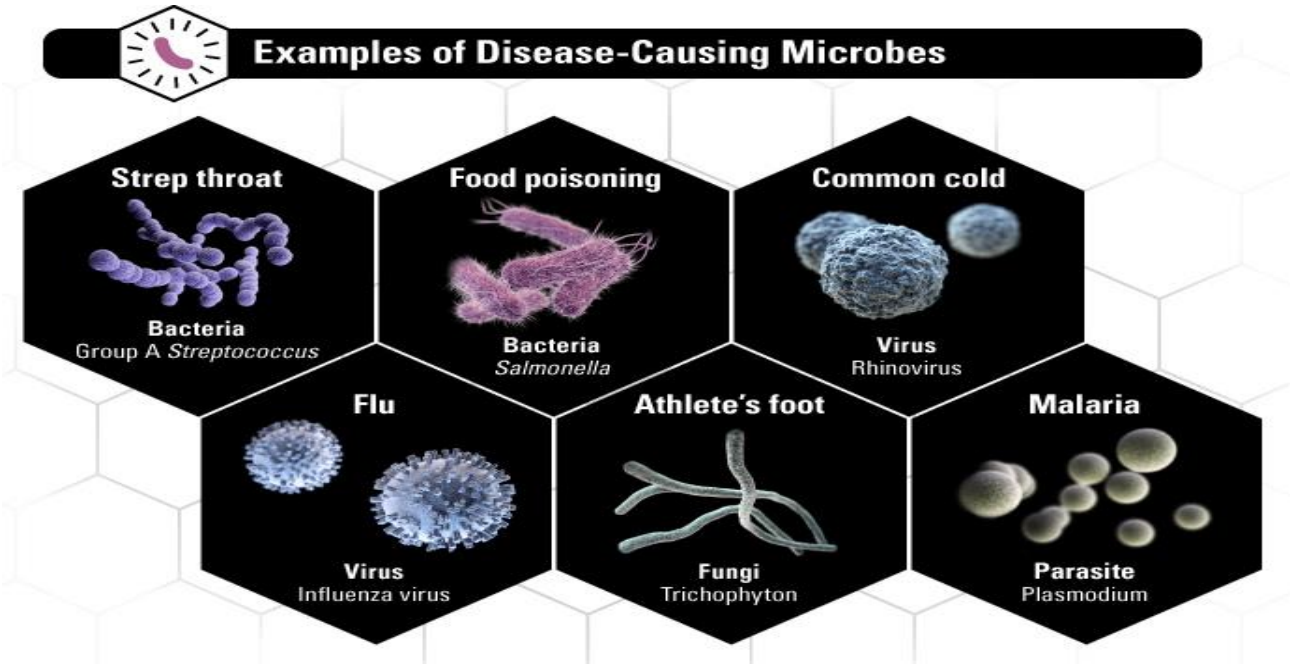
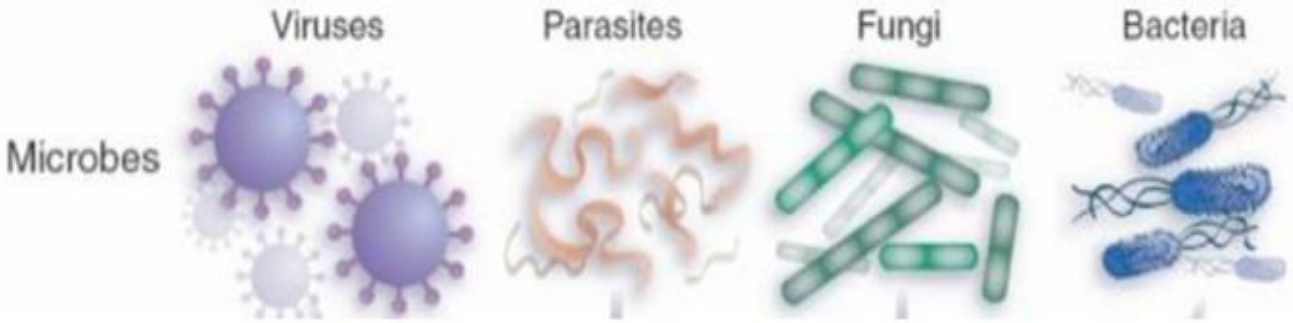
Microbiology
team 436

436
MEDICINE
KING SAUD UNIVERSITY

microbiology

PRACTICAL



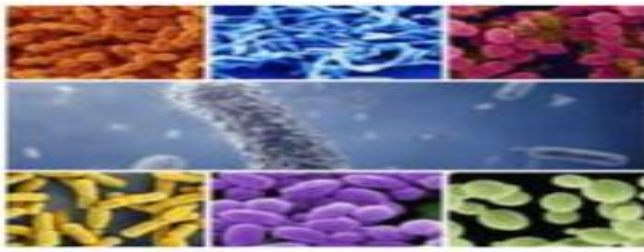


Laboratory diagnosis of infections . ID

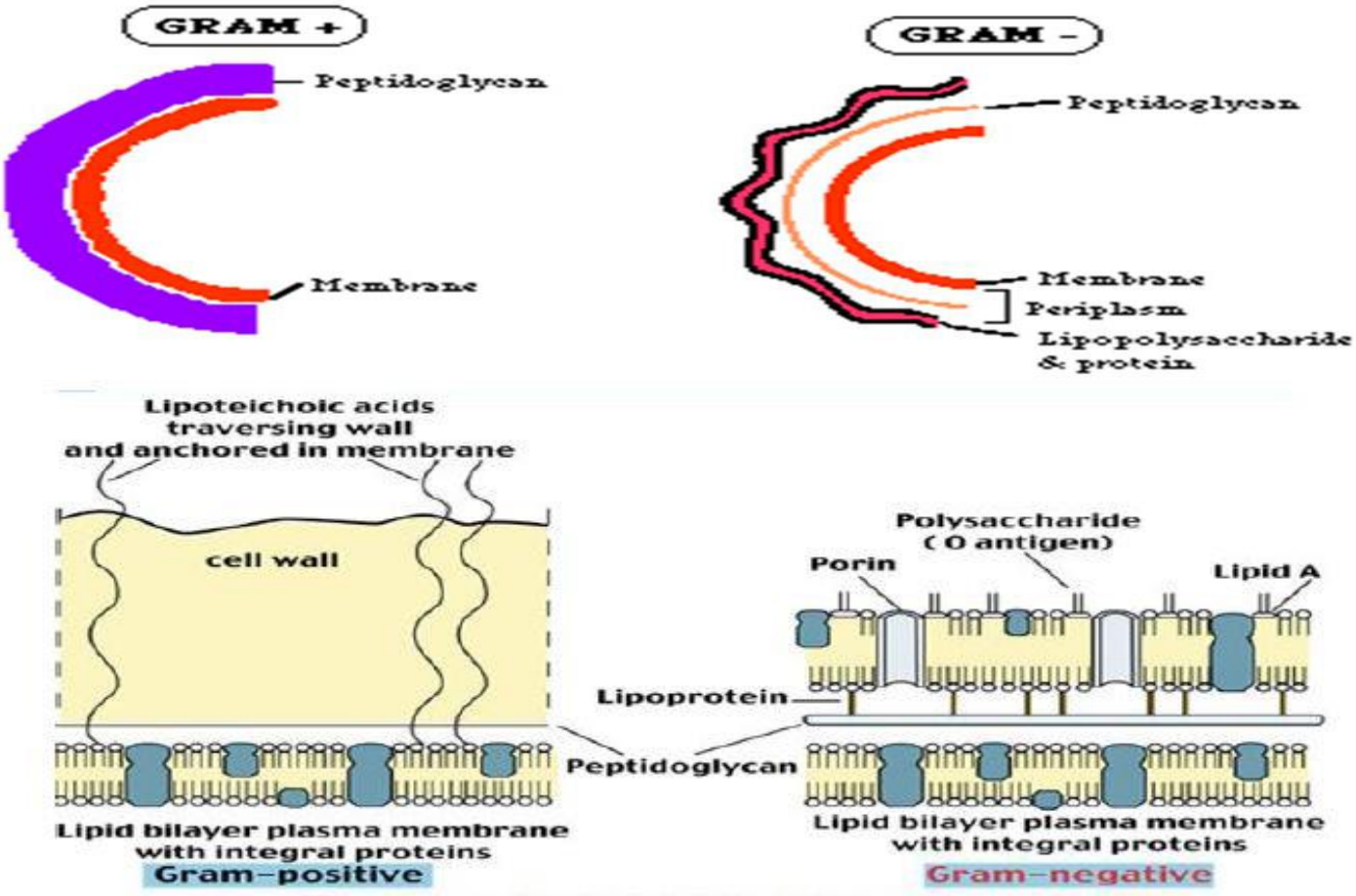
- *Microscopic examination.*
- *culture.*
- *Serological tests (Ab).*
- *Detection of Ag.*
- *Molecular method .*

Types of specimens

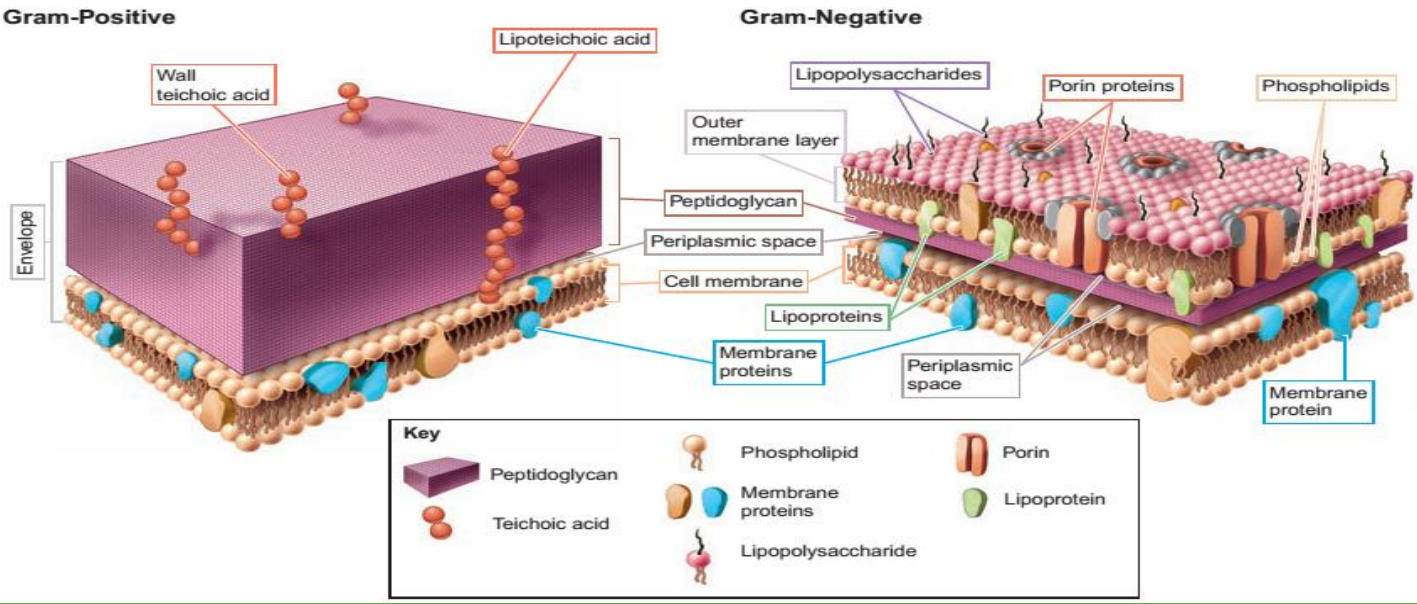
BACTERIOLOGY



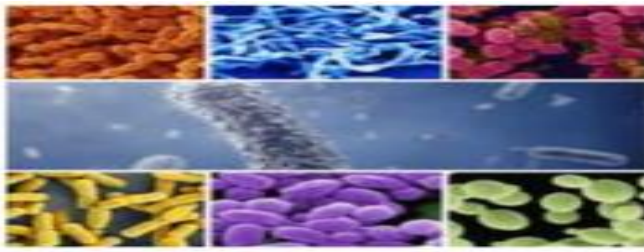
Bacterial cell wall



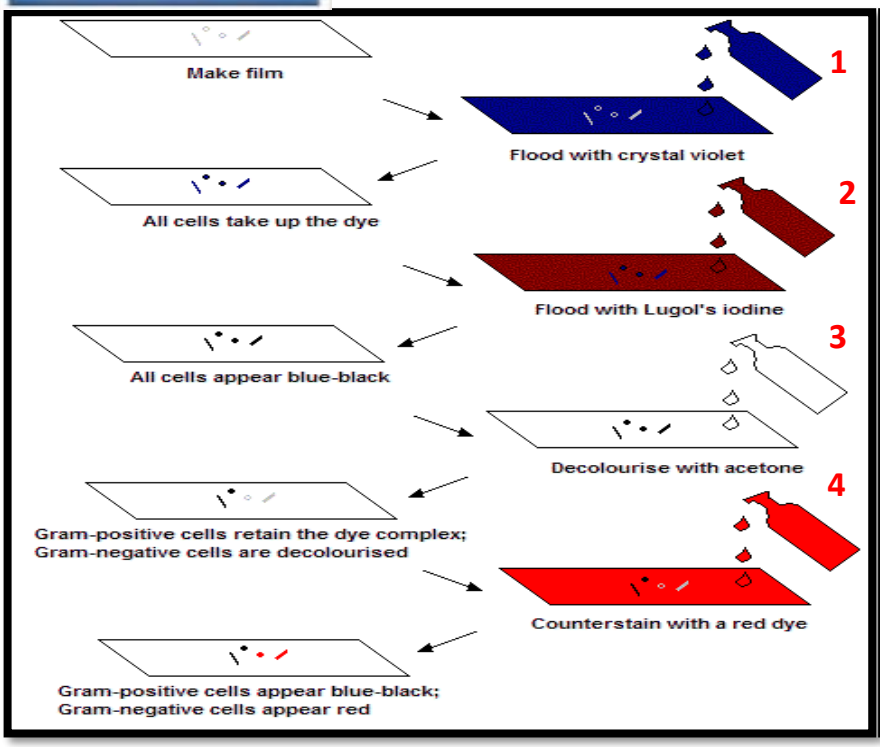
Extra picture just to make it clear

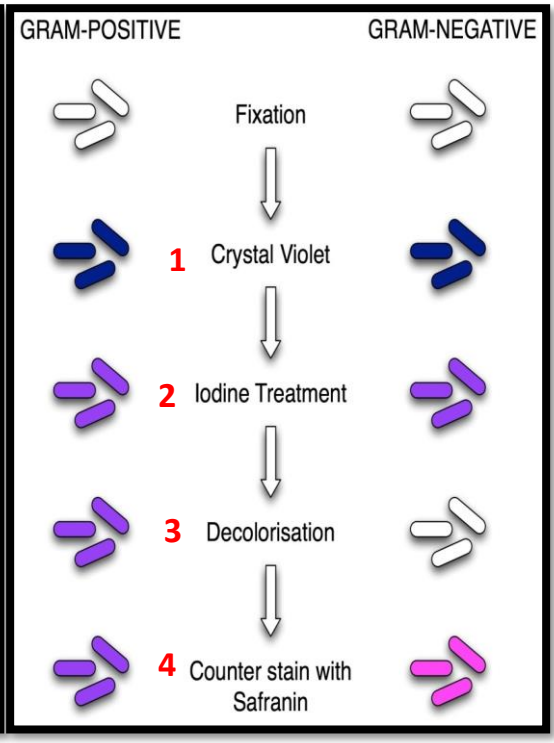


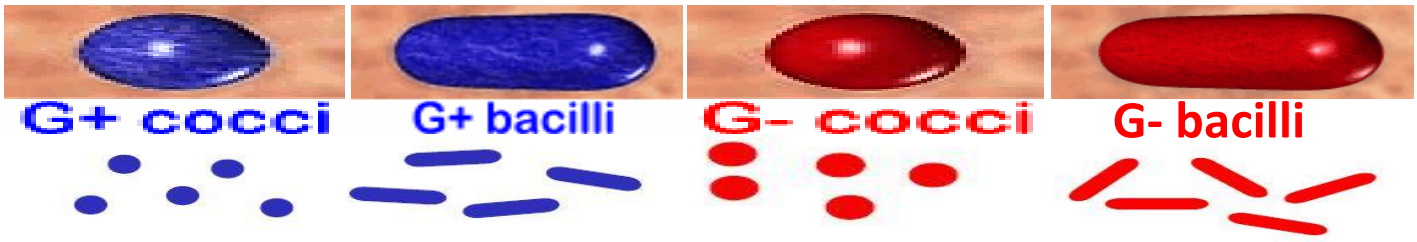
BACTERIOLOGY



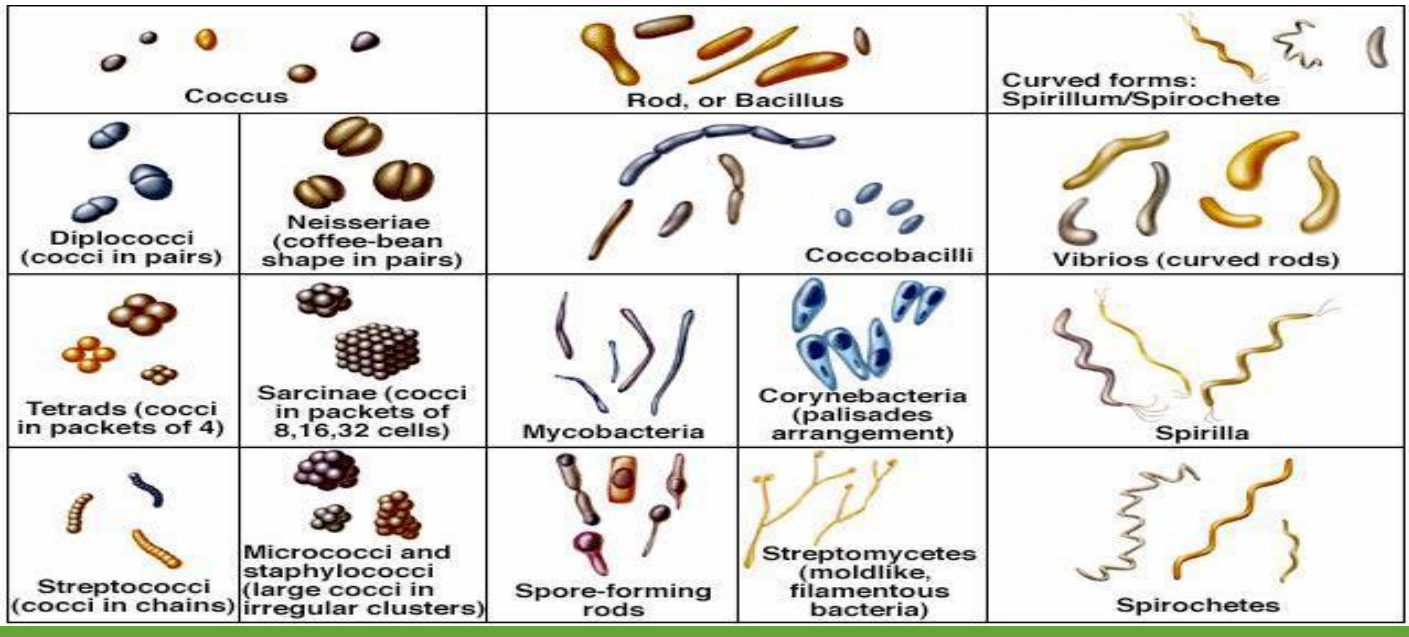
Gram stain





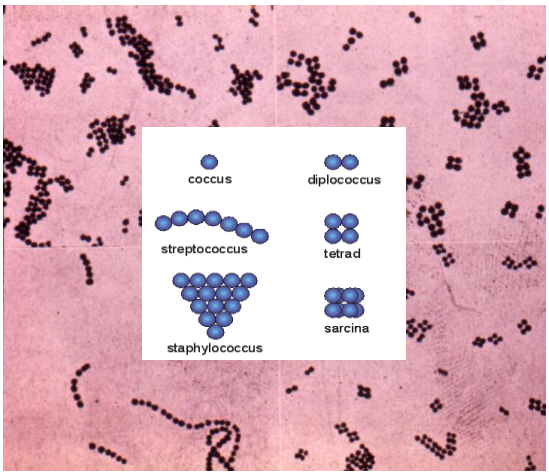


Bacterial shapes and arrangements

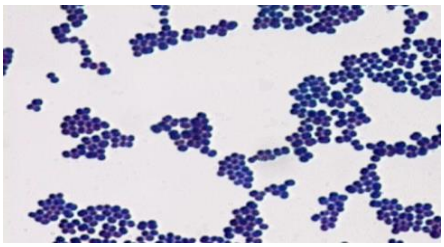


Gram positive bacteria

- cocci
  - Staphylococcus (catalase+)
    - *S. aureus* coagulase+
    - coagulase–
      - *S. epidermidis* — Novobiocin-sensitive
      - *S. saprophyticus* — Novobiocin-resistant
  - Steptococcus (catalase–)
    - β-hemolytic (clear)
      - *pyogenes* — Group A, bacitracin-sensitive
      - *agalactiae* — Group B, bacitracin-resistant
    - γ-hemolytic
      - *Enterococcus* — *E. faecalis*, *E. faecium*
    - α-hemolytic (green)
      - *pneumoniae* — optochin-sensitive, bile-soluble, capsule=> quellung+
      - Viridans — *mutans, sanguis* optochin-resistant, not bile-soluble, no capsule
- bacilli
  - *Corynebacterium*
  - *Clostridium*
  - *Listeria*
  - *Bacillus*

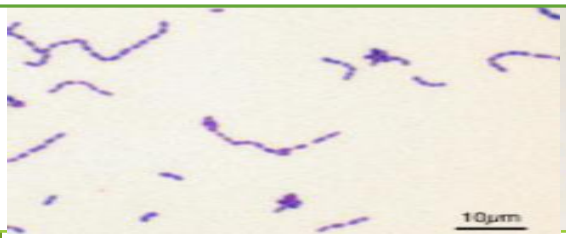


Q. Describe this organism?



Gram positive cocci arrange in clusters Staphylococcus

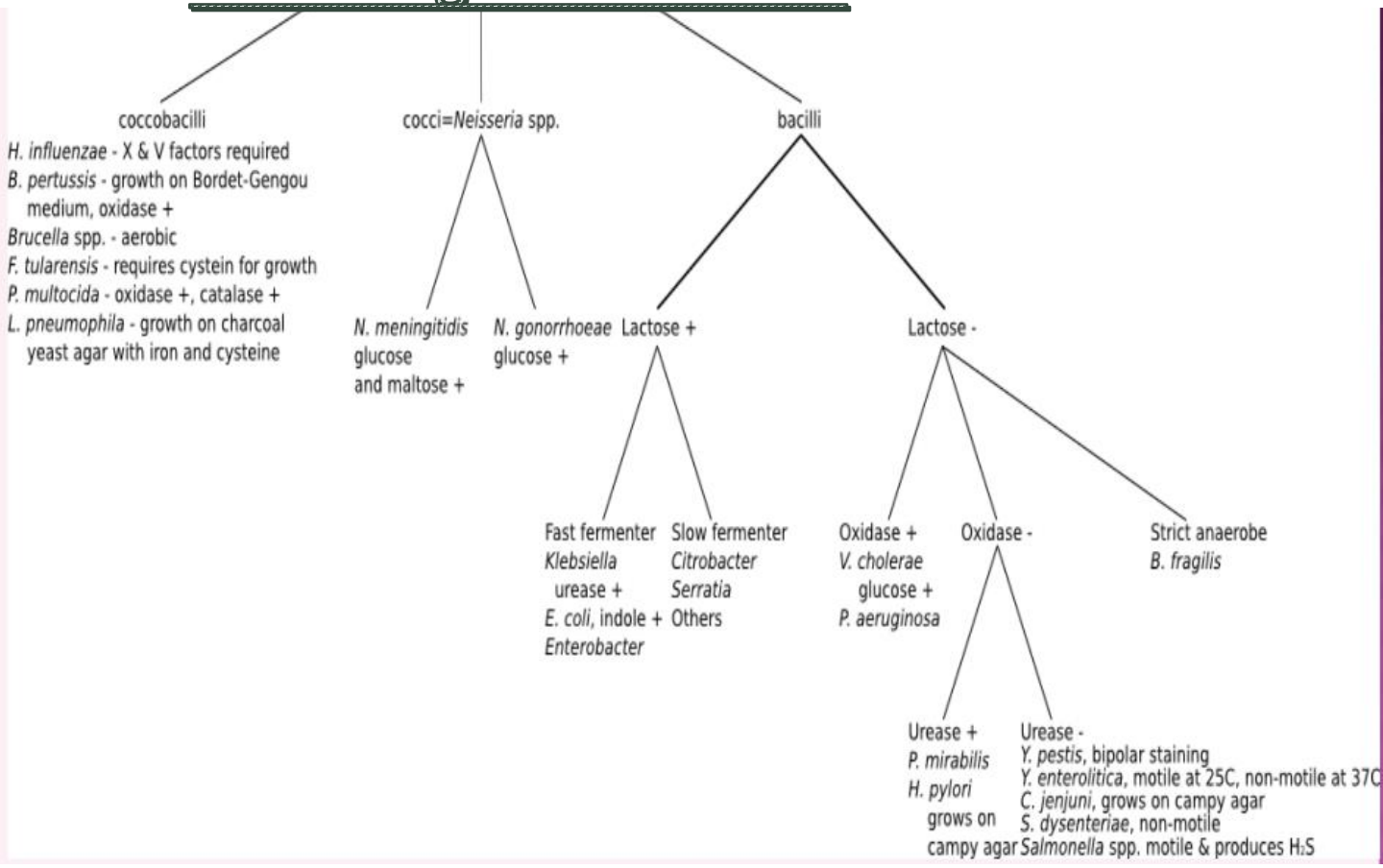
Gram positive cocci arrange in chain Streptococcus



Gram stain	**positive**	**positive**
the shape	cocci	cocci
The arrangement	chain	clusters
Ex:	Streptococci	Staphylococci
RX (Prescription)	Penicillin Cephalosporin	cloxacillin Cephalosporin if MRSA → vancomycin

BACTERIOLOGY

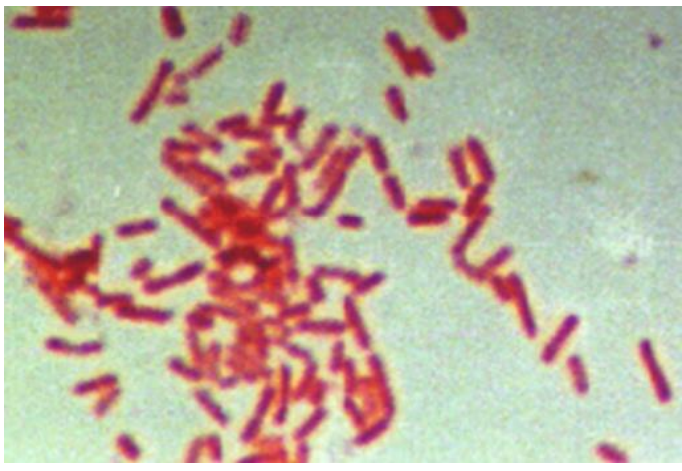
Gram negative bacteria



Gram negative cocci (Diplococci)

e.g Neisseria

Describe the Gram stain of this organism?
Describe its shape ?



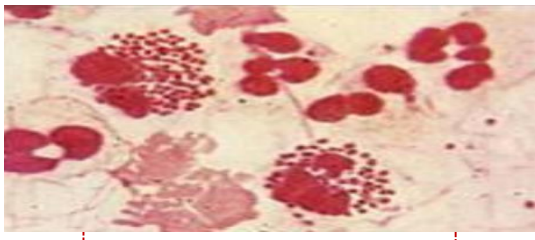
Gram negative bacilli (rods)

e.g E. coli Salmonella

BACTERIOLOGY

Case1:

Following is the Gram-stained smear of from urethra of a 25 –year old male complaining of urethral discharge



Describe the Gram stain of the intracellular bacteria ?

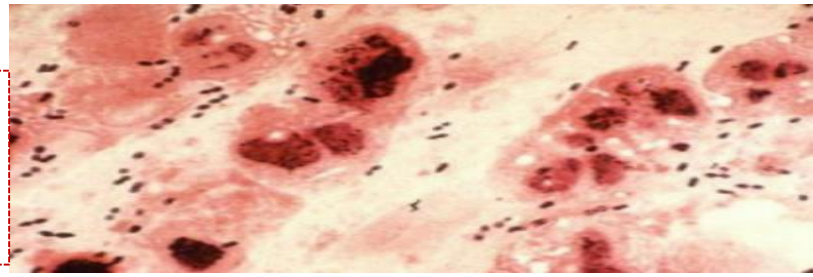
Describe the shape of the bacteria ?

-Gram negative
-cocci (diplococci)

Case2:

A gram-stained smear of a CSF sample from a 3 year old child seen in the emergency department presenting with fever and neck stiffness.

Describe what you see ?



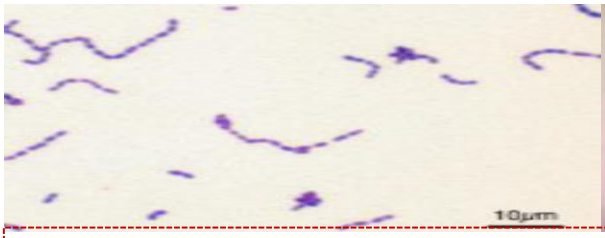
Gram-positive diplococci & pus(neutrophils) cells
Streptococcus pneumoniae

Case3:

This is a bacterium isolated from a child with sore throat and tonsillitis .

A: Describe the Gram stain

B: Describe the shape and arrangement of the bacteria?



Gram positive Cocci in chains

Case4:

Following is the Gram stained smear of an organism isolated from a wound infection.

Describe what you see in this slide ?
What is the likely organism ?

Gram-positive cocci in clusters
Staphylococcus aureus

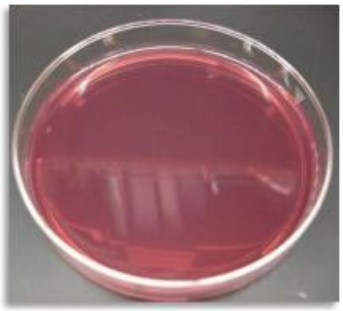
BACTERIOLOGY

Bacterial Culture Media

Type of Media	Purpose
Selective	Suppression of unwanted microbes; encouraging desired microbes.
Differential	Differentiation of colonies of desired microbes from others.
Enrichment	Similar to selective media but designed to increase number of desired microbes to detectable levels.

Blood agar

This is a general culture medium used for culture of bacteria.

General culture medium (Blood Agar)	Enriched medium (Chocolate Agar)
	
Differential medium (MacConkey Agar)	**Selective medium (Thiosulphate citrate bile salt sucrose TCBS)**
	

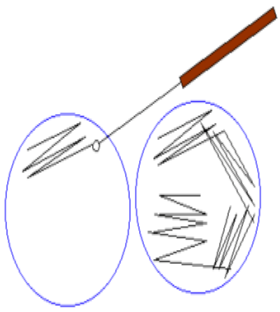
BACTERIOLOGY

Bacterial Culturing

Inoculation | Streaking | Incubation

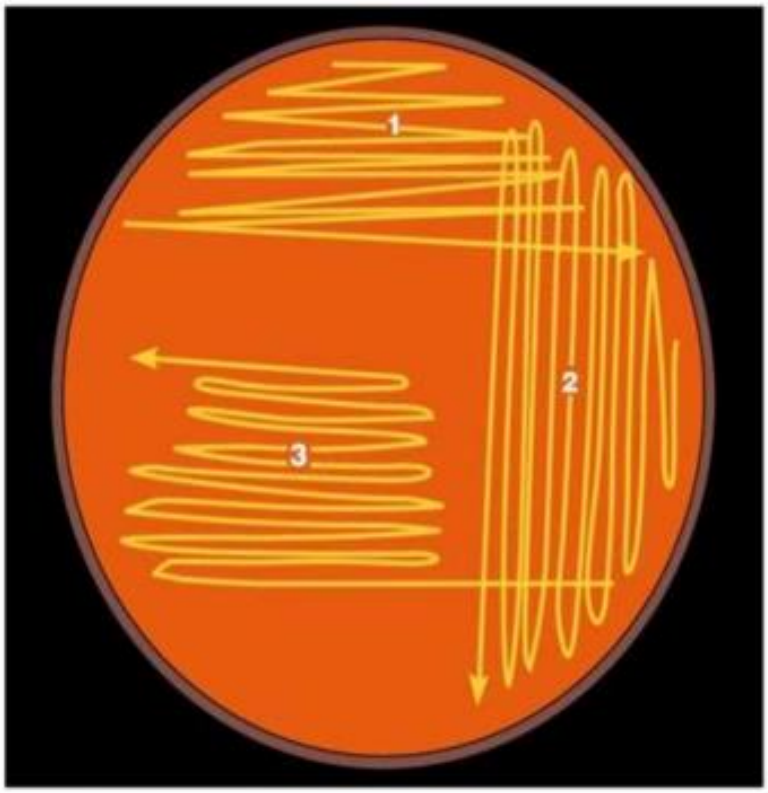
1-INOCULATION

2-STREAKING

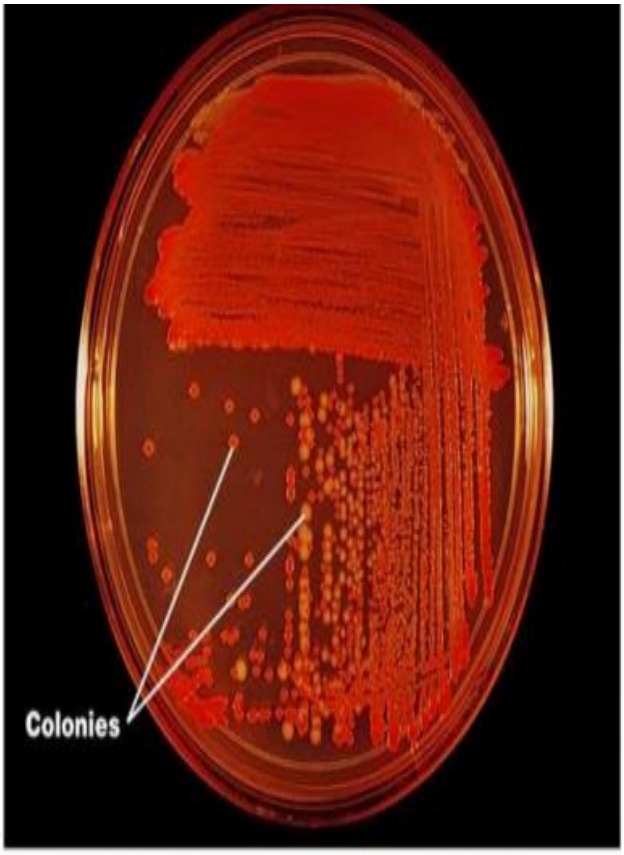


3-INCUBATION

Laboratory Incubator



(a) The direction of streaking is indicated by arrows. Streak series 1 is made from the original bacterial culture. The inoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns.



(b) In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and yellow, have been obtained.

BACTERIOLOGY

GRAM POSITIVE COCCI

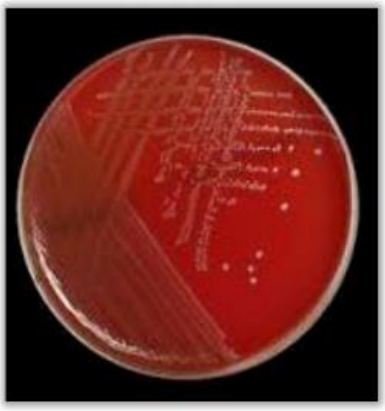
Note: *Strep. viridans* are alpha hemolytic and negative for all the tests below

Catalase

- (+) *Staphylococcus* (Clusters) → **Coagulase**
  - (+) *S. aureus* — 6 hemolytic, mannitol yellow
  - (−) *S. epidermidis* — nonhemolytic (usually), mannitol white
- (−) *Streptococcus* (pairs & chains) → **Hemolysis**
  - (1) BETA: Bacitracin (+) → *S.pyogenes* (group A)
  - CAMP/Hippurate (+) → *S. agalactiae* (group B)
  - (2) ALPHA: Optochin/Bile Solubility (+) → *S. pneumoniae*
  - (3) GAMMA: Bile Esculin (+) 6.5% NaCl (+) → Group D* *Enterococcus*
  - Bile Esculin (+) 6.5% NaCl (−) → Group D* *Non-Enterococcus*

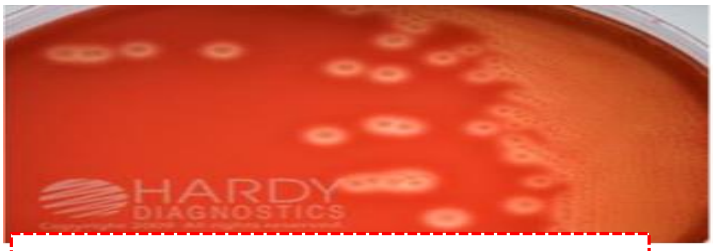
Hemolytic reaction

Identification of streptococci by hemolytic reaction

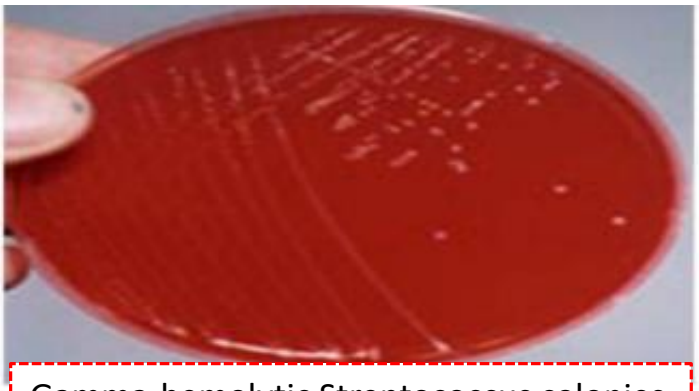
		
Colonies are surrounded by clear zone of hemolysis complete hemolysis	***Colonies are surrounded by partial hemolysis with greenish color***	***No haemolysis***
Beta-hemolytic Streptococcus colonies *St. pyogenes*	**Alpha-hemolytic Streptococcus colonies** St. pneumoniae	**Gamma-hemolytic Streptococcus colonies** ***Enterococcus faecalis***

BACTERIOLOGY

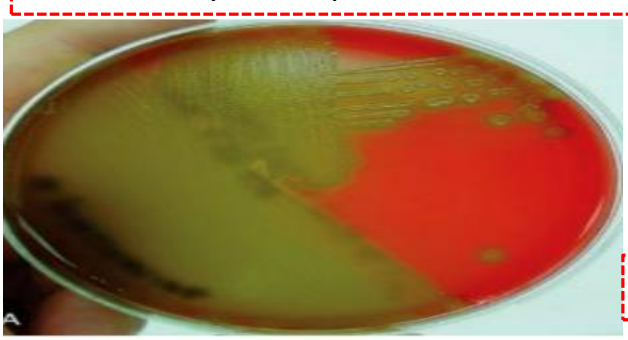
Identification of streptococci by Hemolytic reaction



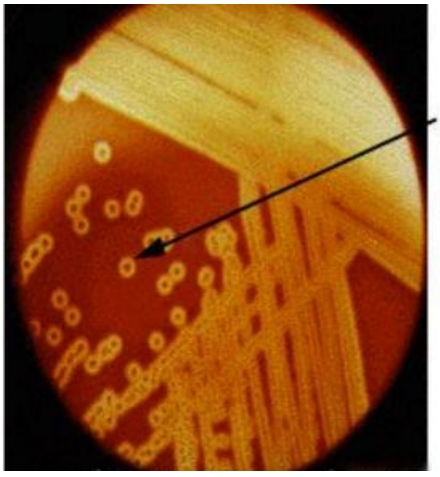
Beta-hemolytic Streptococcus colonies



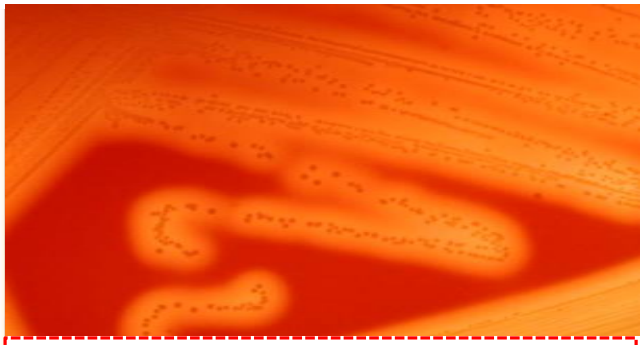
Gamma-hemolytic Streptococcus colonies



Alpha-hemolytic Streptococcus colonies



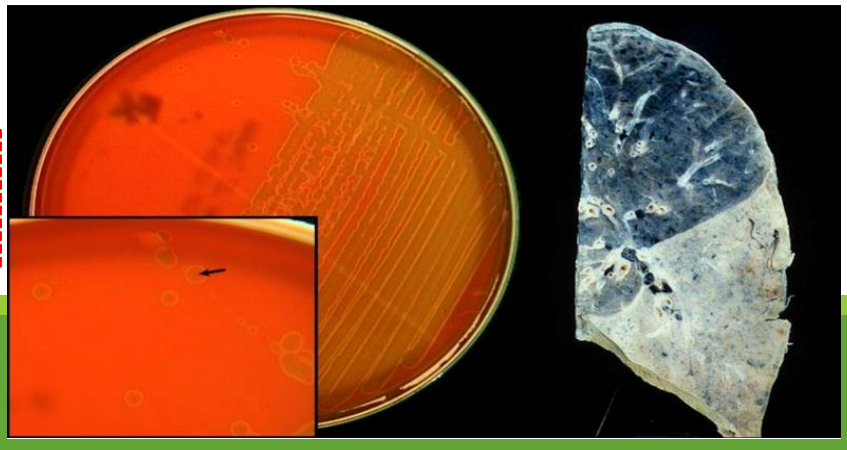
Note the clear zone of beta-hemolysis surrounding the *Streptococcus* colonies when grown on blood agar.



This is a blood agar growing beta hemolytic streptococci.

Case:

This culture was grown from a sputum specimen of a 60 year old man complaining of cough, fever and chest pain



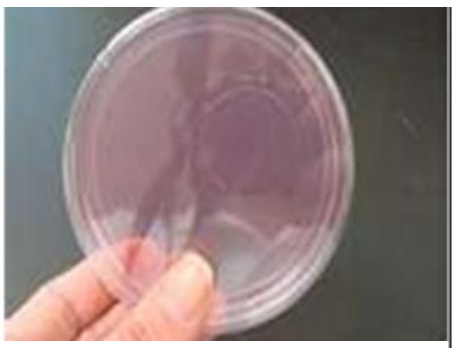
Alpha-hemolytic streptococci on blood agar

BACTERIOLOGY

Gram negative bacteria

- **coccobacilli**
  - *H. influenzae* - X & V factors required
  - *B. pertussis* - growth on Bordet-Gengou medium, oxidase +
  - *Brucella* spp. - aerobic
  - *F. tularensis* - requires cystein for growth
  - *P. multocida* - oxidase +, catalase +
  - *L. pneumophila* - growth on charcoal yeast agar with iron and cysteine
- **cocci=*Neisseria* spp.**
  - *N. meningitidis* glucose and maltose +
  - *N. gonorrhoeae* glucose +
- **bacilli**
  - Lactose +
    - Fast fermenter: *Klebsiella* urease +, *E. coli*, indole +, *Enterobacter*
    - Slow fermenter: *Citrobacter*, *Serratia*, Others
  - Lactose -
    - Oxidase +: *V. cholerae* glucose +, *P. aeruginosa*
    - Oxidase -
      - Urease +: *P. mirabilis*, *H. pylori* grows on campy agar
      - Urease -: *Y. pestis*, bipolar staining; *Y. enterolitica*, motile at 25C, non-motile at 37; *C. jenjuni*, grows on campy agar; *S. dysenteriae*, non-motile; *Salmonella* spp. motile & produces H_2S
    - Strict anaerobe: *B. fragilis*

MacConKey's agar (Deferential Medium)



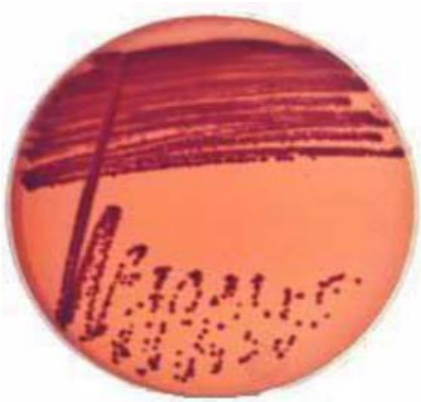
MacConkey's agar

Lactose fermenting colonies

E. coli

non-lactose fermenting colonies

salmonella

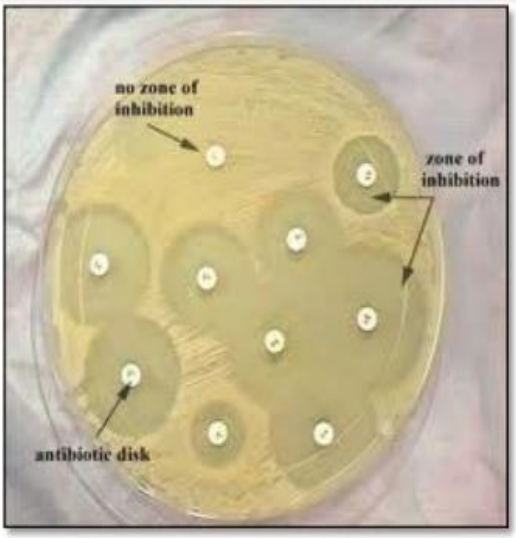


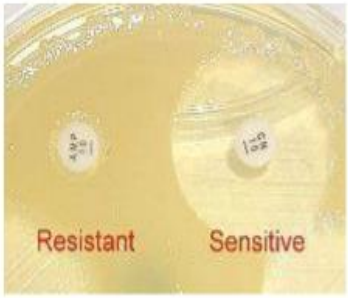
BACTERIOLOGY

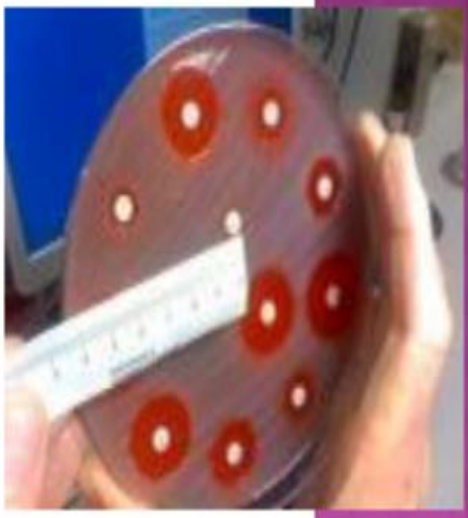
Biochemical testings

To confirm the identity of bacteria.

Antibiotic susceptibility testings



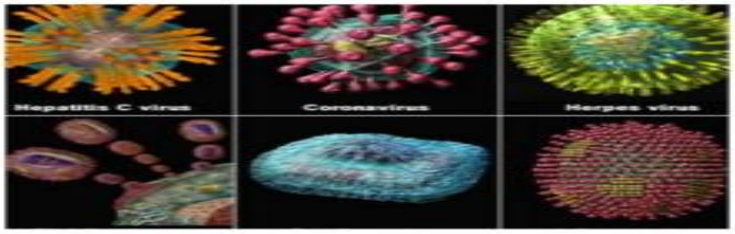




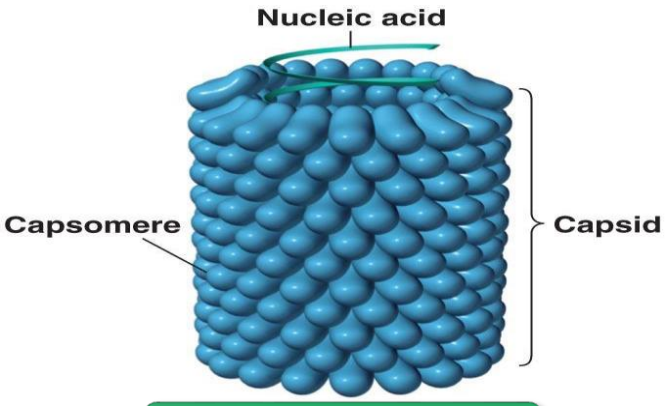
Automated instrument for identification and susceptibility testings

VITEK

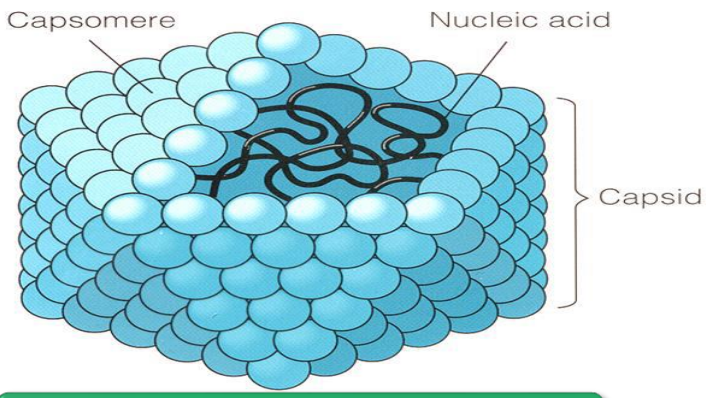
VIROLOGY



VIRAL STRUCTURE

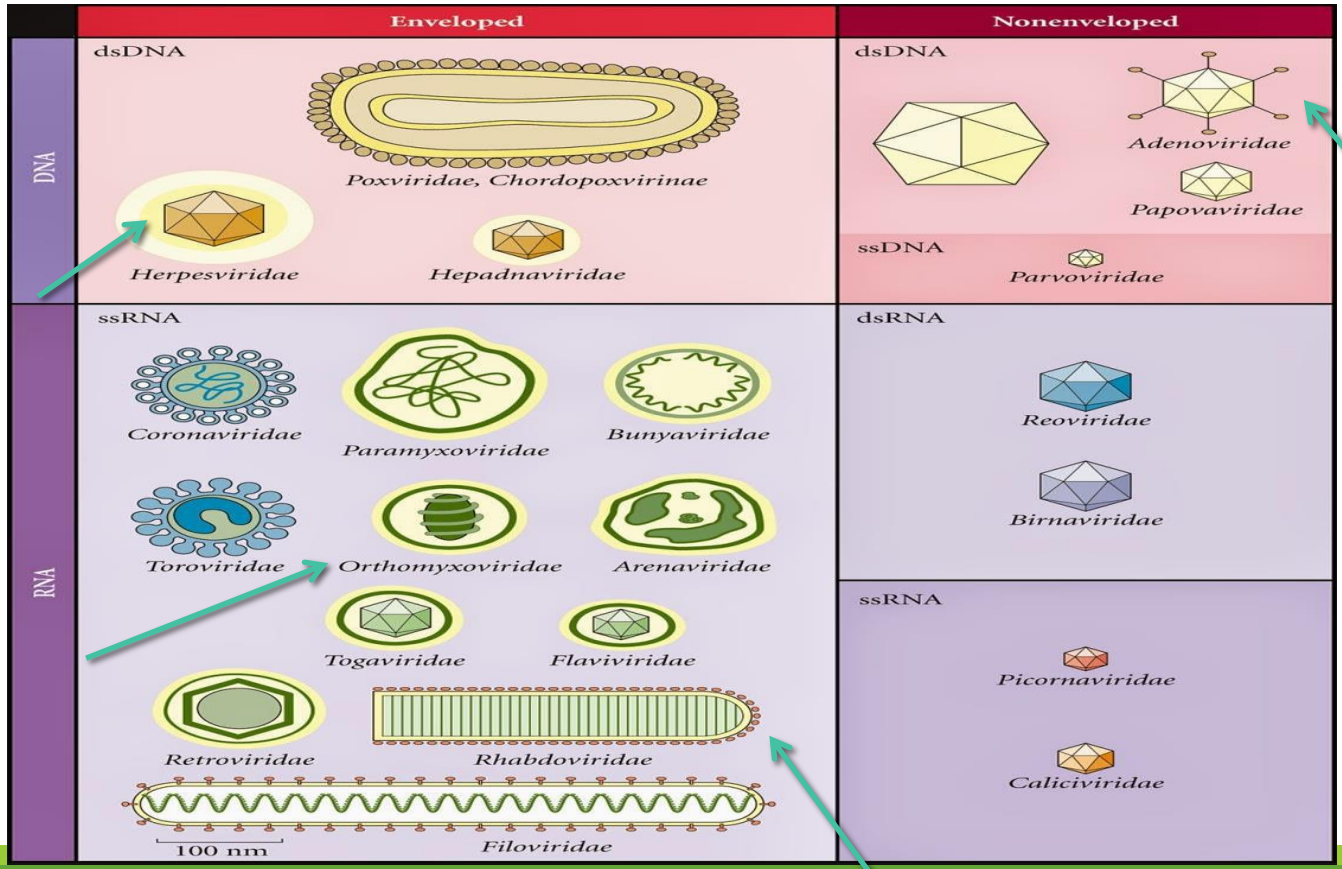


Helical Virus

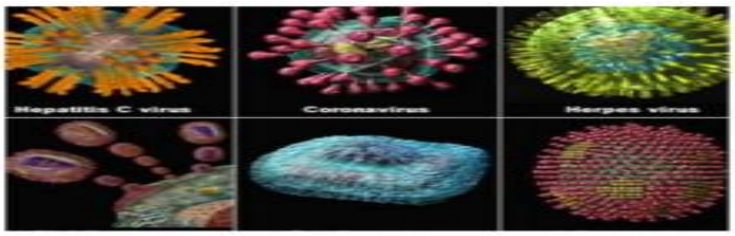


Icosahedral Virus

VIRAL CLASSIFICATION



VIROLOGY



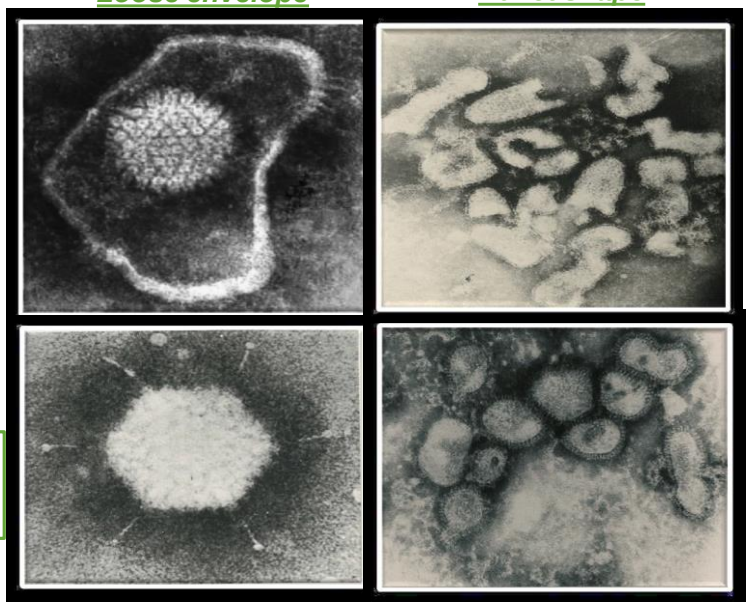
VIRAL ELECTRON MICROGRAPH

Herpes virus: Herpesviridae

1. Enveloped virus
2. Icosahedral capsid
3. d.s DNA genome

Adenovirus : Adenoviridae

1. Nonenveloped virus
2. Icosahedral capsid
3. d.s DNA genome



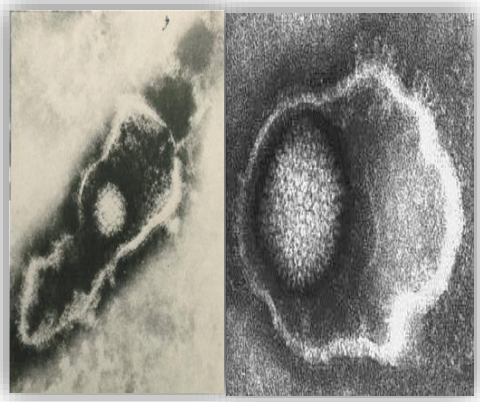
Rabies virus: Rhabdoviridae

1. Enveloped virus
2. Helical capsid
3. s.s RNA genome

Influenza Viruses: Orthomyxoviridae

1. Enveloped V & spikes
2. Helical capsid
3. Segmented s.s RNA

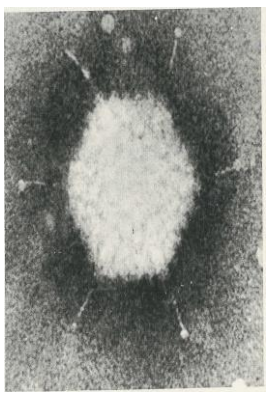
These are electron micrographs of a virus



Q1: Name this virus ?
Herpes virus

Q2: Describe its structure?

1. Enveloped virus
2. Icosahedral capsid
3. d.s DNA genome



Q1: Name this virus ?
Adenovirus

Q2: Describe its structure ?

1. *Nonenveloped virus, with fiber*
2. *Icosahedral capsid*
3. *d.s DNA genome*

Q1: Name this virus?
Influenza Viruses

Q2: Describe its structure ?

1. Enveloped Virus with spikes ,
2. Helical capsid
3. Segmented s.s RNA

Q1: Name this virus?
Rabies virus

Q2: Describe its structure ?

1. *Enveloped virus*
2. *Helical capsid*
3. *s.s RNA genome*

PARASITOLOGY

Classification of Parasites

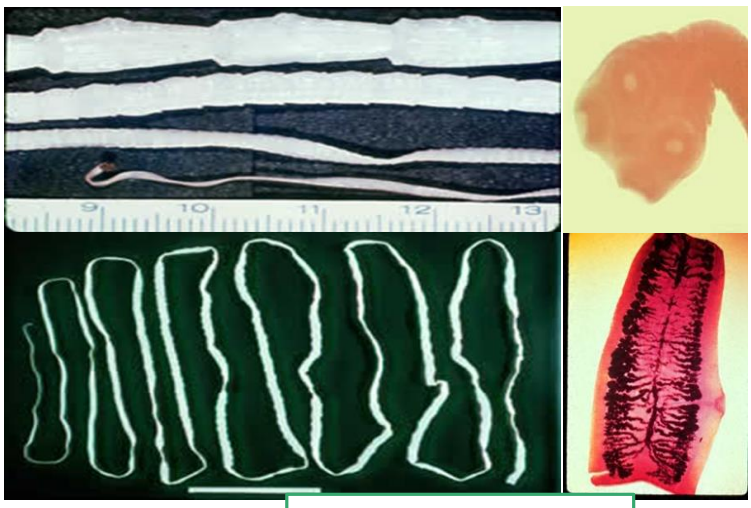
Protozoa	Helminthes
Unicellular **Single cell for all function**	**Mulicellular** **Specialized cells**
Amoebae: move by pseudopodia. **Flagellates:** move by flagella. **Ciliates :** move by cilia **Apicomplexia** (sporozoa) Tissue parasites	**A- *Round worms =*** **Nematodes** cylindrical, un-segmented(Ascaris) **B- *Flat worms*** **1-Trematodes:** leaf-like, un-segmented. **2-Cestodes:** tape-like, segmented

1- Ascaris lumbricoides(round warm)

Ascaris adult

Round worm , un-segmented

2- taetnia saginata (**cestodes**)



Tape-like worm segmented.

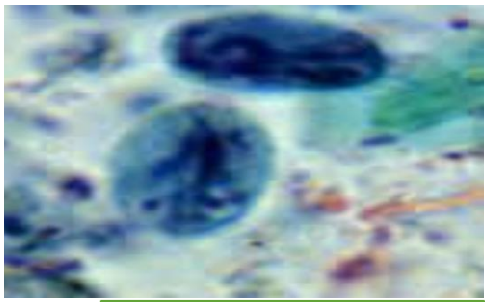
3- fasciolahepatica(**trematodes**)

flat worm , un-segmented ,leaf like

4- giardia lamblia trophozoite

Two nuclei,
each with central karyosome
Four pairs of flagella

5- giardia lamblia **cyst**



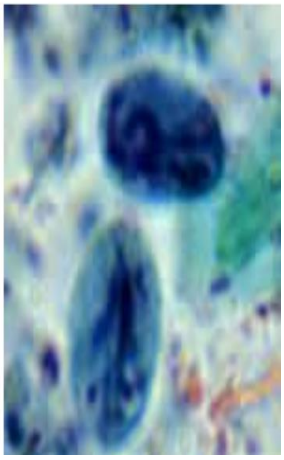
- Mature, infective cyst, containing 4 nuclei
- Note a straight axoneme running longitudinally

PARASITOLOGY

Following is the microphotograph of an organism found in stools

Name the Organism

Giardia lamblia



What is the Stage?

Cyst stage

Following is the microphotograph of an organism found in the upper part of the small intestine .

Name the Organism

Giardia lamblia

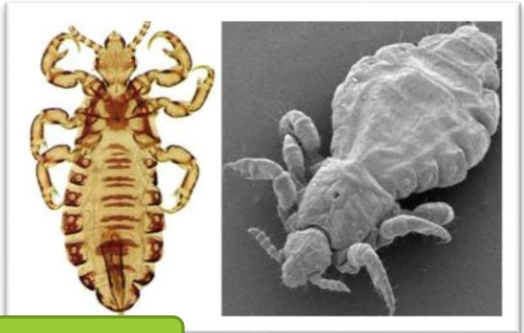
What is the Stage?

Trophozoite stage

ARTHROPODS OF MEDICAL IMPORTANCE

Class *Insecta* الحشرات	Class *Arachnida* العناكب	Class *Crustacea* القشريات
• Muscid flies:housefly,Tsetse fly • Myiasis-producing flies . • Mosquitoes البعوض: *Anopheles, Aedes Culex* • Sandfly ذباب الرمل (*Phlebotomus*) • Black fly (*Simulium*) • Fleas البراغيث • Lice(*Pediculus,Phthirus*) القمل • Bugs: *Cimex, Triatoma* البق • Bees النحل	• Scorpions العقارب • Spiders العناكب • Ticks: القراد hard, soft • Mites السوس -*Sarcoptes scabiei*, -dust mites	• Water flea (*Cyclops*)

1-LICE ,Louse(singular) , Lice (pleural)
Pediculus humanus

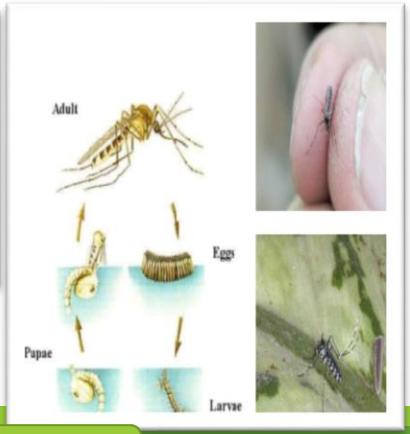


vector

2-Phlebotomus (sand fly)

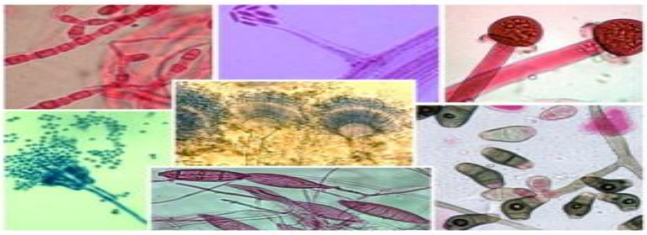
vector

3-Mosquitoes

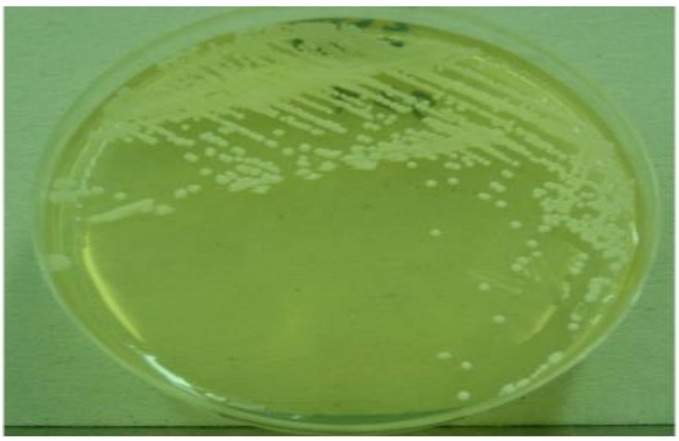


vector

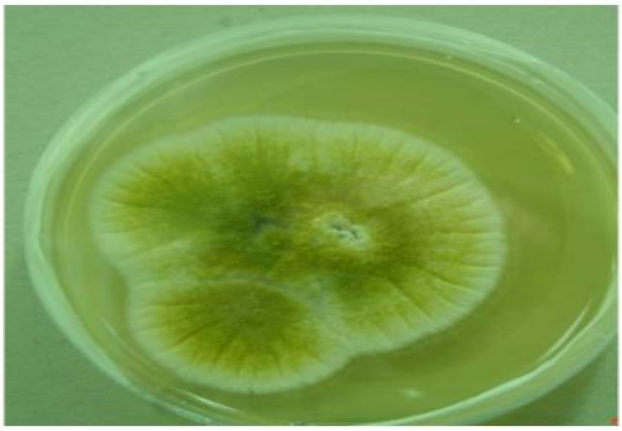
MYCOLOGY



Fungi can be divided to two types based on morphology



A



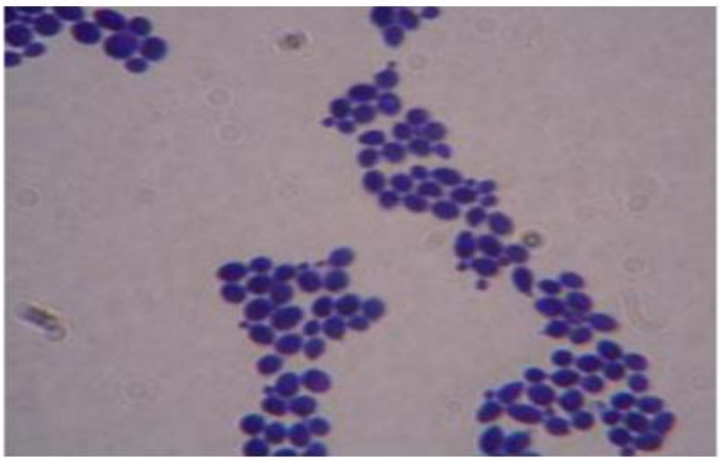
B

Based on morphology, name the two fungal structures in A and B?

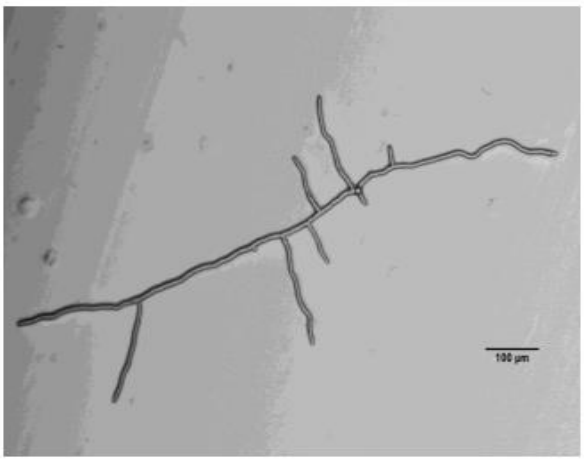
A: Yeast
e.g. *Candida*

B: Mould fungi
e.g. *Aspergillus*

Microscopic appearance of yeast and mould fungi



A



B

Name the two fungal structures in A and B?

A: Budding yeast cells
e.g. *Candida*

B: Branching Fungal hyphae
e.g. *Aspergillus*

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