Sterilization

Sterilization :-is freeing of an object from all living microorganisms (M.O.) including bacteria and their spores , viruses , yeasts , molds (pathogenic or nonpathogenic).

Disinfection : freeing of an object from some or all living pathogenic [microorganism by inhibit growth & multiplication of microorganism .

Methods of Sterilization :-

- Physical methods .
- Chemical methods .
- Mechanical methods

Sepsis : presence of infection (M.O) in living tissue .

Asepsis : Absence of infection (M.O) in living tissue .

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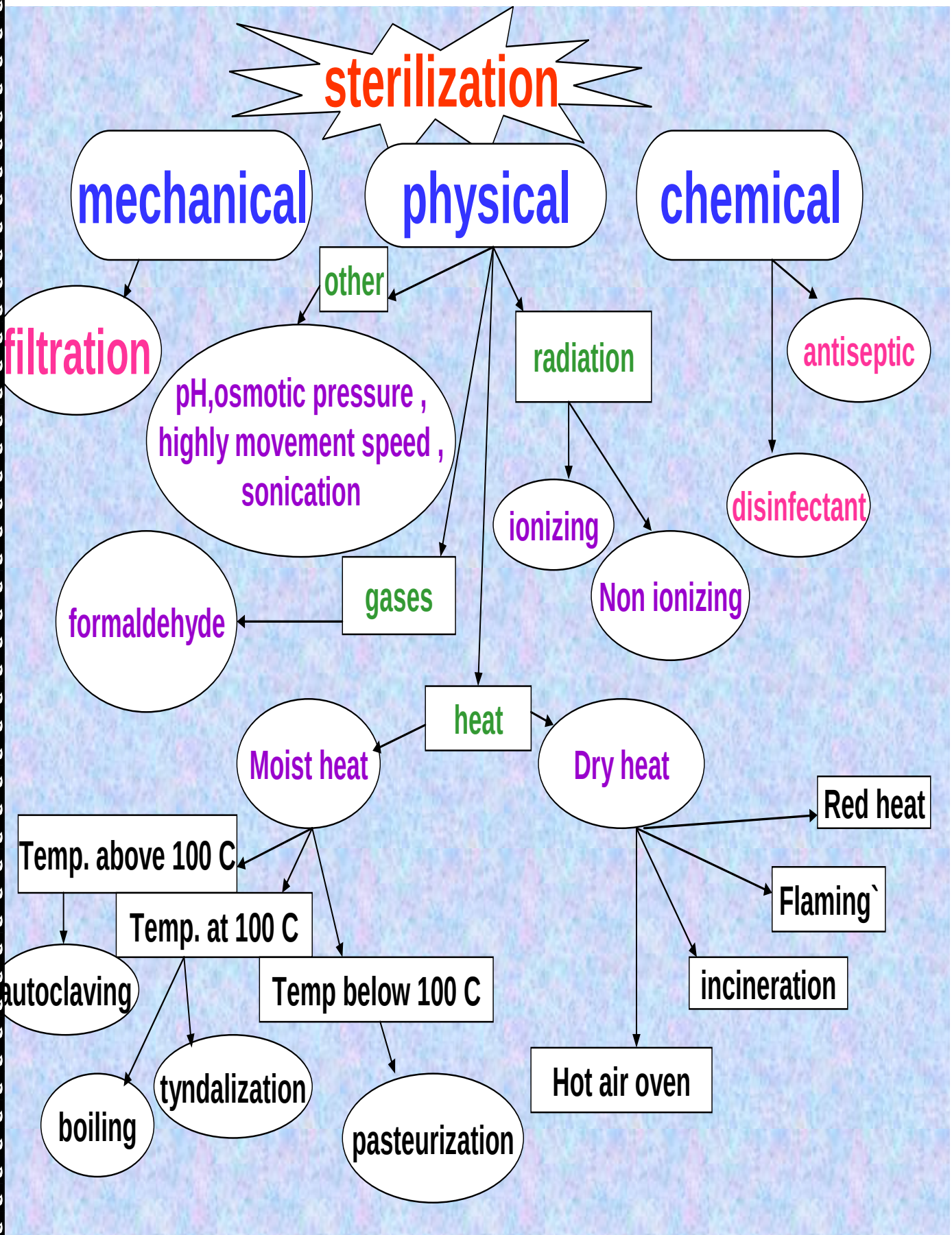


1) Chemical methods of sterilization

A- Antiseptic :-

It is chemical substance that inhibit the growth of M.O on living tissues , ex. 70% **alcohol , heptane , 10% detol** to sterilize bench, hand, floor.

B- Disinfectant :-

It is a chemical substance used to sterilize **non living objects** , ex. Phenol , 5% formalin to sterilize refrigerator.

Other chemical methods

a) Acid and alkalines
b) Metallic Iions
c) Halogens e.g Iodine for skin
d) Oxidizing agent
e) Formaldehyde gas, which killing bacteria spores at vegetative forms and disinfection rooms, blankets, clothing, shoes.
f) Soap and detergents
g) Dyes e.g Iodine
h) Aerosol and gaseous disinfection.

Bacteriostatic:-Any substance which inhibits the growth and multiplication (reduce NO.) of bacteria but do not necessarily kill them **.**

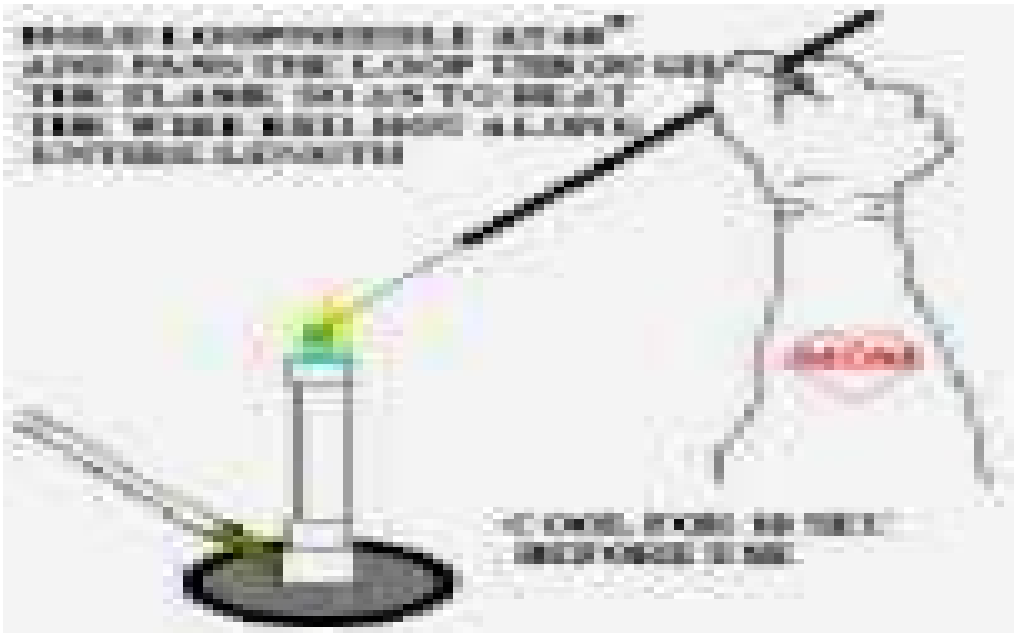
Bacteriocidal :- any substance which kills the bacteria

2) Physical methods of sterilization :-

A -Heat sterilization

1) **Dry heat sterilization**

a. Red heat ,used to sterile wire loops ,point end of forceps and metallic objects



b. **Flaming,** used to sterile mouth of tubes, glass spreaders, needle, cover slips and glass slide (which are flamed in ethanol).

c. **Incineration** ,used in pathological fuming materials .

d. **Hot air oven** (160-180 ˚C) for 2-4 hr., used to sterile glass wares (pipette , syringes , flask , Petri dish, scissors, swabs, test tubes, powder, oil and fats.

2) Moist heat sterilization

a. Temperature below 100˚C

1) pasteurization (63˚C for 30 min) , to sterilize milk .

2) Inspissation at 80 ˚C e.g serum and lowenstain jonsen media.

b. Temperature at 100 ˚C

- **Boiling** (5-10 min) to sterilize rubber tubes , glass syringes, cylinders, forceps, scissors (kills all non spore forming bacteria).
- Steaming (tyndillization) steam 30 min for 3days ,used to sterilize **gelatin media , sugar media** .

c .Temperature **above** 100 ˚C (autoclaving) the condition used in this instrument (15 lb ,121˚C ,15 min),used for sterilization of surgical tools and clothes, culture media and equal solution.

** the material is effect by heat (serum , protein , sugar,..) sterilized by filtration or autoclave for 5 min .

B -Radiation sterilization

- Non ionizing type, like ultra violate rays , infra red rays
- Ionizing type, like Gamma rays , X ray , Beta rays

U V light sterilize room operation . lab. Room ….

Infra red sterilize Water bath.

Mechanical methods: as **filtration**, which method sterilized through specific filters which holdback any bacteria present e.g plasma, vitamins, carbohydrates solution and antibiotics.

Culture media

Basic requirement of culture media :

1- Energy source : (carbon , amino acid ,..)
2- Carbon source : (glucose ,..)
3- Nitrogen source
4- Mineral salt
5- Osmatic pressure , PH
6- Oxidation – reducing potential
7- Growth factor

Common ingredients of culture media :-

1- Water
2- Agar : (Agar Agar) polysaccharide extract from sea weedy (red alga) , used for solidification of culture media , its solidify at 42 C^0 & melted at 95 C^0 .
3- Peptone : an intermediate product formed in the digestion of lean meat by pepsin , trypsin
4- Casein : milk protein consist of Amino acid .
5- Yeast extract : bakery yeast removal cell wall of yeast considered as a source of growth factor .
6- Blood : contain all nutrient material .
7- PH

Indicator	Acid media	Alkaline media
Neutral red Methyl red	Pink	Yellow
Phenol red	Yellow	Pink
Bromo thymol blue	Green –yellow	blue

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Classification of media

A/ according to solidity :

1 – liquid media (broth media) :contain all ingredient except agaragar (0%).

2 – semi solid media :contain all ingredient and 0.2 – 0.4% agaragar .

3 – solid media :contain all ingredient and 1.5 – 2% agaragar.

B/ according to function :

1 – Minimum media :contain CHO source ex. (Glucose) &inorganic N source and hydrogen .

2- Ordinary media : contain simple nutrient ex.(nutrient agar ,nutrient broth , lactose broth).

3- Enriched media : contain basic nutrient with additional material ex.(blood) to enhance the growth of bacteria .

ex: basic nutrient + blood = blood agar

basic nutrient +heated blood = Choclate agar .

4- Differential media : used to differentiate between two group of bacteria , ex.(on blood agar =type of hemolysis), (on MacConky agar =lactose fermenter from non lactose fermenter).

5- Selective media : in this media inhibit some bacteria & promote others , by add certain chemical substance . ex(S.S agar = to salmonella-Shigella , Manitol salt agar for staph. aureus).

6- Differential & Selective agar : ex MacConky agar differ lactose ferment from nonlactose ferment &its selective for G-ve bacteria.

7-Enrichment media : used to increase the small number of bacteria to plenty ex (tetra thionate broth).

8-Special media : ex:

a/Brucella agar for Brocella

b/Bordet Gengou agar for Bordetellla

c/ Loeffler media for Corynbacterium diphtheria

d/Lowenstain –Jensen media for Mycobacteria tuberculosis .

preparation of media :-

1-Nutriant agar or Nutrient broth :No. gram of media + Distal water = dissolve by heating &then sterilized with autoclave &then the solid media (N.agar)in plate & the broth media (N.broth) in test tube .

2- Blood agar : No. gram of media (N.agar or blood agar) + Distal water = dissolve by heating &then sterilized with autoclave &then cool to $55c^0$ &then add 3-4 % blood .

3- Chocolate agar :- : No. gram of media (N.agar or blood agar) + Distal water = dissolve by heating &then sterilized with autoclave &then cool to $80c^0$ &then add 3-4 % blood (blood is hemolysis release X , V factor).

4- sugar :- 100ml of N.broth + 1% (1 gram)sugar (Lactose , Maltose , Glucose) +dissolve with gently heat = sterilize by autoclave for 5 min or by filtration.

5-Slant media : ex . (Triple sugar iron /TSI) its diagnosis media ,

No. gram of media + Distal water = dissolve by heating &then sterilized with autoclave & then put in test tube & make slant .

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Cultivation of Bacteria

Colony : A macroscopic vascular growth of microorganism on a solid media .

Culture : A population or cultivation of microorganism.

Subculture : the streaking of isolated colony from mixed or confluent growth on fresh media .

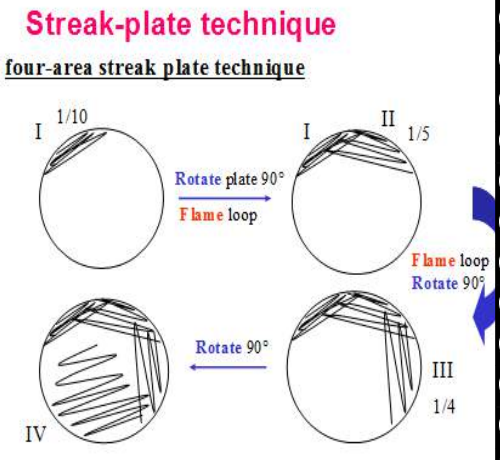
Stock culture : species or strain of Bacteria (pure culture) , unidentified , stored in stock media (enriched media +5%glycerol) in freezing for study .

Techniques used to isolated bacteria :

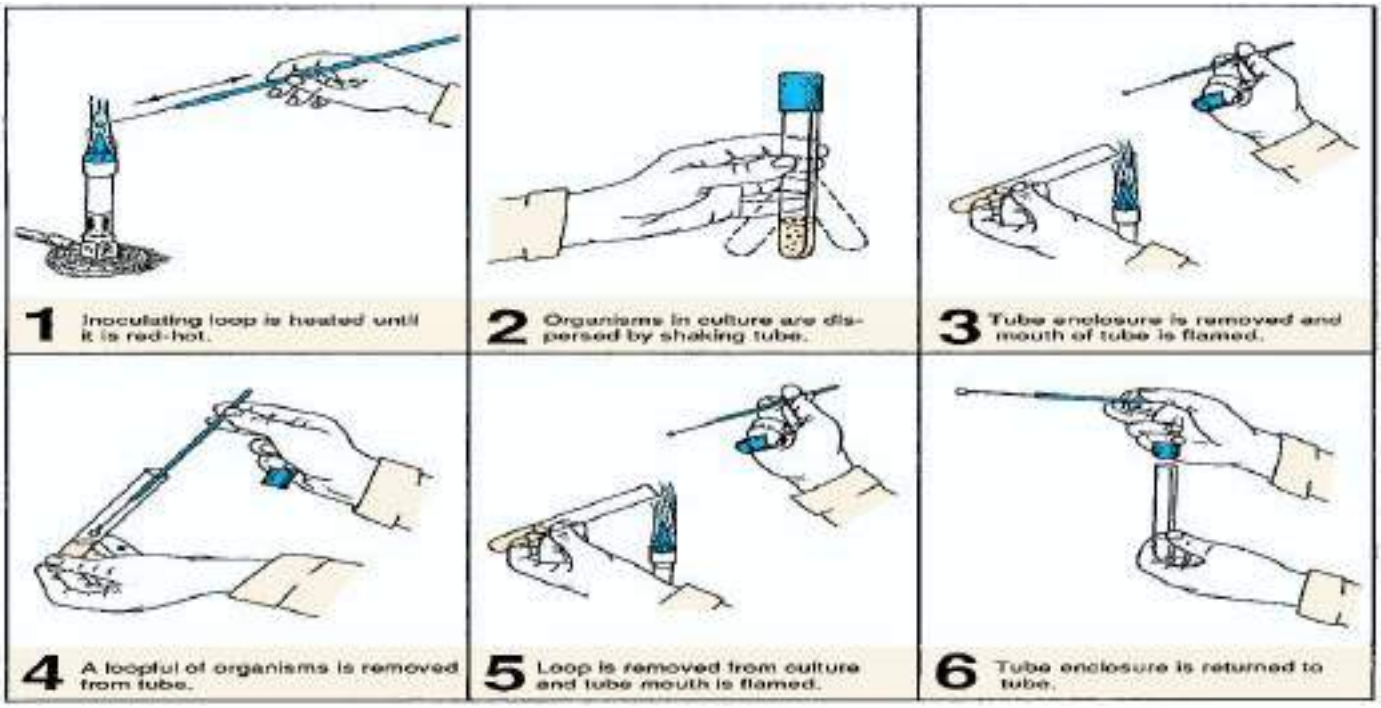
A -streaking on solid media

-procedure:

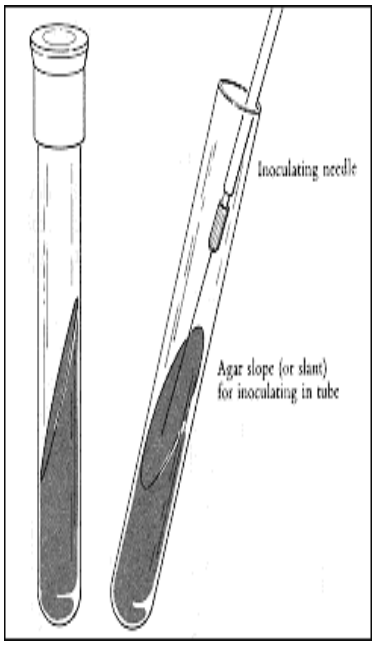
1-prepane Solid media in a Petri dish.
2-sterilize the loop by flaming .
3-cool it by touching the loop on side of medium.
4-hold single of colony by loop and transfer it to a new media .as in A this area termed inoculum area.
5-Re sterilizes the loop and repeat point 3.
6-make 4 parallel line as in B
7-repeat point (2.3)
8-repeat point 6 as in c
9- repeat point 2.3 .
10- repeat point 6 as in D
11- repeat point 2.3 .



B- Inoculation :bacteria is transferred from (broth to broth) or (broth to solid) …



C-Stabbing or stabbing &streaking : make on slant media or solid media in tube or semisolid



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4- Pouring method : liquid sample in dish + warm media volat then solidification =incubater at $37c^0$,identification of bacteria , shape (staining) of bacteria .

Colonies of bacteria are described as follows:

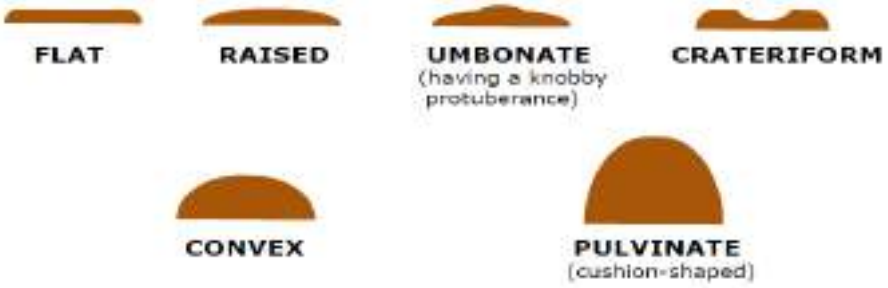
a. **Shape:** circular, irregular, radiating or rhizoid.

b. Surface: Bacterial colonies are frequently shiny and smooth in appearance. Other surface descriptions might be: **veined, rough, dull, wrinkled (or shriveled), glistening.**

c. Color – It is important to describe the color or pigment of the colony. Also include descriptive terms for any other relevant optical characteristics such as: **opaque, cloudy, translucent, iridescent.**

d. Size: Surface colonies are measured in millimeter, they are 2-3 mm in diameter. Smaller ones may be less than (about 0.5-1 mm)

e. Elevation: may be raised, low convex, dome shape



f. Edges: mostly edges are entire, sometimes undulate, lobate, curled, filiform.

g. **Color(pigmentation):** some organism may produce pigmented colonies (*Staphylococcus,Pseudomonas*)
h. **Opacity:** colonies on nutrient agar may be transparent, translucent or opaque.
i. **Consistency: Mostly** soft and butyrous and may be hard, firm, mucoid, tenacious, dry, adherent to medium, friable and membranous.

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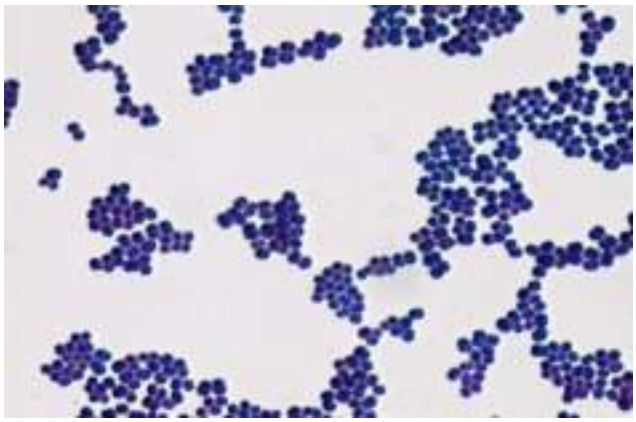
Genus Staphylococcus

***General characters*:-**

G +ve cocci grape like appearance or cluster some time appear in short chain pairs or single, non motile, non spore non capsulated **.**



Cultural characters:-

a. Grow on ordinary media.
b. Aerobic and facultative anaerobic.
c. Ferment sugar & produce lactic acid.
d. Some species (pathogenic) grow on media contain 7.5%NaCl.(mannitol salt agar)
e. Best media blood agar.
f. Optimum temperature 37Cº, PH 6.8.

Incubation period 18-24 hr

Species:-

1- Staphylococcus aureus.
2- Staphylococcus epidermidis.
3- Staphylococcus saprophyticus.

1) Staphylococcus aureus.

On blood agar:-

- **Golden** to **cream** or occasionally **white** colonies

2-4 mm in diameter and slightly raised

- Beta hemolysis

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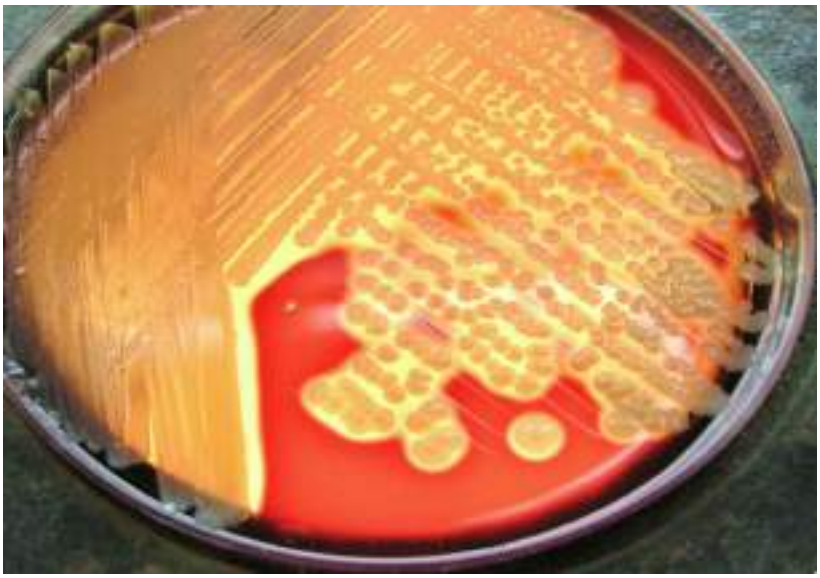
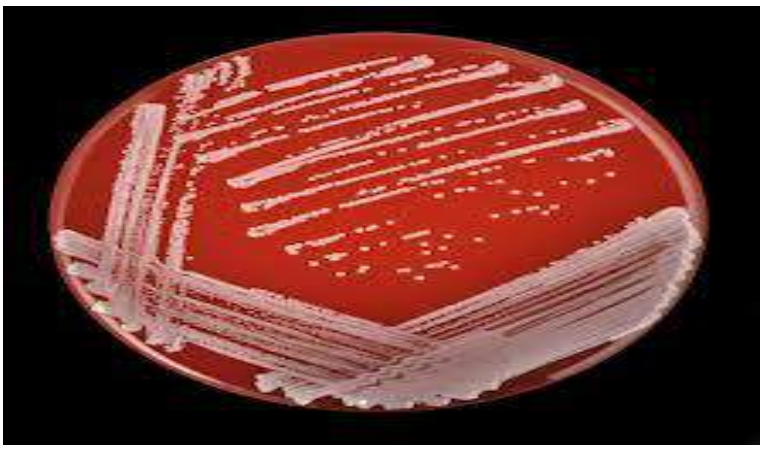


2- Staphylococcus epidermidis.

On blood agar:-

- non-hemolytic
- White colonies



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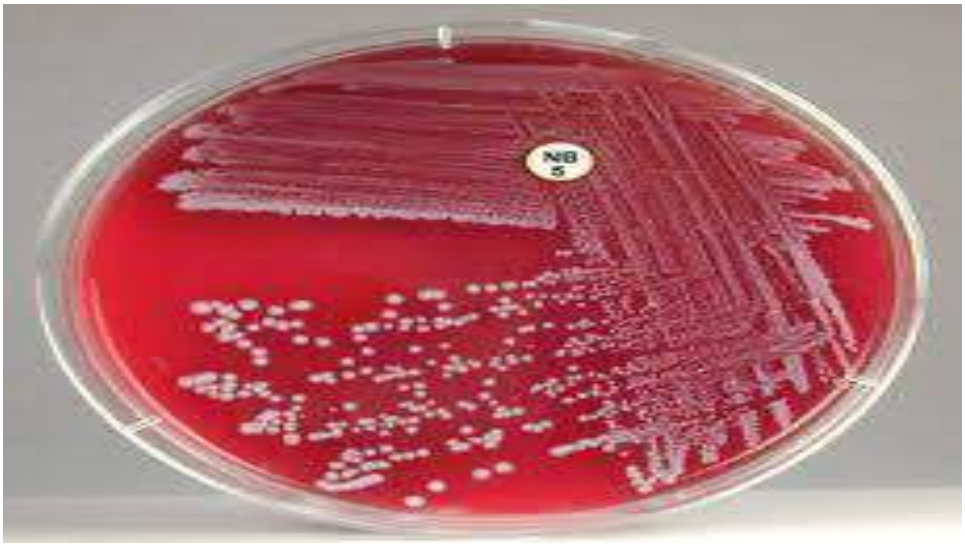
3) Staphylococcus saprophyticus.

On blood agar:-

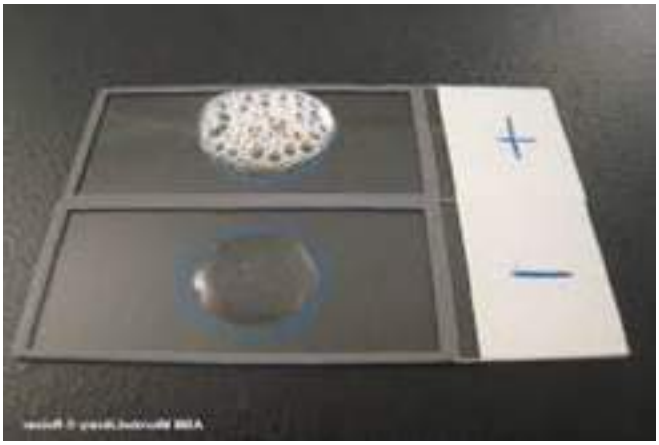
- non-hemolytic
- bright white
- creamy colonies



Lab diagnosis

1-Catalase positive for staph. aureus, epidermidis & saprophyticus.

2 H2O2 + coloney catalase H2O+O2.



- **Positive test:** Appearance of gas bubbles
- **Negative test:** NO bubble production

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2- coagulase positive for pathogenic species (staph. aureus) which convert fibrinogen to fibrin (clot) but **coagulase is negative for staph. epidermidis & saprophyticus.**

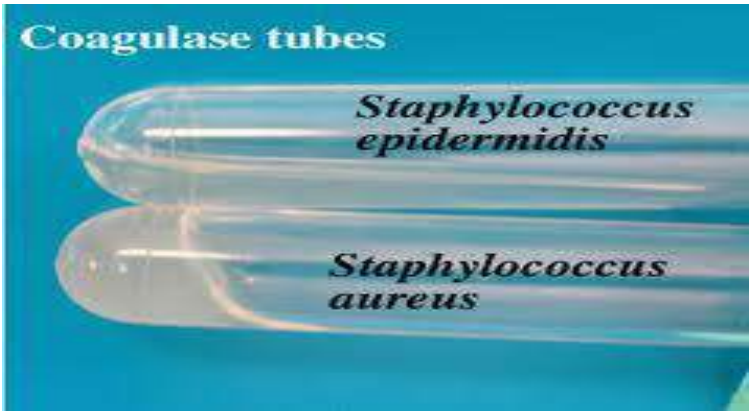
Plasma + colony ⟶ clot

a/bound coagulase : done by slid method clump cell visible aggregation .

➢**Positive test:**
Macroscopic clumping in 10 seconds or less in plasma drop.

➢**Negative test:**
No clumping in plasma drop.

b/ free coagulation : done by tube method tested each 30min for 1-4 hours to detect delay +ve.



negative test (non clotting on the plasma)

positive test (clotting of the plasma)

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3-Haemolysis : β-hemolysis -----**staph. aureus on blood agar around the colony appear clear zone due to the production of hemolysine** & **non hemolysis for staph. epidermidis & saprophyticus**

4- selective media (mannitol salt agar ,7.5% NaCL)only pathogenic species is grow with golden colony .

Staph. aureus ferment mannitol —— acid— phenol red ——yellow. and non fermenter for Staph epidermidis & saprophyticus

.

➢On Mannitol Salt Agar (MSA):

Acid production due to fermentation of mannitol turns the phenol red indicator yellow.

+ve for S.aureus

5- colony morphology :Large colony about 2-4mm, entire edge, convex elevation showing golden-white in color, pigmentation is less marked or absent in anaerobic condition.

Pathogenicity :- boil, carbuncle, pneumonia bronchitis, meningitis, urinary tract infection, food poisoning, osteomyelitis, tonsillitis & pharyngitis.

Clinical samples: Urine, sputum, C.S.F, swab of boil, wound, ear pus, samples from food poisoning or from cases of septicemia, meningitis & endocarditis .

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Streptococcus Genus

General characters : G+ve cocci arranged in chains ,non motile ,non spore formation ,aerobic & facultative anaerobic , **catalase -ve .**

Species :

1-streptococcus Pyogenes

2- streptococcus agalactiae

3- streptococcus viridance

4- streptococcus pneumonia

1-*streptococcus Pyogenes*

lab. Diagnosis :

1-Gram stain is G+ve cocci

2-Small colonies 0.5-1 mm semi transparent, low convex, pin point, required enriched media with blood or serum.

3- culture on a blood agar with crystal violet 1/500,000 +Na azide as a (selective media).

4- ferment sugar with acid only

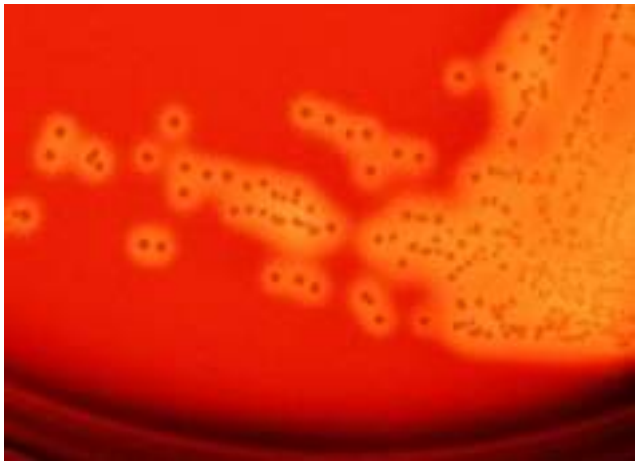
5- serology test (A.S.0.T)

6- Dick test for scarlet fever

7-CAMP test (-ve)

8- β-hemolysis

9-**sensetive to Bacitracin 0.04** unite



On blood agar

*Colonies 0.5-1 mm in diameter , colorless, dry, shiny or mucoid

*B hemolysis

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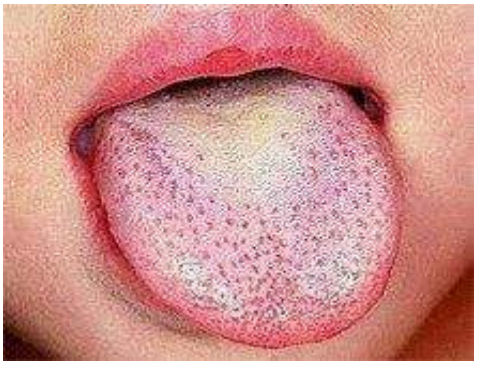

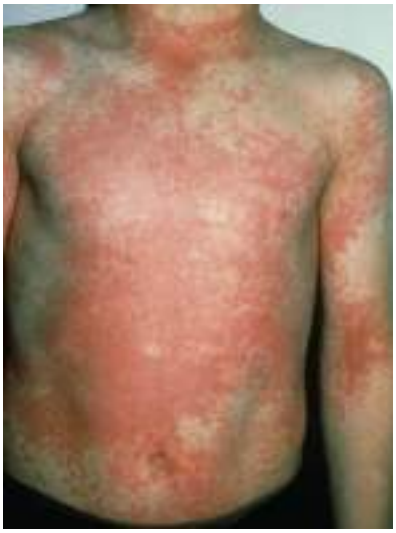

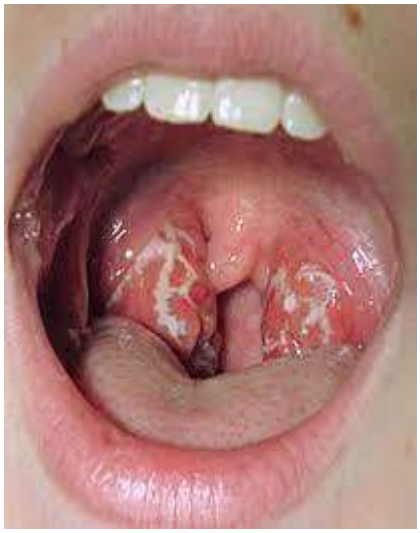

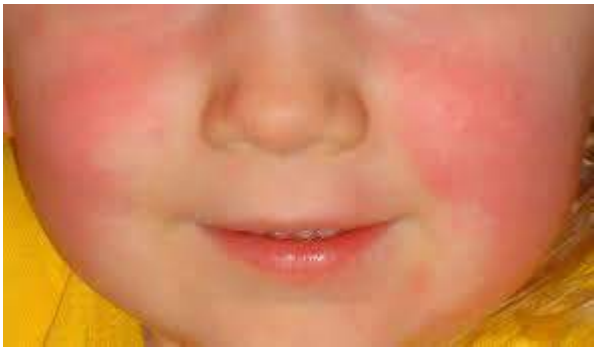

Scarlet fever

CAMP factor is an extracelluar diffusible protein produced by *S. agalactiae

***This protein interacts with the staphylococcal beta-lysin producing enhanced haemolysis.**

***arrow-head shaped area of haemolysis is produced where the staphylococcal organism meets test organism.**

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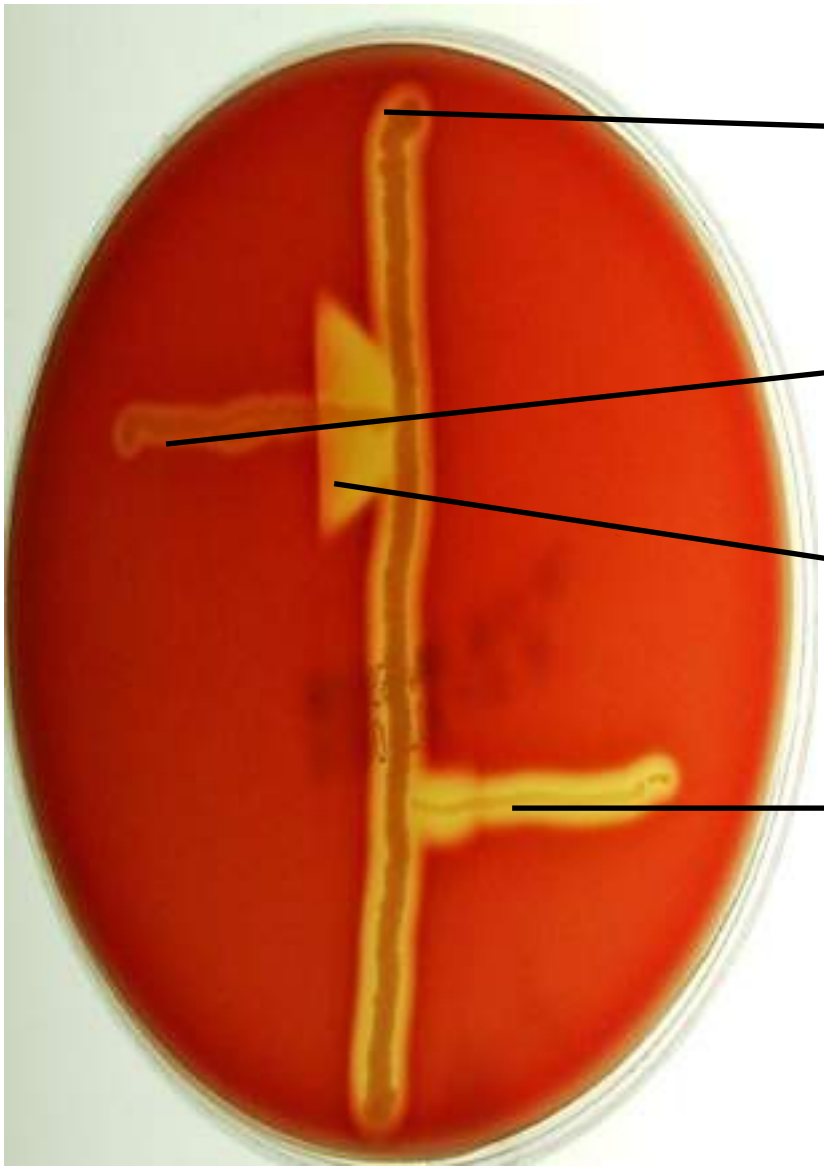

S.aureus
Indicator

S.agalactiae
(+ve CAMP test)

Arrow head zone of haemolysis

s.pyogenes
(-ve CAMP test)

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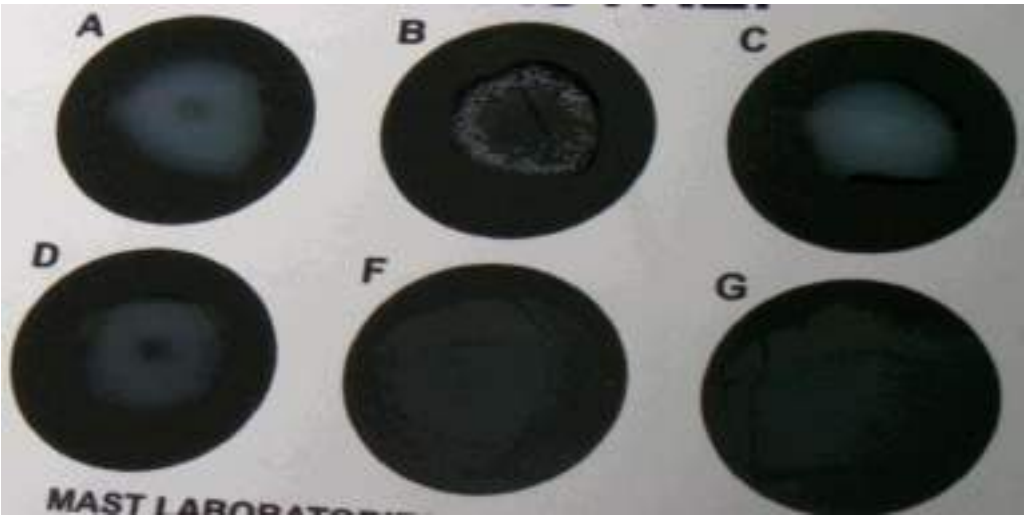
ASOT for (serology test) e.g latex agglutination test

Latex slide agglutination test for streptococcal grouping



The clinical isolate showed agglutination in Group streptococcal antisera.

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Bacitracin sensitivity Test

Pathogenicity of pyogenes is :

1-respiratory infection

2- tonsillitis , pharangitis (scarlet fever)

3-wound , burn infection

4-Otitis media

5- Abscess of the organ (brain, lung, liver).

6- Non suppurative complication

a) Acute Rheumatic fever
b) Acute glomerulonephritis

Clinical sample:

1-Swabs (throat , ear, vaginar)

2- C.S.F

3- sputum

4-swab from wound and burns

5- Urine

6- Blood

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2- streptococcus agalactiae

1-β-hemolysis

2- resistance to Bacitracin 0.04 unite

3-colony : small , dull , pin point .

4- CAMP test +ve

On blood agar:

Pathogenesis:

Meningitis, neonatal sepsis, pneumonia, endocarditis, urinary tract infection, endocarditis, arithritis

3- streptococcus viridance

1-alpha-hemolysis with partial hemolysis (greenish discoloration , convert bilirubin to bilivirdin green)

2- colony : small , pin point .

3- non pathogenic

4- resistance to optochin

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4- streptococcus pneumonia

Diplococcus pneumonia

1-G+ve cocci pair , flattened edge

2-capsolated

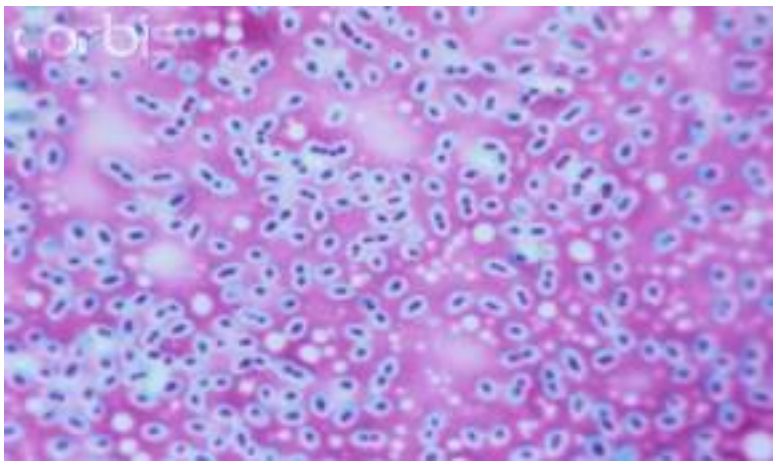
3- alpha-hemolysis

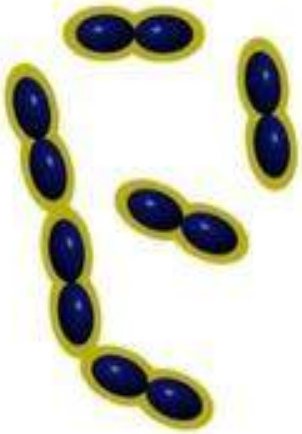
4-sensetive to Optochin

To isolate need:

1-enriched media 2- (5_10%) CO_2

Streptococcus pneumoniae:
Capsule staining





On blood agar:

***Translucent or mucoid colonies**
***1-2 mm in diameter**

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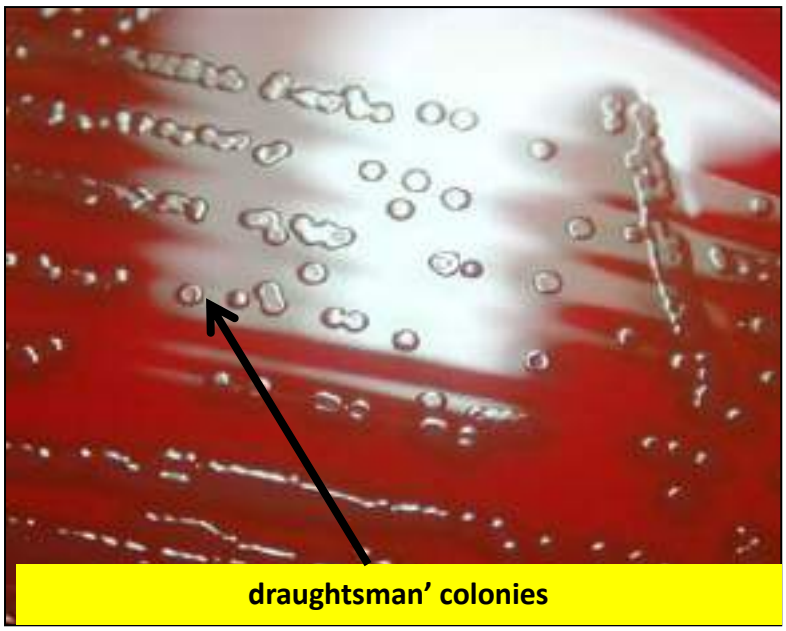

On blood agar:

*** Raised colonies in young cultures but later become flattened with raised edges**

On blood agar:

α haemolytic

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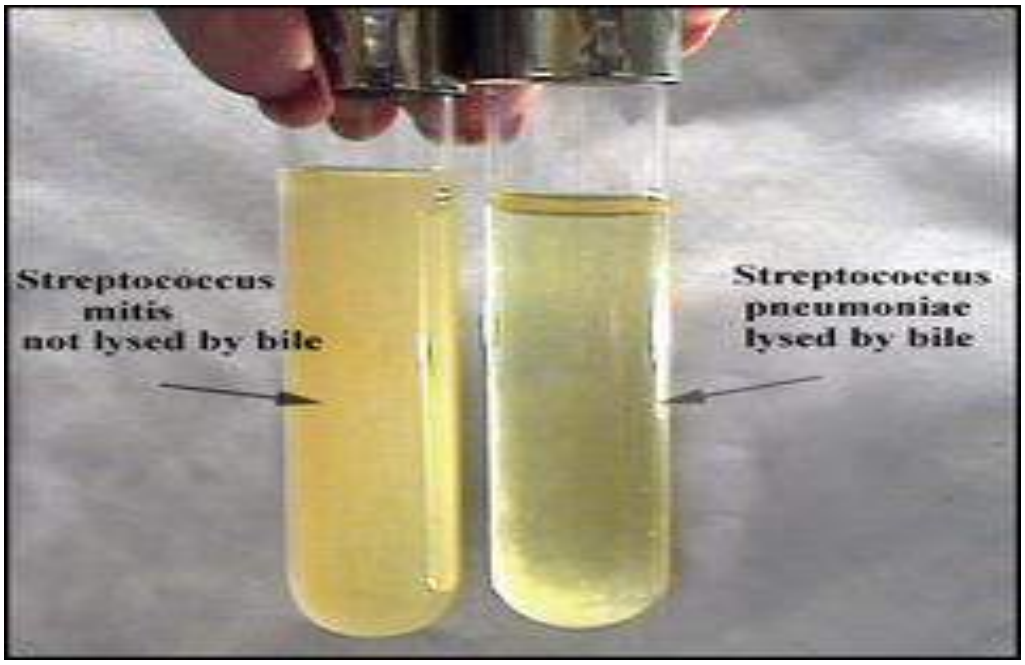
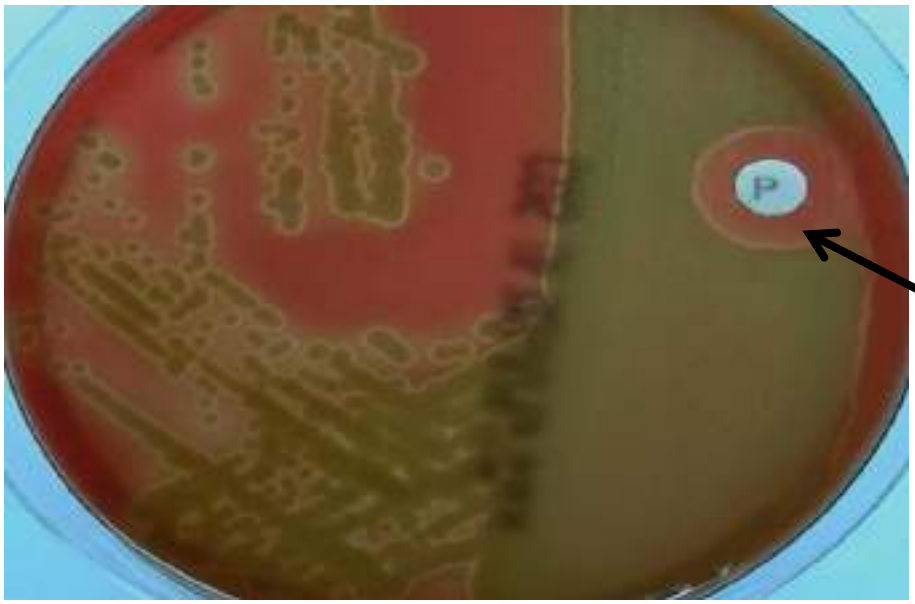
Bile solubility test



Optochin sensitivity Test
(S.pneumoniae is sensitive to Optochin)



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Pathogenicity

1-lobar pneumonia (80%)

2-bronchopneumonia (60%)

3- pneumococcal meningitis

4- otitis media , sinusitis , conjunctivitis

Clinical sample:

1-sputum 2- C.S.F

3-swab (ear , throat , skin…..)

4- blood

5-urine.

Streptococcus pneumonia	Streptococcus viridance
1-α hemolysis on blood agar	Same
2-morphology G+ve lanceolate diplococcic	G+ve short chain rounded or oval shape
3-capsulated by capsular swelling(quelling test)	Non capsulated and give negative test
4-flat colonies, later with raised run called(Druaghtsman)	Raised colonies
5-bile solubility is +ve	-ve
6-optochine sensitivity is +ve	-ve
7-pathogenic for laboratory animal	Non pathogen
8-growth on broth show uniform turbidity	Granular growth