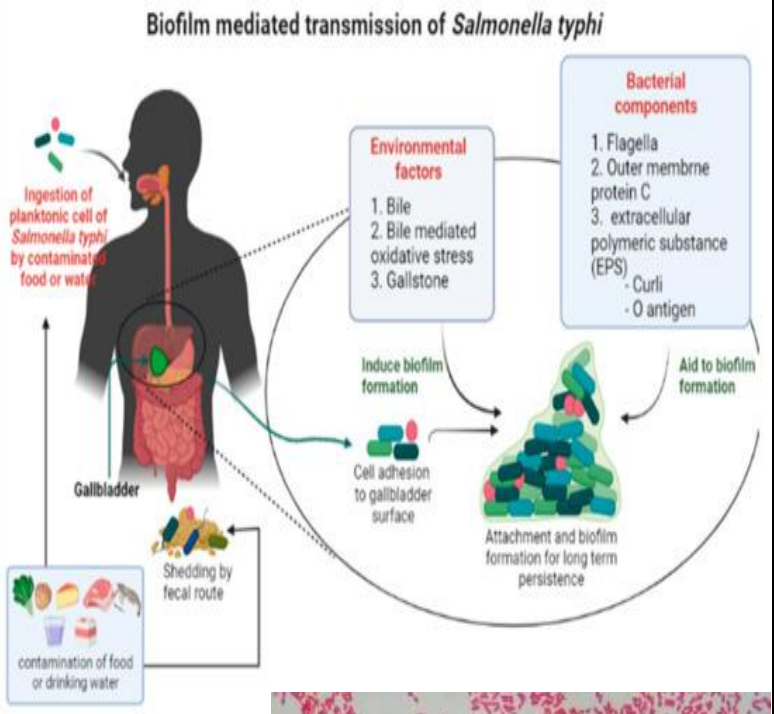
- Meat contamination occurs generally as a result of improper handling of the infected organs, such as the gut and liver, during carcass processing

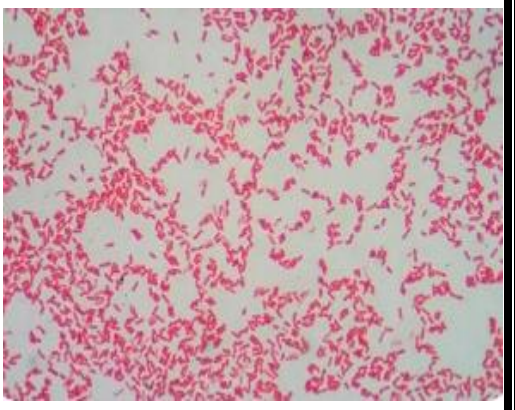
Genus shigella : Gram-negative, facultative anaerobes belong to *Enterobacteraceae* family .

General characteristics:

1. Slender gram-negative, Non motile rods
2. Facultative anaerobes
3. All shigellae ferment glucose with the (exception of ***Shigella sonnei***) without gas production.
4. Grow on MacConkey's agar, non lactose fermenter
5. The natural habitat of shigellae is limited to the intestinal tracts of humans and other primates, where they produce bacillary dysentery.







***Shigella* under light microscope**



***Shigella* under electronic microscope**

Laboratory diagnosis

1. Specimens

A -Salmonella specimens: according to disease.

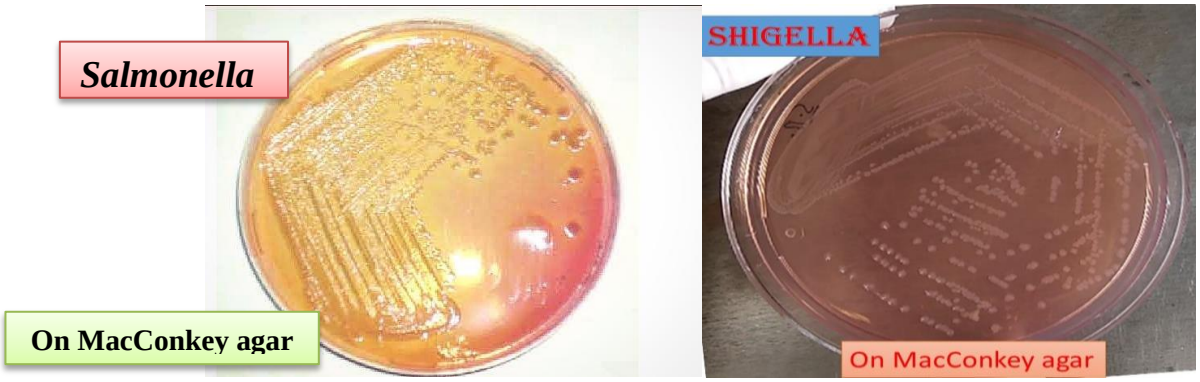
- Gastroenteritis → Stool
- Bacteremia → Blood (during high fever)
- Enteric fever →
  - Blood (+ve in the 1 st week of disease)
  - Stool (+ve from 2nd week on; -ve earlier in disease)
  - Serum (+ve 2nd & 3rd week)

B. Shigella specimens → **Fresh stool**, **mucus** or **rectal swabs** for culture

2. Culture media / (Differential & selective) Media:

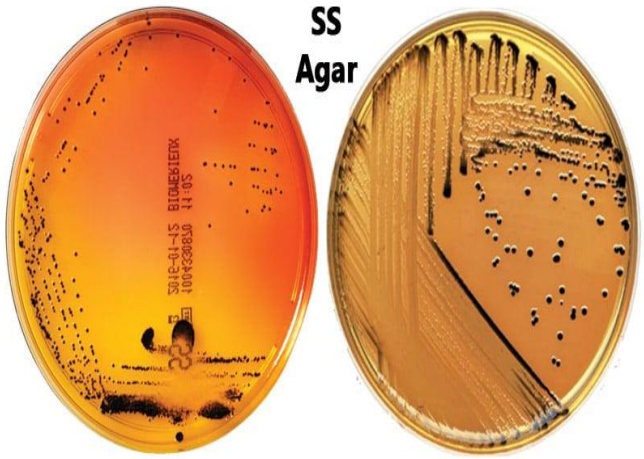
A. MacConkey's agar: non-lactose fermentor . at 37C for 24-48h

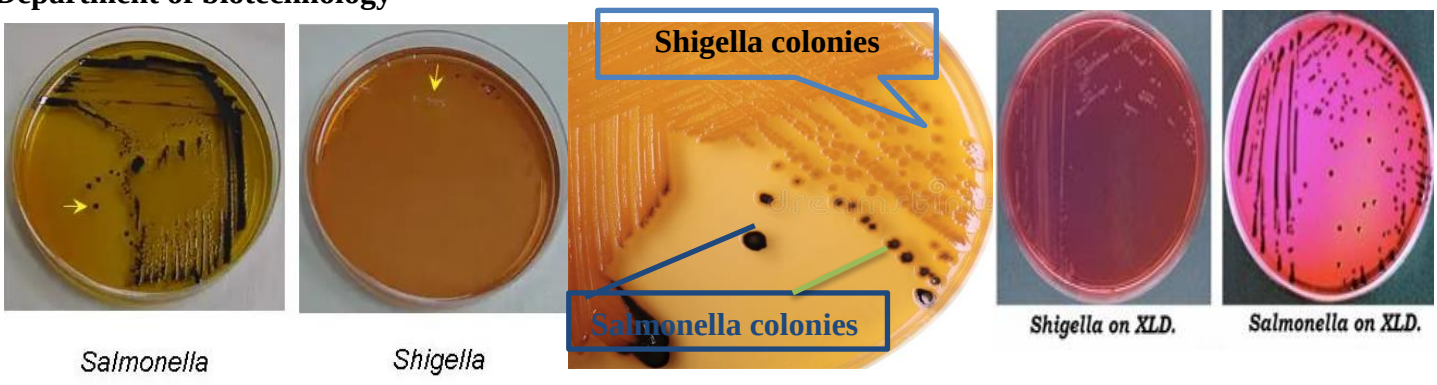
This medium both selective & differential medium



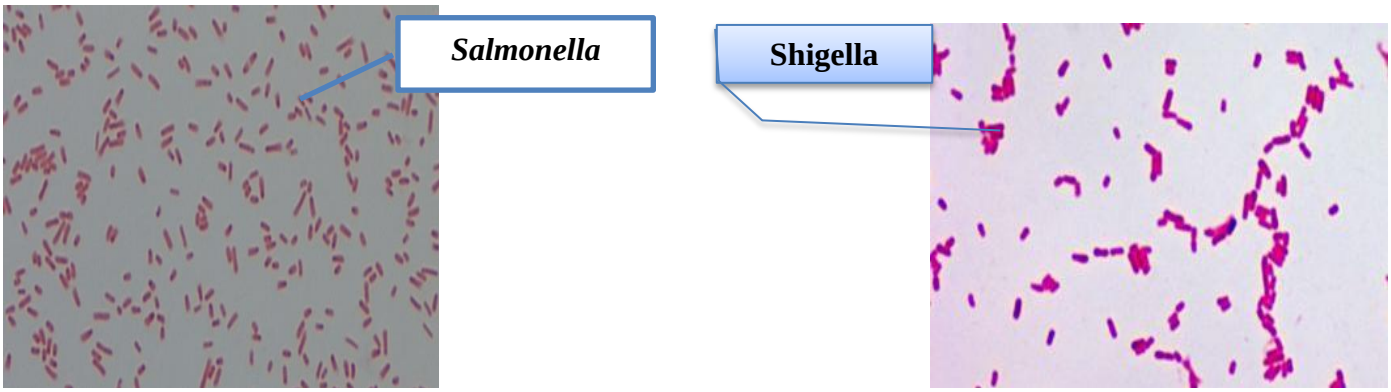
B. S.S. agar (salmonella ,shigella agar): This medium both selective & differential medium

- **Selective** because of bile salt & brilliant green inhibit G+ve and G-ve other than ***Enterobacteriaceae***
- **Differential** : because it contain lactose, Salmonella & Shigella non lactose fermenter, colorless colonies
- Salmonella produce **H2S (black colonies)**
- Other G-ve appear red colonies.





3- Gram staining : both salmonella & shigella are gram negative rods .



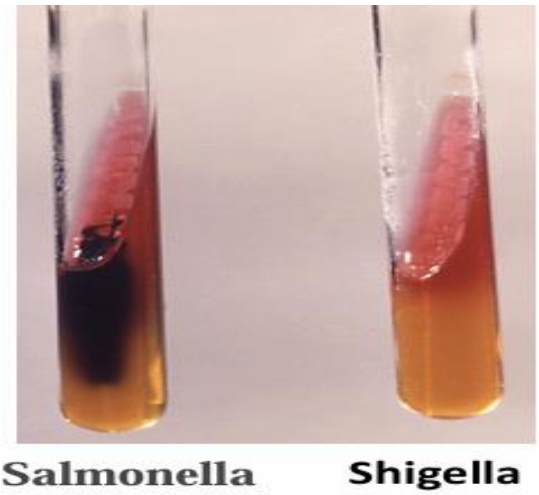
4. Biochemical tests :

A. TSI agar (triple sugar iron): results are read as follow: **Slant/ butt - or + gas , - or + H2S**

► **Salmonella alk /acid + gas**, +H2S The black color in the tube indicates the production of H2S.

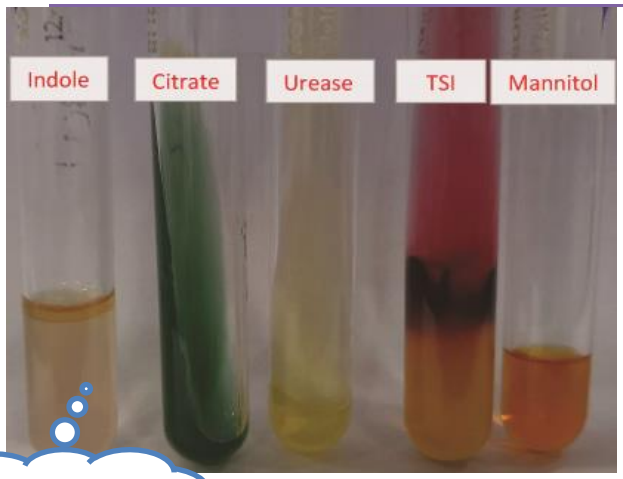
►**Shigella alk / acid –gas –H2S**

►**Interpretation of TSI results**: Bacteria ferment only glucose with acid produced the butt will be yellow (acidic), the slant (surface) (alkaline) red (the bacteria are not capable of utilizing the other two sugars will switch to peptones present in the medium and splitting it to amino acids and then to ammonia as end product). If H2S produced by bacteria it will bind iron with black color of the butt which can mask the yellow butt.

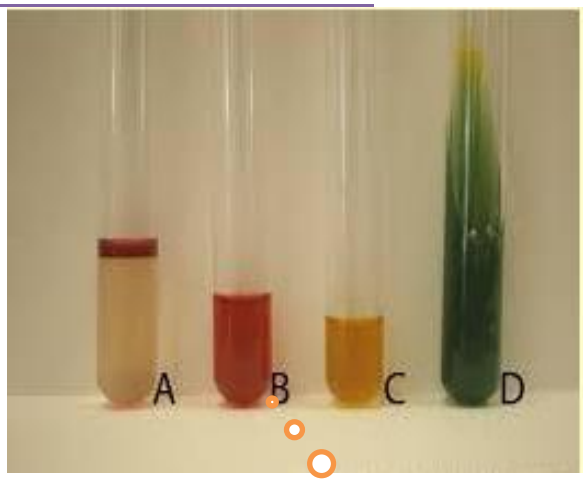


B. IMViC test:

Bacteria	Indole	Methyl red	Vogues proskour	Citrate utilization
Salmonella	-ve	+ve	-ve	+ve
Shigella	±	+ve	- ve	- ve



Salmonella IMViC TEST



Shigella IMViC TEST

5. Serologic methods:

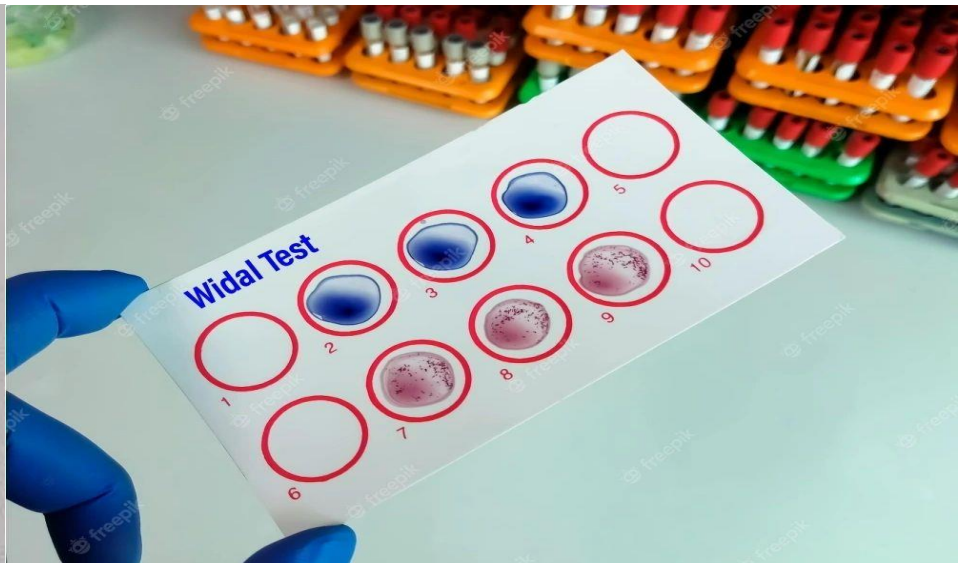
►Widal Test for salmonella enteric fever

- The Widal Test is a serological test used to diagnose typhoid fever, a bacterial infection caused by Salmonella typhi.
- The test detects the presence of antibodies in the blood that are produced in response to the bacteria that cause typhoid fever.
- The Widal Test can be used to confirm a suspected diagnosis of typhoid fever based on a patient's symptoms and other laboratory tests.
- This test is based on the agglutination, where the antibodies in the blood clump together with the bacterial antigens in a visible reaction that can be detected in a laboratory.
- Serum agglutinins rise sharply during the second and third weeks of salmonella infection. At least two serum specimens, obtained at intervals of 7–10 days, are needed to prove a rise in antibody titer. Serial (twofold) dilutions of unknown serum are tested against antigens from representative salmonellae. The results are interpreted as follows:

- **If (H) Ab titer ≥ 1:160 → Past immunization or past infection.**
- **If (O) Ab titer ≥ 1:160 → Active Infection.**

- If (Vi) Ab titer ≥ 1:80 ⟶ Carrier.

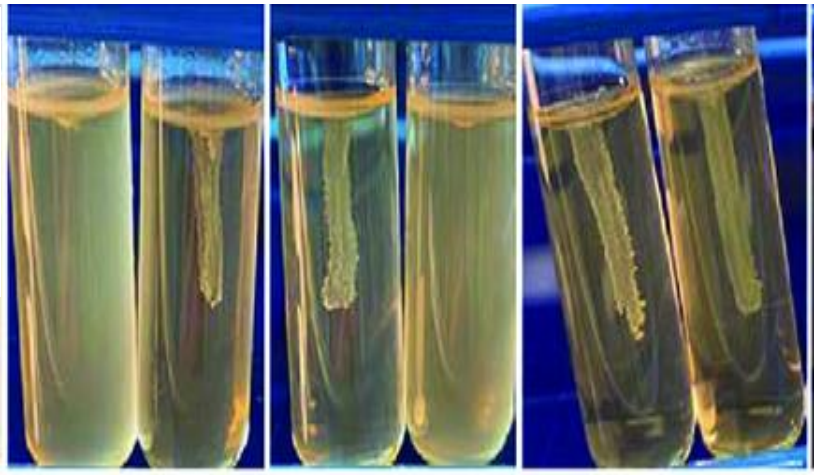




6-Motility test:

By semisolid agar

- ***Salmonella*** **(motile)**
- ***Shigella*** **(non motile)**



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Lab 7 Gram positive Bacilli *(genus clostridia) :*

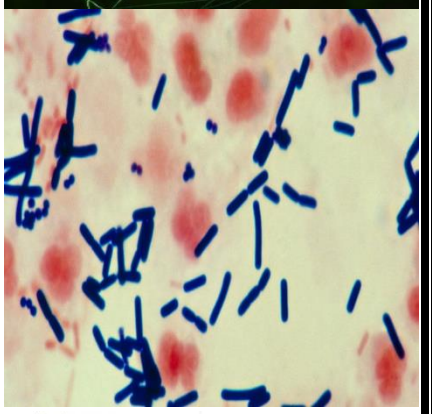
- ***Clostridium*** is G+ve, obligate anaerobic spore-forming bacilli. Clostridium is an anaerobic pathogenic bacterium that is primarily found in soil and animal intestinal tracts. Four main species responsible for disease in humans:



- ***Clostridium perfrigens;***
- Gram +ve, non-motile, obligate anaerobe, spore-forming, capsulated in tissues or young cultures, rounded end bacilli.
- Spores are oval, central or subterminal, not bulging.
- The strains that produce more spores produce less toxins, and vice versa.
- Grow best on media containing carbohydrates such as; glucose agar, or glucose blood agar.
- Spores of type A (of types A-F) resist boiling for few minutes, while strains of food poisoning resist boiling for 1-3 hours. Other strains can resist boiling for several hours.
- Produce narrow zone of complete hemolysis (theta toxin), while α-toxin produce a wide zone of hemolysis.
- Direct smear from deeper parts of the wound may show 3 types of Clostridia;

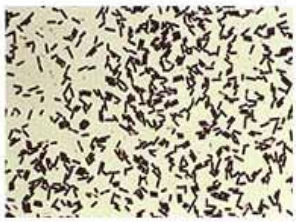


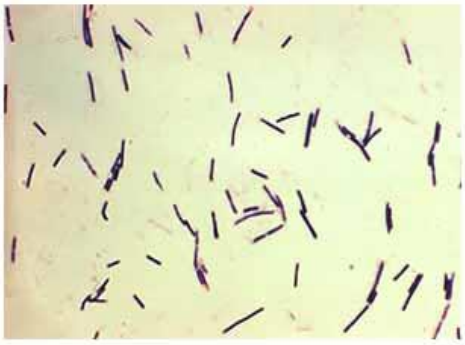
1- C. perfringens; thick Gram +ve bacilli
2- C. septicum; citron bodies, boat-shape, pleomorphic, with irregular staining.
3- C. novyi; Large bacilli, with oval sub terminal spores.

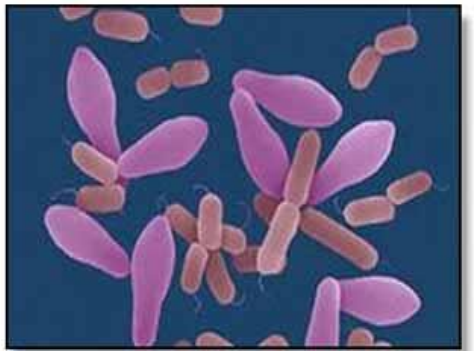


Morphology of *Clostridium perfringens*





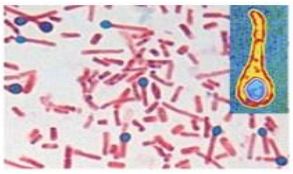




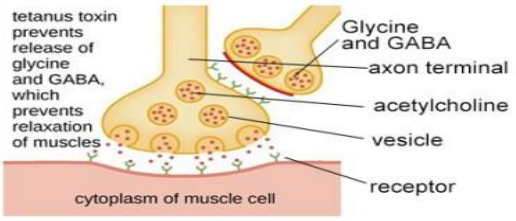


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'Drum Stick' or
'Tennis Racket' appearance



Mechanism of Tetanus Toxin

Clostridium tetani



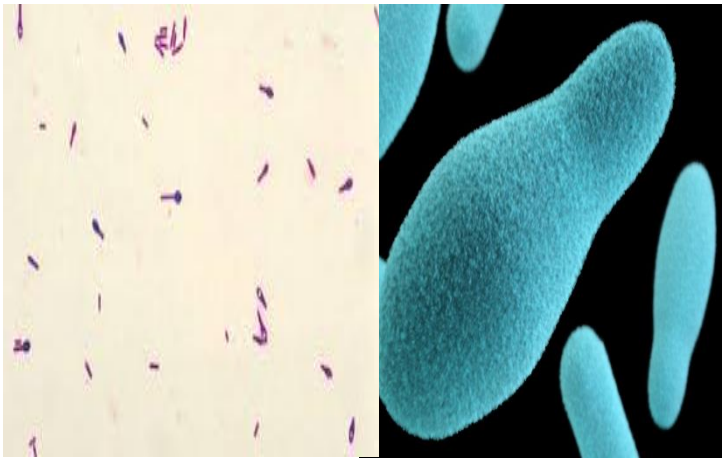
Opisthonus

microbeonline



Risus Sardonicus

- ***Clostridium botulinum;***
  - Rod-shaped, single or in pairs or in chains Spores are oval, subterminal, bulging Spores formed best in sugar-free medium.
  - Motile with 4-8 peritrichous flagella Form large irregular, glistening colonies with fimbriated border.
  - Meat is digested in cooked meat medium with putrid odour.
  - Spores are destroyed at 180oC, and in dry heat for

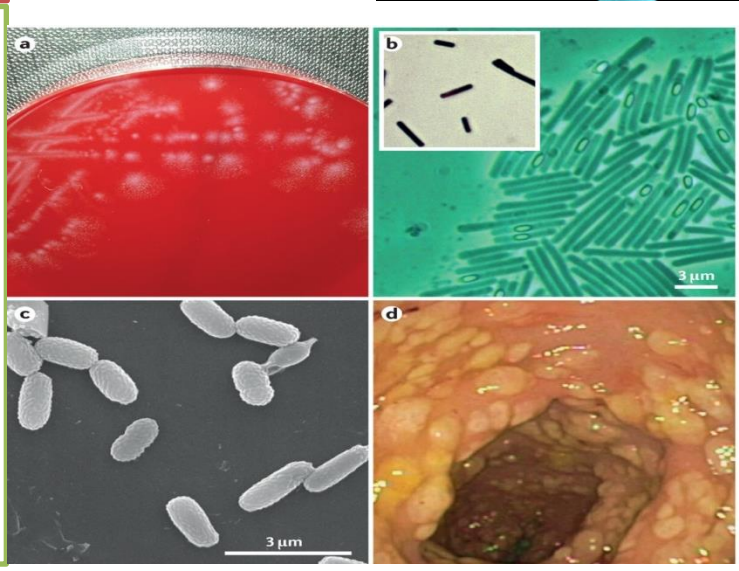


- ***C. difficile*** which overgrows during antibiotic therapy, also may lead to pseudomembrane colitis. Clostridium spp. could be divided into;

1. Saccharolytic organisms; C. perfringens, ferments carbohydrates, produces acid and gas

2. Proteolytic organisms; C. tetani, digest proteins with blackening and bad smell production.

3. Mixed saccharolytic & proteolytic; certain groups of ***C. botulinum***.



Pathogenicity :

► ***C. perfringens:*** secretes a variety of exotoxins, enterotoxins, and hydrolytic enzymes that facilitate the disease process.

A. **Exotoxins:** ***C. perfringens*** secretes at least 12 exotoxins. The most important of these required for virulence in tissue, is alpha toxin. Alpha toxin is a lecithinase that degrades lecithin in mammalian cell membranes, causing lysis of endothelial cells, as well as RBC, WBC and platelets.

B. **Enterotoxin:** a small protein, acts in the lower portion of the small intestine, and causes loss of fluid and intracellular proteins.

C. **Degradative enzymes:** it is produces of hydrolytic enzymes, including proteases, DNases, hyaluronidase, and collagenases, which liquefy tissue and promote the spread of infection.

- **Myonecrosis (gas gangrene):** Clostridial spores are introduced into tissue, for example, by contamination with infected soil, or by endogenous transfer from the intestinal tract. Severe and open wounds are a prime predisposing condition. Alpha toxin and other exotoxins are secreted and extensive cell killing follow (ensues). Production of enzymes that break down ground substance facilitates the spread of infection.



Figures (A&B) show gas gangrene caused by *C. perfringens*

Clostridium botulinum	*Clostridium tetani*
causes flaccid paralysis.	**causes spastic paralysis**
affected the neurons of PNS	**Tetanus exotoxin, called tetanospasmin (A&B)**
The botulinum toxin constitute a set of proteins whose neurotoxicity arises from proteolytic cleavage of specific synaptic vesicle peptides, causing subsequent failure of neurotransmission. botulinum toxins affect peripheral cholinergic synapses by blocking the neuromuscular junction and inhibiting release of the neurotransmitter, acetylcholine, preventing contraction and causing flaccid paralysis.	**The A fragment blocks neurotransmitter release at inhibitory synapses, and thus causes severe, prolonged muscle spasms. The A fragment has been shown to be a protease; it cleaves a small synaptic vesicle protein (synaptobrevin) and prevent the flow of inhibitory neurotransmitters. tetanus toxin, which causes contraction (spasms)**

Table 1 main differences between Clostridium tetani & Clostridium botulinum Pathogenicity

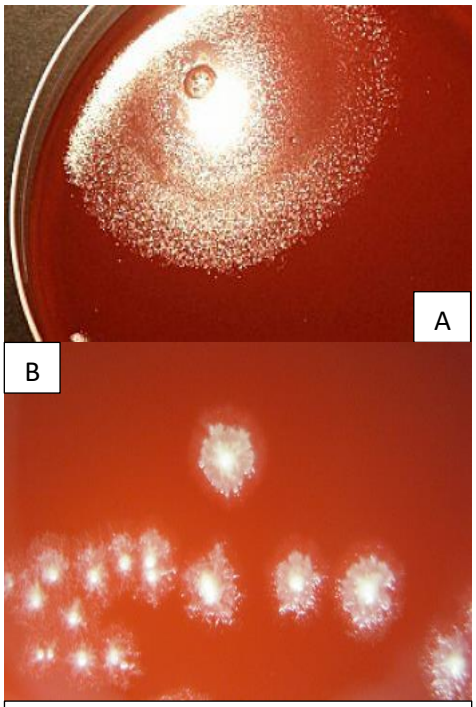
- **Laboratory diagnosis :**

1. **Specimen; Wound, food, stool.**
2. **Culture media : (cultre media should incubate in anaerobic conditions**

►**Blood agar,** most clostridia produce flat, gray, spreading colonies somewhat resembling those of Bacillus sp. They are also usually hemolytic. Clostridium perfringens produces raised, entire colonies with double zone hemolysis, the complete zone round the colony is due to theta toxin, and the wider zone around it is due to alpha toxin.

►**Robertson's medium (cooked meat medium);** Cooked Meat Medium provides a favorable environment for the growth of anaerobes, since the muscle protein in the heart tissue granules is a source of amino acids and other nutrients. The muscle tissue also provides reducing substances, particularly glutathione, which permits the growth of strict anaerobes. The sulfhydryl groups, which exert the reducing effect, are more available in denatured protein; therefore, the meat particles are cooked for use in the medium. Cooked Meat Medium is still widely used for the cultivation and maintenance of clostridia and for determining proteolytic activity of anaerobes. On Robertson's cooked meat medium → blackening of meat will be observed with the production of H2S and NH3 (in the case of ***C. tetani***).

► **Brewer Thioglycollate Medium ;** contains highly nutritious proteose peptone and beef infusion which support the growth of fastidious bacteria. Sodium thioglycollate helps to create anaerobic conditions. Methylene blue indicates oxygen content of the medium by exhibiting a bluish-green color of the medium in the presence of oxygen. Brewer's anaerobic jar is used for growing bacteria under anaerobic condition.

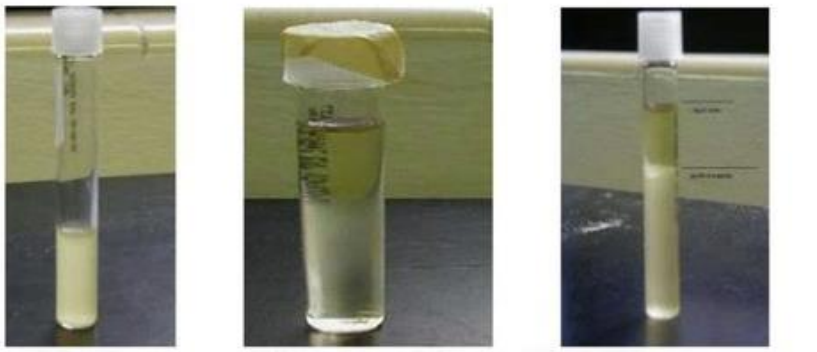


A&B colonies of C. tetani on blood agar



Cooked Meat Medium Tubes (Growth & gas produced are evident). Gas produced lifted the cooked meat particles to the surface

Growth Pattern in Thioglycollate Medium

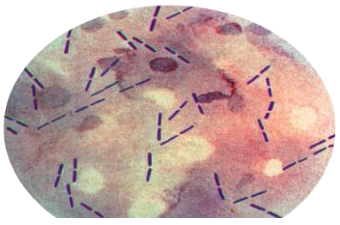


E. coli
Facultative Anaerobic

Micrococcus luteus
Microaerophile

Clostridium sporogenes
Strict anaerobic

3. Gram staining : G+ve rods with spore site according to *spp*



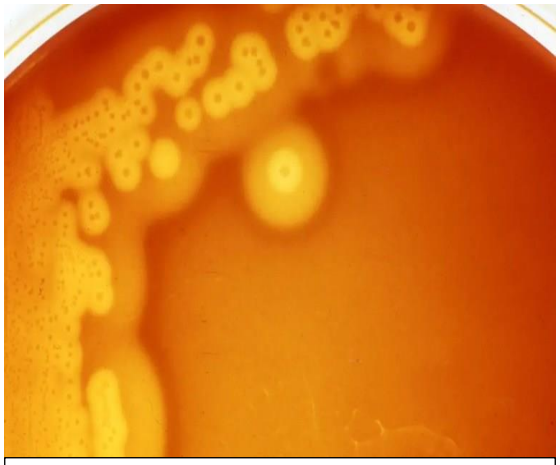
4- Biochemical tests; stormy fermentation In anaerobically-grown Litmus Milk cultures, enzymes of ***C. perfringens*** will attack the proteins and carbohydrates of the milk producing a "stormy fermentation" with acid production (the indicator, litmus turns pink), clotting of milk proteins, and gas formation.

5- Serology test; Nagler's reaction for the detection of lecithinase activity; A test for the identification of alpha toxin of Clostridium perfringens; the addition of antitoxin to cultures on egg yolk agar prevents visible opacity, due to lecithinase action which is normally observed around colonies. An egg-yolk plate is divided into two halves. Over one half is spread with a specific antitoxin. The culture to be tested, together with a positive control is streaked across the plate, going from the untreated area of the plate to the area that is covered with antitoxin. The culture is then incubated anaerobically. Following incubation, lecithinase activity, caused by the action of the ***Clostridium perfringens*** a-toxin is seen as a precipitate in the medium on the side of the plate that did not receive the antitoxin, but not on the side that was treated.



Lecithinase activity of *C. perfringens* in Egg Yolk Agar



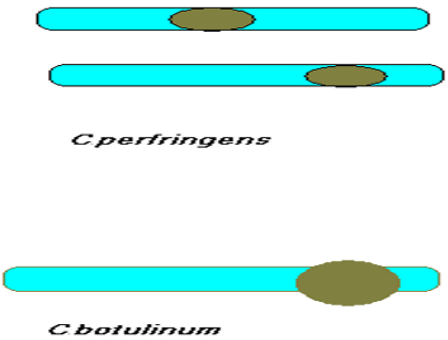


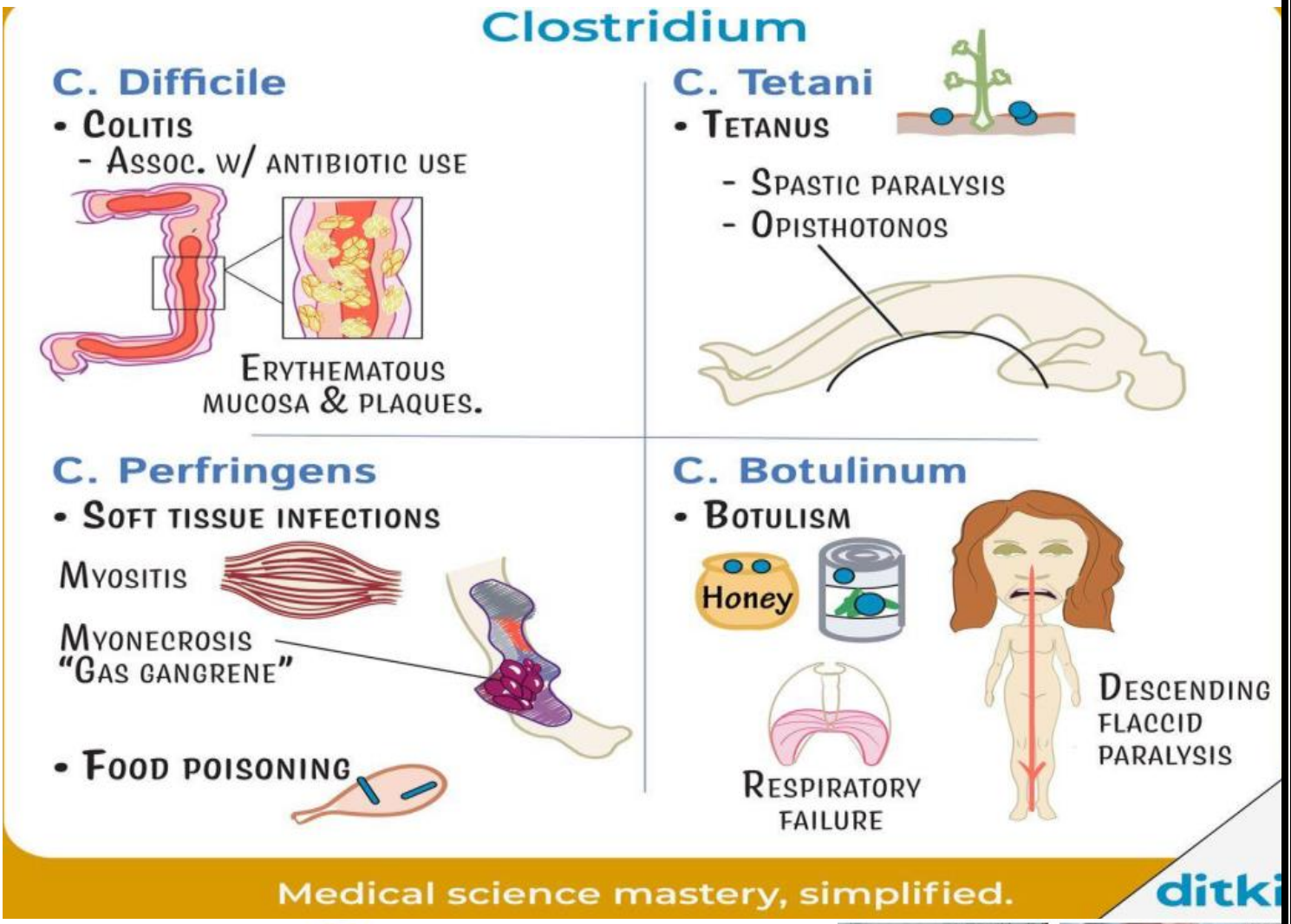
Target hemolysis (Double zone hemolysis)

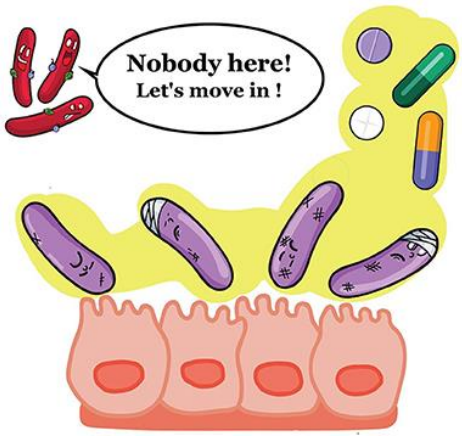


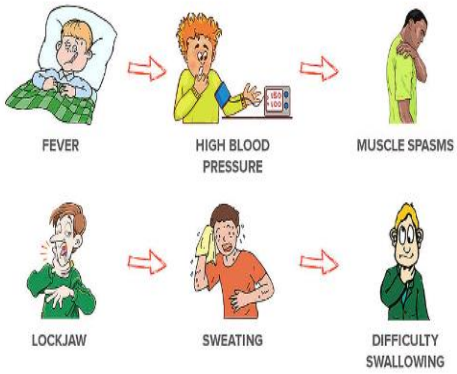
Litmus milk test with 'stormy clot reaction'















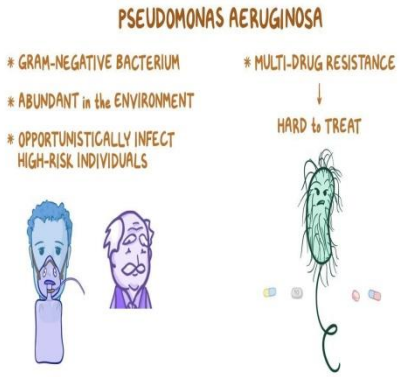


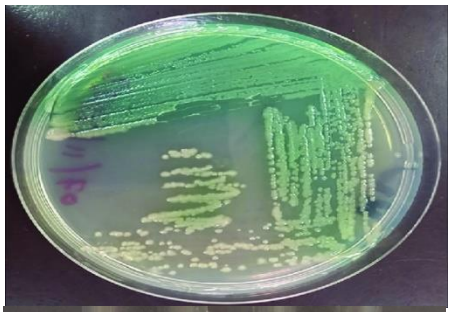
Clinical symptoms of tetanus

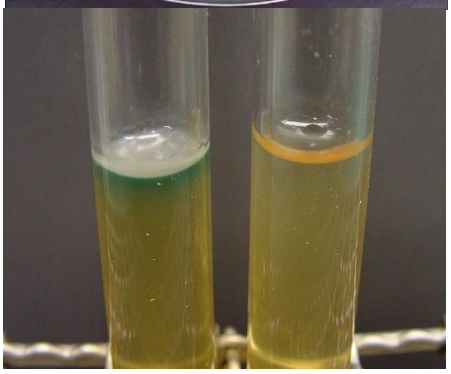
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Lab8 : Genus *Pseudomonas* :









- ***Genus Pseudomonas:*** *Pseudomonas spp*. Are gram negative rods aerobic widely distributed and play relevant ecological roles, with biotechnological applications; several species are commensal or pathogenic to humans, animals and plants. The genus *Pseudomonas* contains more than 140 species, most of which are saprophytic. More than 25 species are associated with humans. Most pseudomonads known to cause disease in humans are associated with **opportunistic infections**. These include ***P aeruginosa, P fluorescens and others.*** The nonfastidious growth requirements of *Pseudomonas* allow a wide distribution in nature. They may be part of normal human flora but are predominantly isolated as a result of nosocomial opportunistic infection. It is a ubiquitous free-living bacterium and is found in most moist environments. Although it seldom causes disease in healthy individuals, it is a major threat to hospitalized patients, particularly those with serious underlying diseases such as cancer and burns. The high mortality associated with these infections is due to a combination of weakened host defenses, bacterial resistance to antibiotics, and the production of extracellular bacterial enzymes and toxins.
- ***Pseudomonas aeruginosa :*** ***Pseudomonas aeruginosa*** can resist high concentrations of salt, dyes, weak antiseptics, and many commonly used antibiotics. Most pseudomonads known to cause disease in humans are associated with opportunistic infections. *Pseudomonas aeruginosa* and ***Pseudomonas maltophila*** are mostly responsible for disease conditions.

- **Characteristics of *Pseudomonas aeruginosa***

This opportunistic pathogen may infect virtually any tissue. Infection is facilitated by the presence of an underlying disease. It is a major threat to hospitalized patients, particularly those with serious underlying diseases such as cancer and burns (burning causes breakdown of nonspecific host defenses).

Sites of infection by *P. aeruginosa*

1. **Central nervous system infections**
2. **Localized infections of the ear and sinus**
3. **Skin and musculoskeletal tissues, burn wounds, surgical wounds**
4. **Respiratory tract: chronic infections in cystic fibrosis patients, acute pneumonia in other patients.**
5. **Bacteremia**
6. **Endocarditis**
7. **Urinary tract infections**

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Laboratory diagnosis of *P. aeruginosa* :

1. **Samples** ⟶ **urine, pus, blood, ear swabs, tissue biopsies, body fluids, etc.**
2. **Culture media :**

a. Basic media : nutrient agar ⟶ produce greenish-blue pigment called **pyocyanin**

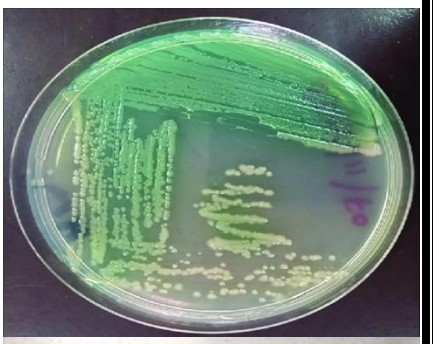
b. Differential media :

- **MacConkey agar** ⟶ **non lactose fermenter**
- **Simon citrate agar** ⟶ **utilize citrate as a source of energy and turned media color** ⟶ **from green to blue .**

c. Special media (Chrome agar) : Colonies of *Pseudomonas spp.* appear as **intense blue-green**, providing rapid and reliable visual identification—even in mixed cultures.

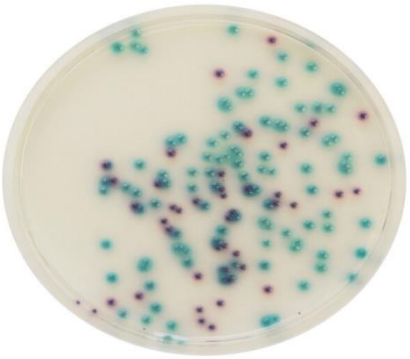
d. Selective media :

- **Pseudomonas agar:** The presence of blue-green or brown pigmentation may be considered as presumptive evidence of *P. aeruginosa*, *Alteromonas* spp. may form brown or pink colonies on the medium.
- **Cetrimide isolation agar:** colonies are typically shiny, opaque, and greenish-yellow, while the medium around them turns light blue due to the production of pigments like pyocyanin and pyoverdine.

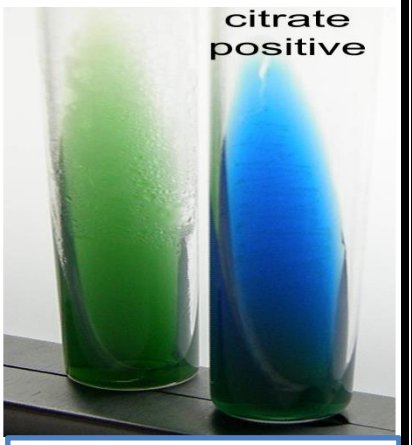




MacConkey agar



Chrome agar



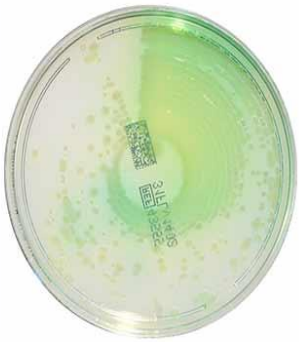
Simmon citrate agar



Colonies of *P. aeruginosa* on Pseudomonas agar



Pseudomonas aeruginosa on Cetrimide Agar
Source: Wikipedia



Incubation of *P. aeruginosa* ATCC® 27853 for 48 hours on Cetrimide agar.
Source: Biomerieux

3. Gram stain: *Pseudomonas aeruginosa* is a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm.

4. Biochemical characteristics:

►*Pseudomonas aeruginosa* is often preliminarily identified by its typical odor in vitro**. The smell is described as grape-like, tortilla-like, or "Philadelphus coronarius-like" (production of aminoacetophenone).**

►Catalase-positive

►Rapid oxidase-positive within 10 seconds (exception *P. luteola* and *P. oryzihabitans)*

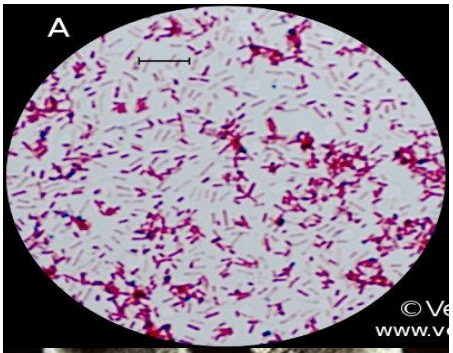
►Motile by means of one or more polar flagella.

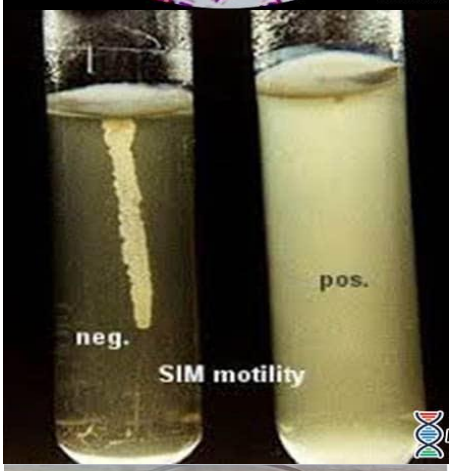
►It is not an active fermenter of carbohydrates and produces acid, but no gas, in glucose and is lactose-negative. Alkaline slant/alkaline deep (K/K) reaction in TSI or KIA agar.

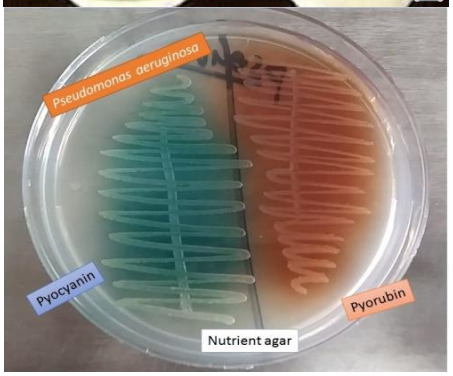
►Few strains of *P. aeruginosa* secret a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), pyomelanin (brown to black), and pyorubin (red-brown).

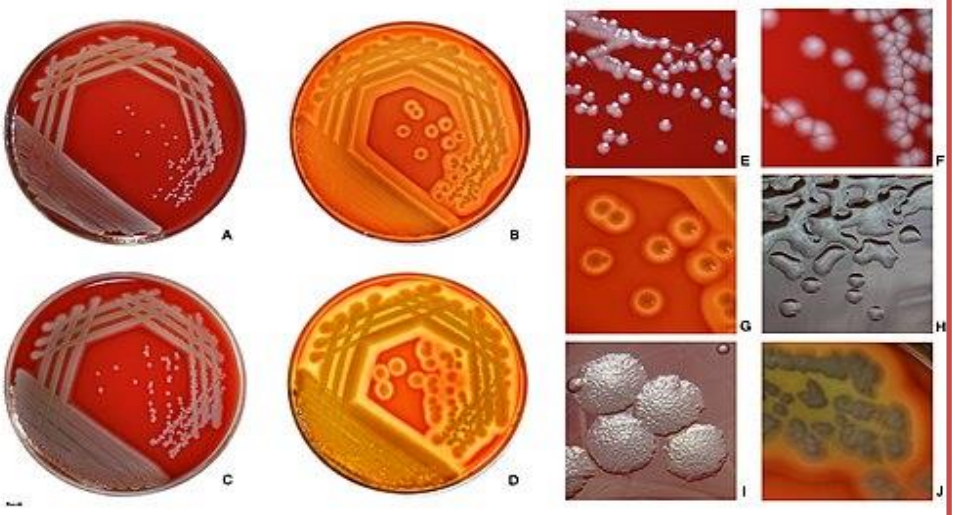
►Can grow in temperatures up to 42°C.The combination of pyocyanin production and the ability to grow at 42°C is sufficient to distinguish *P.aeruginosa* from other *Pseudomonas* spp. (e.g., *P.fluorescens, P.putida, P.stutzeri, P.putrefaciens*).

►**Hemolysis test :** On blood agar, *Pseudomonas aeruginosa* typically appears as large, gray-white colonies with a **distinctive metallic sheen** and irregular margins. These colonies are often **β-hemolytic** (causing a clearing of the red blood cells) and can produce **diffusible pigments** (like pyocyanin, which is blue-green), which may be more visible on other media. A **"grape-like" or fruity odor** is also a characteristic feature due to the production of aminoacetophenone.



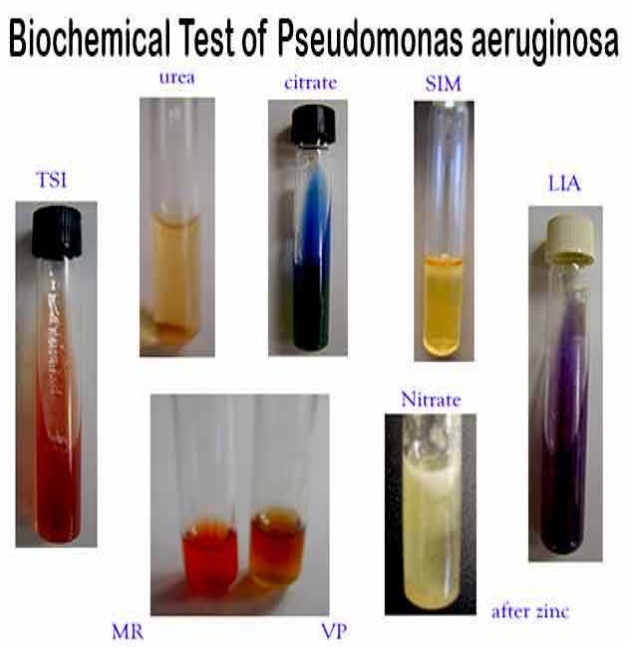






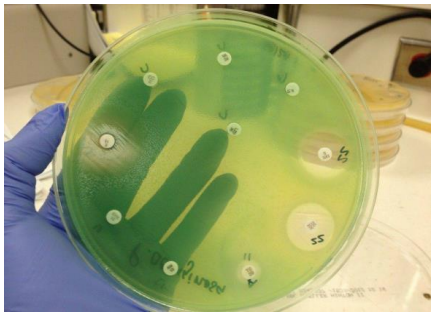
***Pseudomonas aeruginosa* growing on sheep blood agar at 37 °C after 24 h (A, C, E, F) and 48 h (B, D, G, H, I, J). *P. aeruginosa* strains are frequently haemolytic (B, D, G, J; reflected + transmitted light). This species gives rise to a variety of colony types. Most of the colonies are glanzing, but varying grades of roughness may occur. Figure I shows an image of colonies with a "beaten-copper" surface and slightly irregular edge.**

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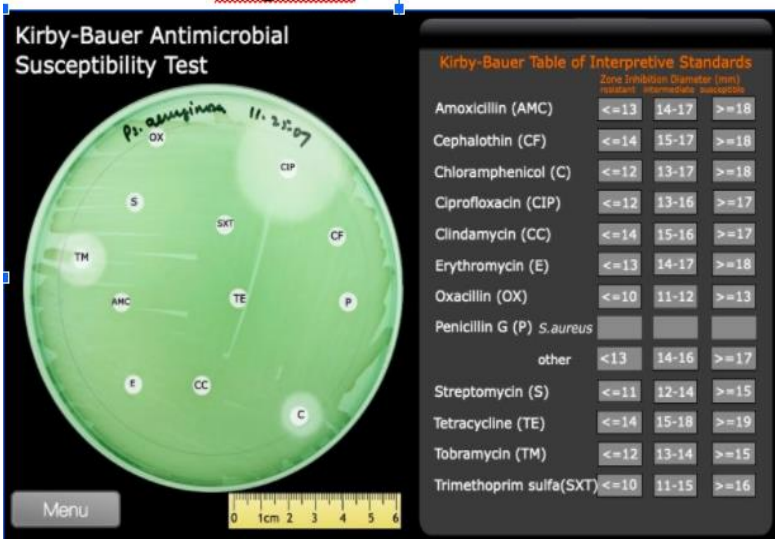
► **Antibiotic resistant** ***Pseudomonas aeruginosa*** is intrinsically resistant to a variety of antimicrobials and can develop resistance during anti-pseudomonal chemotherapy both of which compromise treatment of infections caused by this organism. Resistance to multiple classes of antimicrobials (multidrug resistance) in particular is increasingly common in *P. aeruginosa*. Some types of multidrug-resistant (MDR) .

P. aeruginosa are resistant to nearly all antibiotics, including carbapenems. ***P. aeruginosa*** carry a mobile genetic element that makes a carbapenemase enzyme. This enzyme makes carbapenem antibiotics ineffective. Mobile genetic elements are easily shared between bacteria, rapidly spreading resistance that destroys these important drugs.



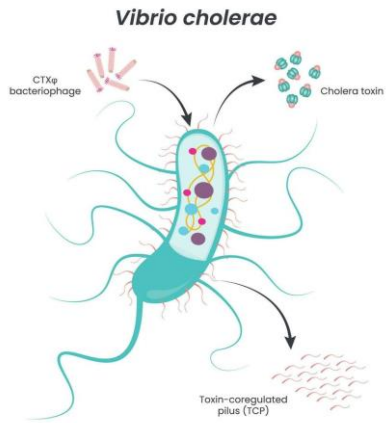
Characteristics	*Pseudomonas aeruginosa*
Gram Staining	**Negative**
Shape (Cocci/Diplococci/Rods)	**Rods**
Motility (Motile / Non-Motile)	**Motile (Unipolar)**
Catalase	**Positive (+ve)**
Oxidase	**Positive (+ve)**
MR	**Negative (-ve)**
VP	**Negative (-ve)**
Indole	**Negative (-ve)**
Citrate	**Positive (+ve)**
Urease	**Negative (-ve)**
H2S	**Negative (-ve)**
Gas	**Positive (+ve)- From Nitrate**
Pigment	**Positive (+ve)**
Cetrimide Test	**Positive (+ve)**
DNase	**–**
Lactose	**Negative (-ve)**
Mannitol	**Positive (+ve)**
Hemolysis	**β**

Pseudomonas aruginosa:



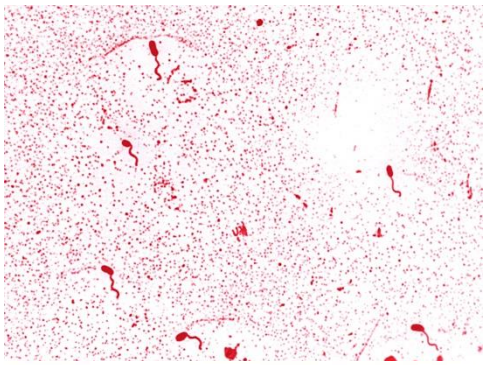
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Lab 9: *Vibrio cholera* :



- ***Genus vibrio* :** There are more than 35 species recognized for the genus vibrio, only 12 of them are responsible for human infection (gastrointestinal and extra-intestinal infections).
- ***Vibrio cholera:***
  - is the most medically important species which is responsible for the cholera disease
  - They are Gram-negative curved rods
  - aerobic with a wide distribution in the nature (Found in marine and surface waters)
  - Actively motile using polar flagellum.
  - The organisms produce an enterotoxin that lead to cause cholera (which is a watery diarrhea that can rapidly led to dehydration and even death if it is not treated)
  - Only two serogroups (O1 and O139) cause cholera in human
  - Other serogroups (more than 200) may cause enteritis and sepsis
  - Other species that can cause infection to human :

V. parahaemolyticus, V. vulnificus, V. minicus and V. alginolyticus.



- **Laboratory diagnosis :**

1. Specimens : ⟶ **Stool, rarely vomit.**

2. Culture media :

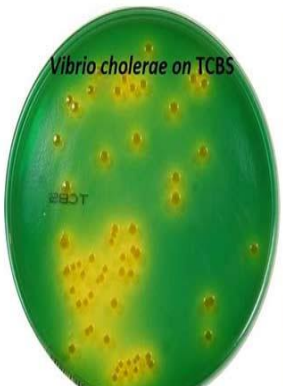
a. Transport media :

- **Sea salt medium**
- **Alkalin peptone water enrichment broth**

b. Selective media ⟶ **TCBS (Thiosulfate citrate bile salt sucrose medium).**

► Indicator – bromothymol blue .Control is green

(prepared as plates) .



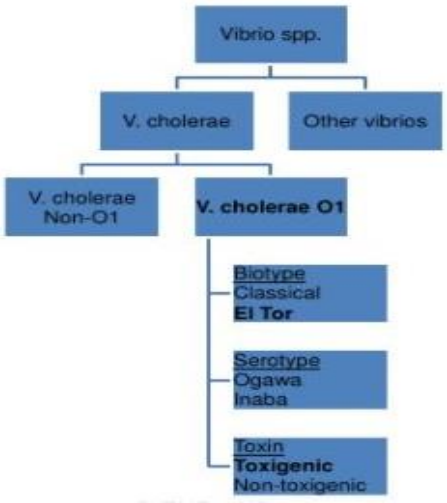
Vibrio cholerae on TCBS Agar


Vibrio parahaemolyticus on TCBS Agar

►After inoculation with suspected bacteria and a 18 to 24 hours' incubation at 35° to 37°C on TCBS agar, colonies of ***V. cholerae*** will appear as yellow, shiny colonies, 2 to 4 mm in diameter.

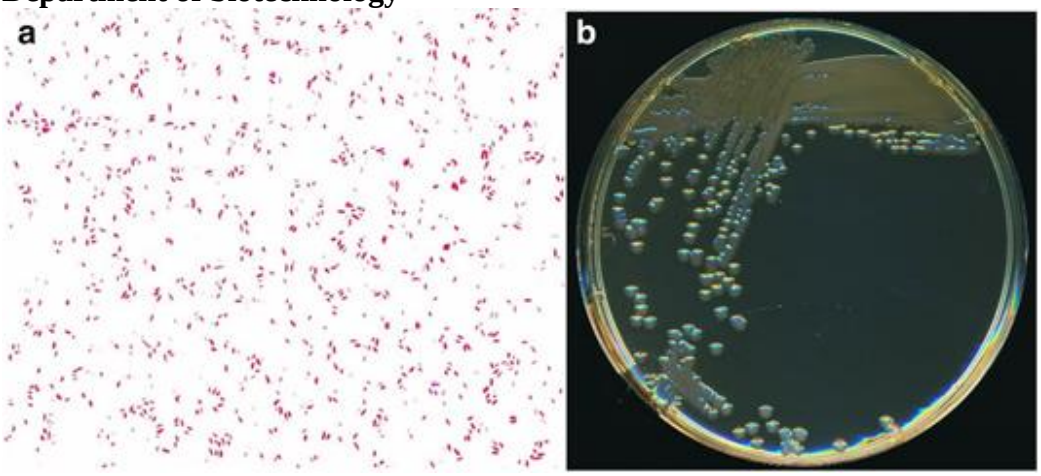
►The yellow color is caused by the fermentation of sucrose in the medium. Sucrose-non-fermenting organisms, such as

V. parahaemolyticus, produce green to blue-green colonies.



3. Staining: Gram negative bacilli or comma shaped.

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a. **Gram stain (magnificence×1000)**
b. **colonial morphology of non-O1, non-O139 *V. cholerae* grown on Trypticase-Soy agar after 18 h of aerobic incubation at 35 °C**

4. Biochemical tests:

► **Vibrio cholerae appears as a late lactose fermenter**

► **Motile**

► **Urease –ve**

► **Oxidase +ve**

► **TSI ; A or k /A**

► **Gas –ve, H2S –ve**

► **IMViC test result :**

Bacteria spp.	I	M	VI	C
Vibrio cholerae	+	+	+ –	+

Test	Result
Oxidase	+
Methyl red	-
Indole	+
Urease	-
Simmon's citrate	+
Glucose and lactose Fermentation on KIA	*A/K, (no gas/no H_2S)
Voges-Proskauer test	+
Growth in 1% NaCl	+

*A: Acid, K: Alkaline, KIA: Kligler Iron Agar

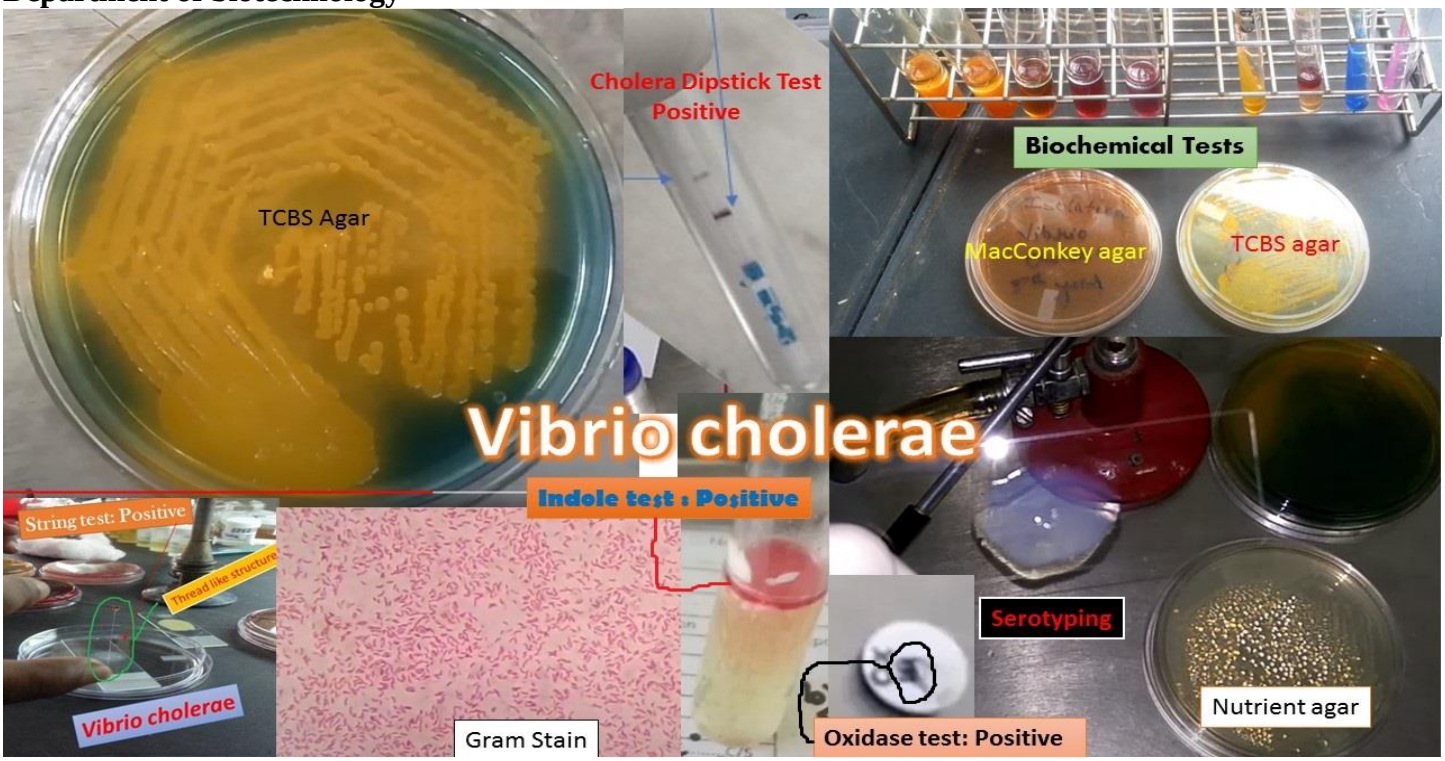


TSI

Bacteria spp.	H2S	Butt	Slant	Gas
Vibrio cholerae	-	Acid	Acid or Alkaline	–

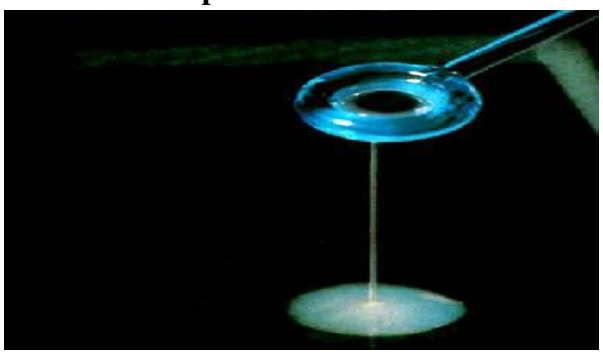
►**Kligler iron agar:** Acid / Alk, no gas, no H2S, Oxidase test : positive. Peptone water: Indol positive (red ring). Urea: negative. Simmon citrate: Positive. Semi solid mannitol: Motile, Mannitol ferment

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5. **String test:** Can be used to separate ***Vibrio cholerae*** from Aeromonas spp. (organism are emulsified in 0. 5% sodium deoxcholate which lyses the vibrio cells. but not those ***Aeromonas spp***. Within 60 sec the cells lyse (loss of turbidity) and DNA strings when a loopful is lifted (up to 2 to 3 cm) from the slide . **positive *V. cholerae* string test**



References

Required Texts	**Lippincott's Illustrated Reviews - Microbiology (Thao Doan, Roger Melvold)**
Recommended Texts	**Jawetz, Melnick and Adellberg's. (2011). Textbook of Medical Microbiology.26th EdiCon.**
Websites	**https://www.microbiologybook.org/book/immunol-sta.htm**

- ***Neisseria spp.*** **:**These organisms are named in honor of **Dr. Albert Neisser** who in 1879 discovered the organism-causing gonorrhea.
- **Morphology :** Gram negative Diplococci Kidney shape with flat opposing edge Oxidase + ve .

PATHOGENIC:

N. gonorrhoea **(gonococci, G.C)** → **Gonorrhoeae.**

N. meningitidis **(meningococci)** → **Meningitis.**

NON PATHOGENIC : ***N. Catarrhalis*** **Normal flora of upper respiratory tract**

- **General characters :**
  - **Aerobic to facultatively anaerobic Gram–ve cocci**
  - **Arrange in pairs (diplococci) with adjacent sides flattened (like coffee beans)**
  - ***Neisseria meningitidis*** **is encapsulated whereas** ***Neisseria gonorrhea*** **is not**

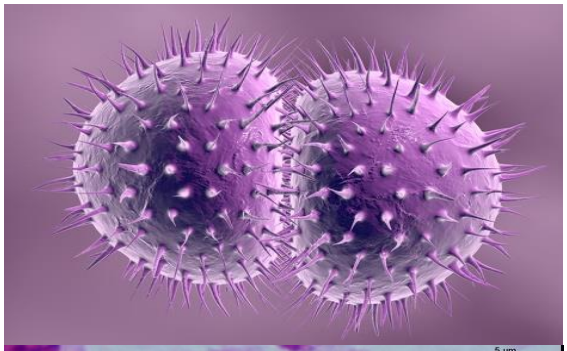


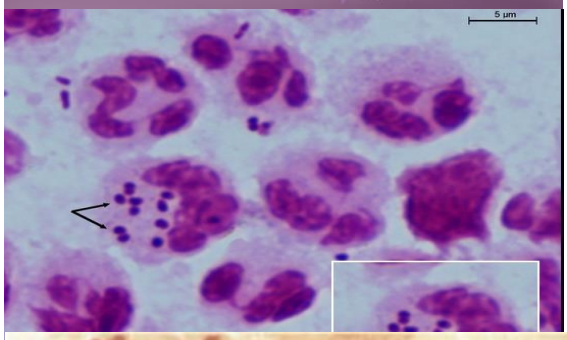
Table (10-1) **Human infections caused by *Neisseria* species**

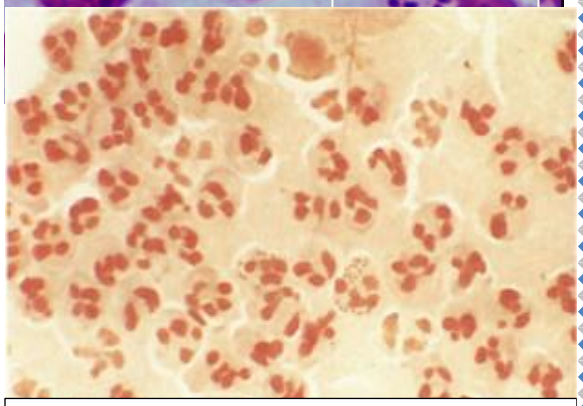
Bacteria	Diseases
Neisseria gonorrhoeae	Gonorrhea, disseminated gonococcal infections, ophthalmia neonatorum; and other gonococcal diseases: anorectal gonorrhea, gonococcal pharyngitis, and acute perihepatitis
Neisseria meningitidis	Meningitis and meningococcemia; other meningococcal diseases: meningococcal pneumonia, septic arthritis, purulent pericarditis, and endophthalmitis
Other *Neisseria* species	Opportunistic infections

1. **Neisseria gonorrhoeae (gonococcus)** :

- Very delicate and fastidious, requiring increased carbon dioxide tension (3% to10%) for cultivation.
- Narrow optimal temperature range, between 35° and 37°C and fails to grow much above or below these temperatures.
- It may remain viable in dried pus for several weeks.







Gram's staining slide for urethral discharge

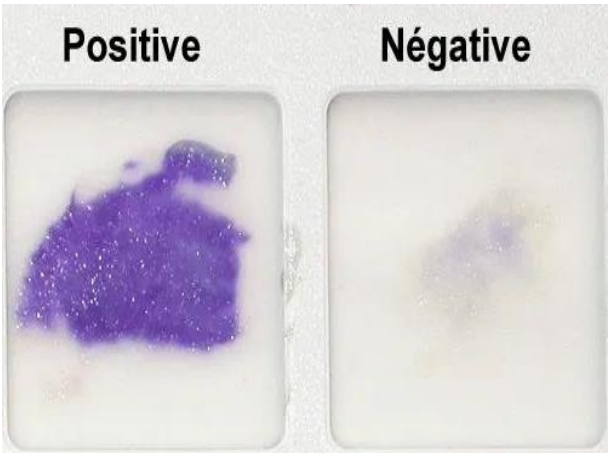
Lab. Diagnosis:

Swab sample: A swab sample from the part of the body likely to be infected (cervix, urethra, penis, rectum, or throat) can be sent to a lab for testing:

1-Gram stain: A sample from the urethra or a cervix is placed on a slide and stained with Gram stain . The cells appear as Gram negative cocci form masses with adjacent cells (i.e. in diploid forms), having a coffee bean-shape, or Kidney-shape. These cells are seen intracellularly (inside polymorphonuclear leucocytes), or extracellularly.

2-Urine test: Gonorrhoea in the cervix or urethra can also be diagnosed with a urine sample sent to a lab.

3- Oxidase Test: It is a hallmark test for the Neisseria. The oxidase test determines the presence or absence of **cytochrome C**. Organisms containing **cytochrome C** as part of their respiratory chain are **oxidase positive** and turn the reagent purple; organisms lacking cytochrome C as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the time limits of the test, and are **oxidase negative.**



4- Culture media



A. Selective media for Neisseria:

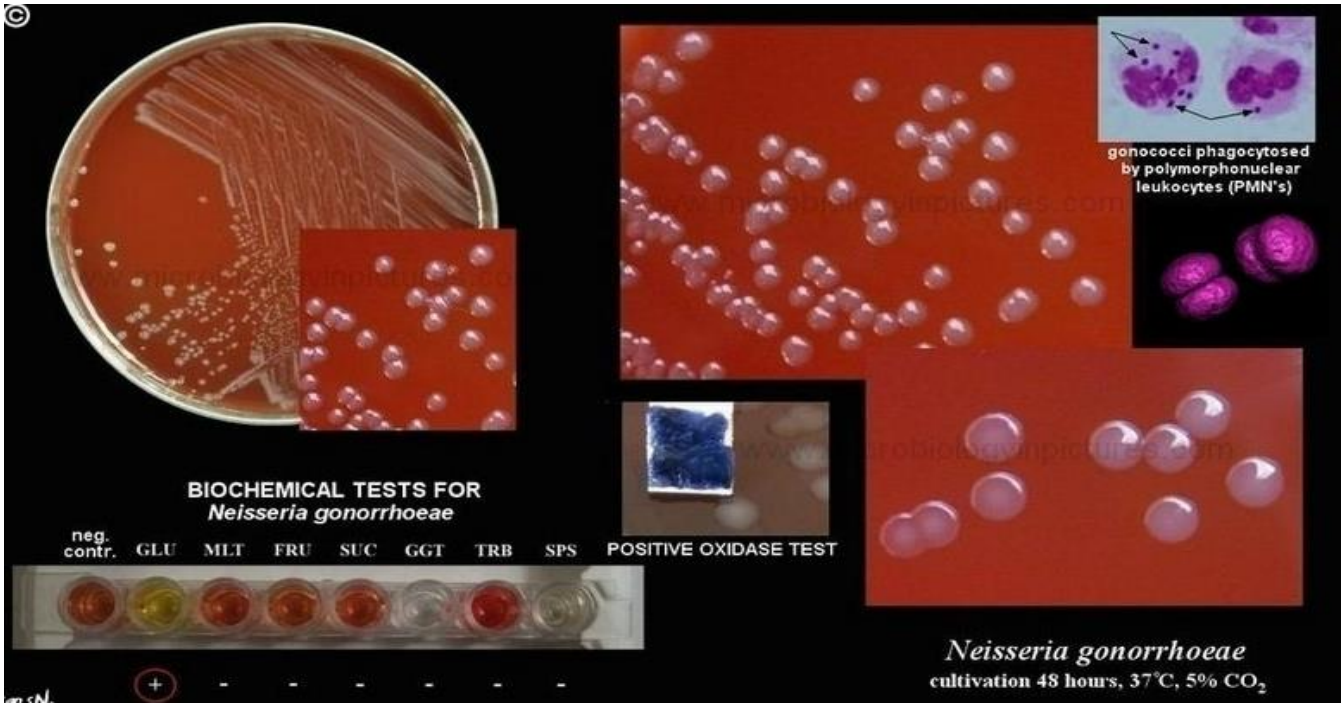
- **Thayer Martin Medium (TM)** :

(Chocolate agar + Enrichment element +Antibiotics :

1. Colistin (against G – ve rods)
2. Vancomycin (against G + ve)
3. Nystatin (against fungi)

- **Modified Thayer Martin**: {same as TM + Trimethoprim (against G –ve especially swarming ***proteus***)}

Non – pathogenic → not fastidious → can grow on nutrient media.

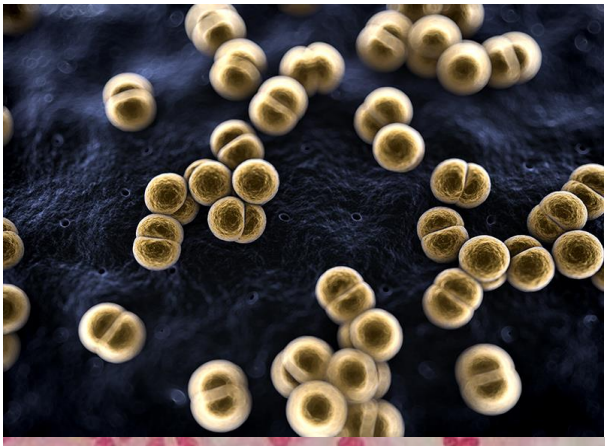


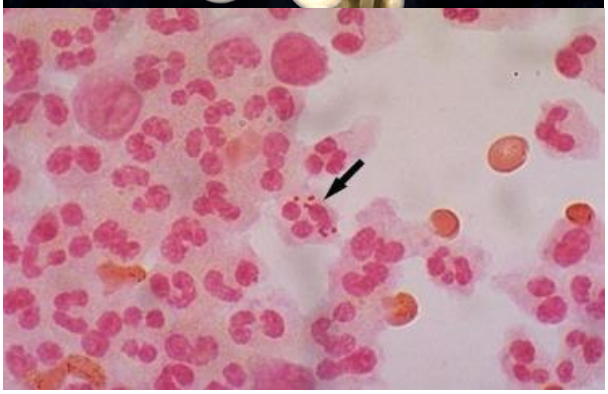
B. Candle jar 5%CO2 : The Candle flame will consume most of the oxygenin the jar producing an elevated level of Carbon Dioxide



Candle jar

2. *Neisseria meningitidis* (Meningococcus): Meningococcus is similar to the gonococcus in morphology and staining reactions.





Neisseria meningitidis is:

- Gram-negative
- diplococcal bacterium
- fastidious in growth requirements and may be cultivated on blood agar or chocolate agar on which it develops relatively large, gray, smooth, raised colonies.
- No hemolysis is produced on blood agar.

Neisseria meningitidis is the Causative agent of meningococcal meningitis, meningococcemia, and bacterial endocarditis. It is an obligate human pathogen.

Diagnosis:

A. Specimen: The gold standard of diagnosis is isolation of ***N. meningitidis*** from sterile body fluid. **Blood, CSF specimen** is sent to the laboratory immediately for identification of the organism. It may be isolated from nasopharynx, joint fluid, and from petechiae of the skin.

1. Gram stain: show adjacent coffee bean-shape, Gram negative cells (diplococci) with flattened surfaces facing each other.

2. Culture: culturing the organism on Thayer-Martin agar.

3. Oxidase test: (all Neisseria show a positive reaction)

4. Sugar fermentation tests: important in differentiation between ***Neisseria spp.***

- **Carbohydrates; maltose**, **sucrose**, and **glucose** test in which ***N. meningitidis*** will utilize the glucose and maltose.

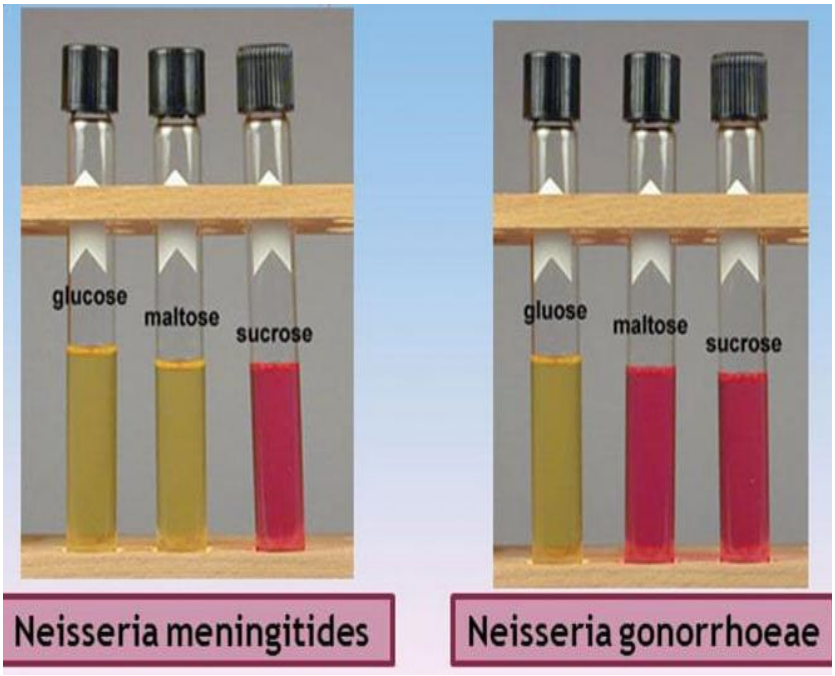


Figure: HISS Medium (CTA + Serum + Sugar + Indicator (Phenol Red)

Result of suger fermentation test

- **Positive reaction:** development of a yellow color change in the inoculated area (stab line) of the medium.
- **Negative reaction:** a red-pink or deep red color in the medium. Growth with a deep red to orange color in the medium indicates that the carbohydrate has not been utilized and that peptone degradation has occurred.

5. Cystine tryptic agar (CTA), also known as cystine trypticase agar: was developed by Vera as a semi-solid growth medium used for the identification and maintenance of microorganisms for extended periods when stored at appropriate temperatures. It is used for the detection of bacterial motility and, with added carbohydrates, it contributes to fermentation reactions and differentiation of fastidious microorganisms such as Neisseria, pneumococci, streptococci, and non-spore-forming anaerobes.



Cystine Tryptone Agar (M159)
1. Control
2. Neisseria gonorrhoeae ATCC 19424
3. Escherichia coli ATCC 25922
4. Neisseria meningitidis ATCC 13090
5. Streptococcus pneumoniae ATCC 6303

Basic Characteristics	Properties (*Neisseria gonorrhoeae*)
Capsule	Negative (-ve)
Catalase	Positive (+ve)
Gas	Negative (-ve)
Gram Staining	Negative (-ve)
H2S	Negative (-ve)
Hemolysis	Negative (-ve)
Nitrate Reduction	Negative (-ve)
Oxidase	Positive (+ve)
Pigment	Negative (-ve)
Shape	Diplococc
Fermentation of	
DNase	Negative (-ve)
Fructose	Negative (-ve)
Glucose	Positive (+ve)
Lactose	Negative (-ve)

Maltose	Negative (-ve)
Mannose	Negative (-ve)
Sucrose	Negative (-ve)

DIFFERENTIATION OF THE NEISSERIAE

Different Neisseria species can be identified by the sets of sugars from which they will produce acid. For example, ***N. gonorrhea*** makes acid from only glucose, however ***N. meningitidis*** produces acid from both glucose and maltose.

Neisseriae	Growth Characteristics	Sugar fermentation			
		Glucose	Maltose	Sucrose	Lactose
N. Gonorrhea	- Growth on enriched media at 37°C in CO2 jar - colonies similar to *N. meningitides*, but smaller and grayish white	+	–	–	–
N. meningitidis	- Growth on enriched media at 37°C in CO2 jar - colonies smooth glistening, translucent, soft, or bluish gray	+	+	–	–

6. **Srology**: determines the group of the isolated organism. If the organism reaches the circulation, then blood cultures should be drawn and processed accordingly.
7. **PCR polymerase chain reaction:** tests can be used to identify the organism even after antibiotics have begun to reduce the infection.

Note about polysaccharide capsule: N. meningitides has a polysaccharide capsule that surrounds the outer membrane of the bacterium. for the bacteria