

Core laboratory

Direct examination and culture



Gram staining



Automated
blood culture
monitoring



Diversified
culture
conditions



Phenotypic identification and antibiotic-susceptibility testing



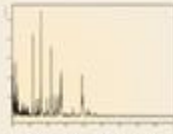
Manual biochemical
phenotype



Antibiogram



Automated biochemical
phenotype and antibiogram



MALDI-TOF MS



Phenotypic
microarray



Unidentified or
unusual bacterium

Molecular detection and identification



PCR

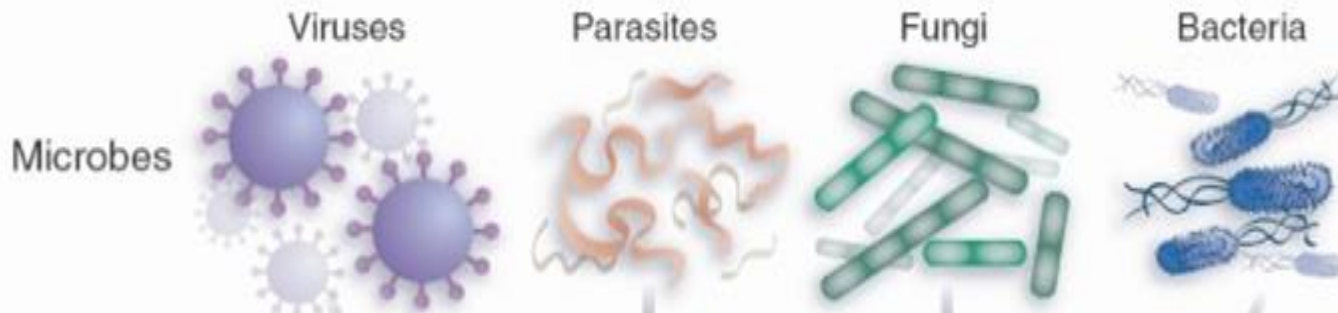


MICROBIOLOGY PRACTICAL CLASS

FOUNDATION BLOCK (2018)

Dr . Malak M. El-Hazmi

MICROBIOLOGY



Laboratory diagnosis of infectious diseases

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

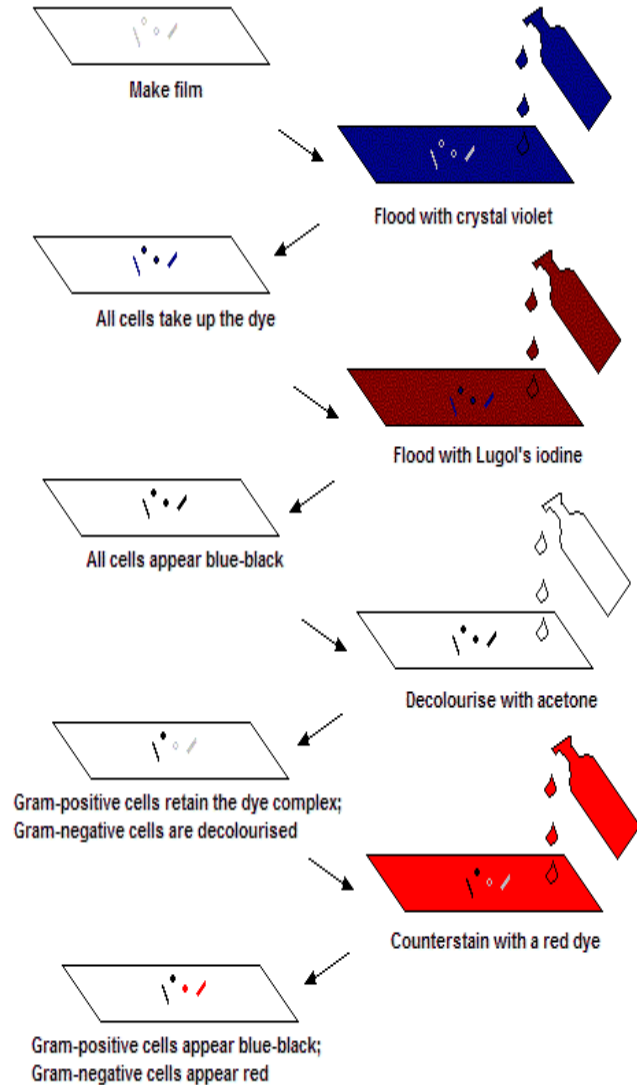
Types of specimens



BACTERIOLOGY



GRAM STAIN



G+ bacilli



G~ bacilli

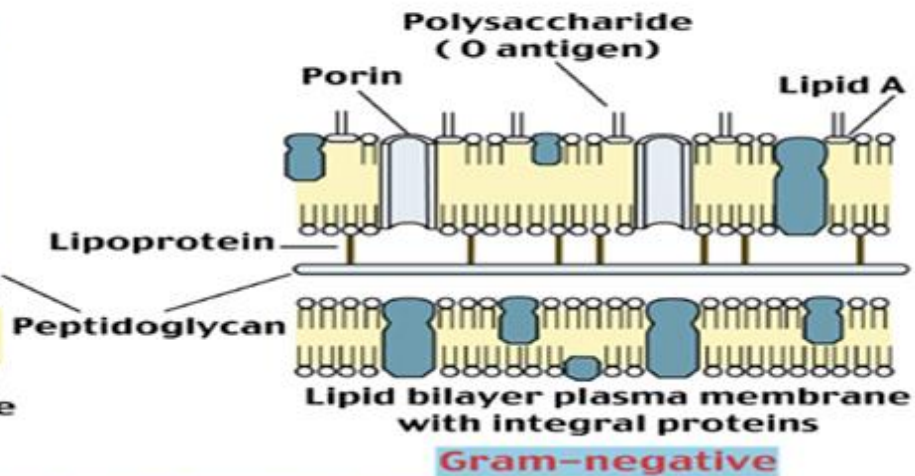
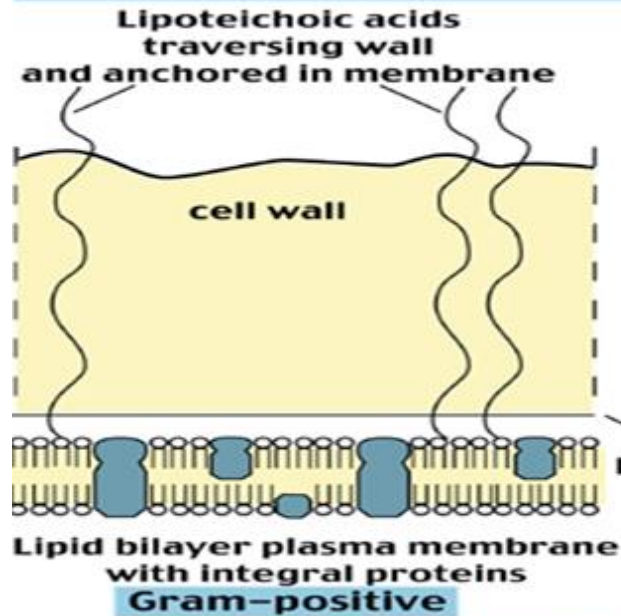
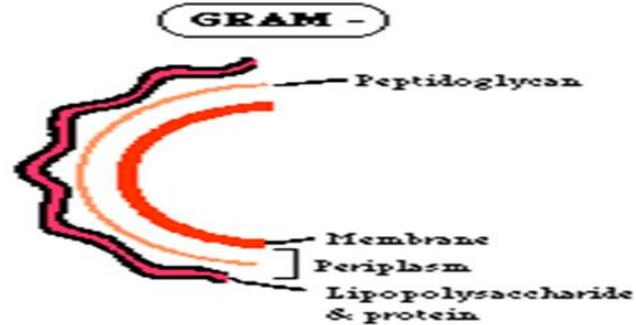
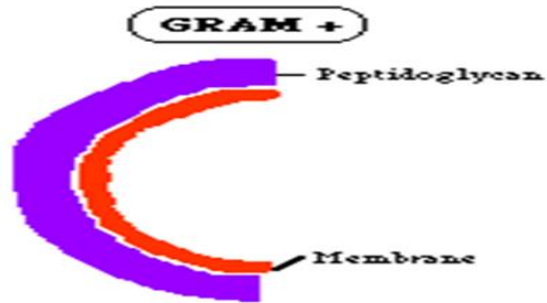


G+ cocci














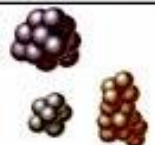





G- cocci

BACTERIAL CELL WALL

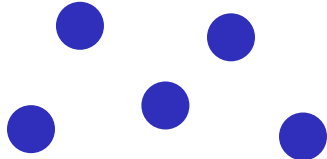


BACTERIAL SHAPES AND ARRANGEMENTS

 <p>Coccus</p>		 <p>Rod, or Bacillus</p>		 <p>Curved forms: Spirillum/Spirochete</p>
 <p>Diplococci (cocci in pairs)</p>	 <p>Neisseriae (coffee-bean shape in pairs)</p>	 <p>Coccobacilli</p>		 <p>Vibrios (curved rods)</p>
 <p>Tetrads (cocci in packets of 4)</p>	 <p>Sarcinae (cocci in packets of 8, 16, 32 cells)</p>	 <p>Mycobacteria</p>	 <p>Corynebacteria (palisades arrangement)</p>	 <p>Spirilla</p>
 <p>Streptococci (cocci in chains)</p>	 <p>Micrococci and staphylococci (large cocci in irregular clusters)</p>	 <p>Spore-forming rods</p>	 <p>Streptomycetes (moldlike, filamentous bacteria)</p>	 <p>Spirochetes</p>

GRAM STAIN

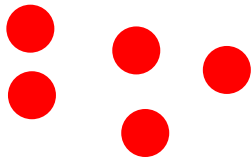
Gram-positive cocci



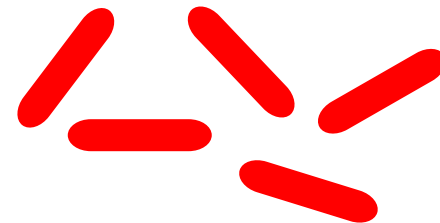
Gram-positive bacilli



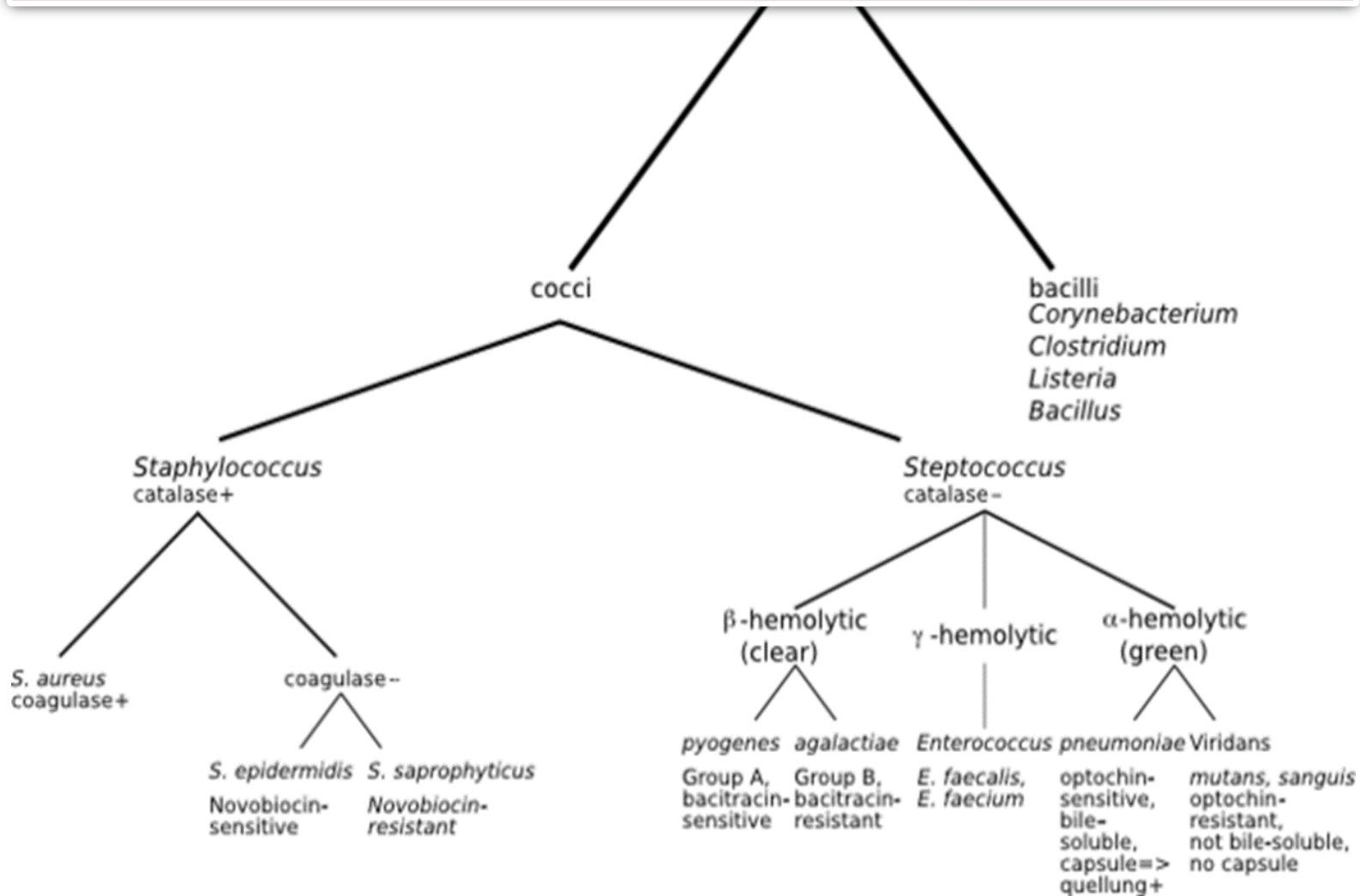
Gram-negative cocci



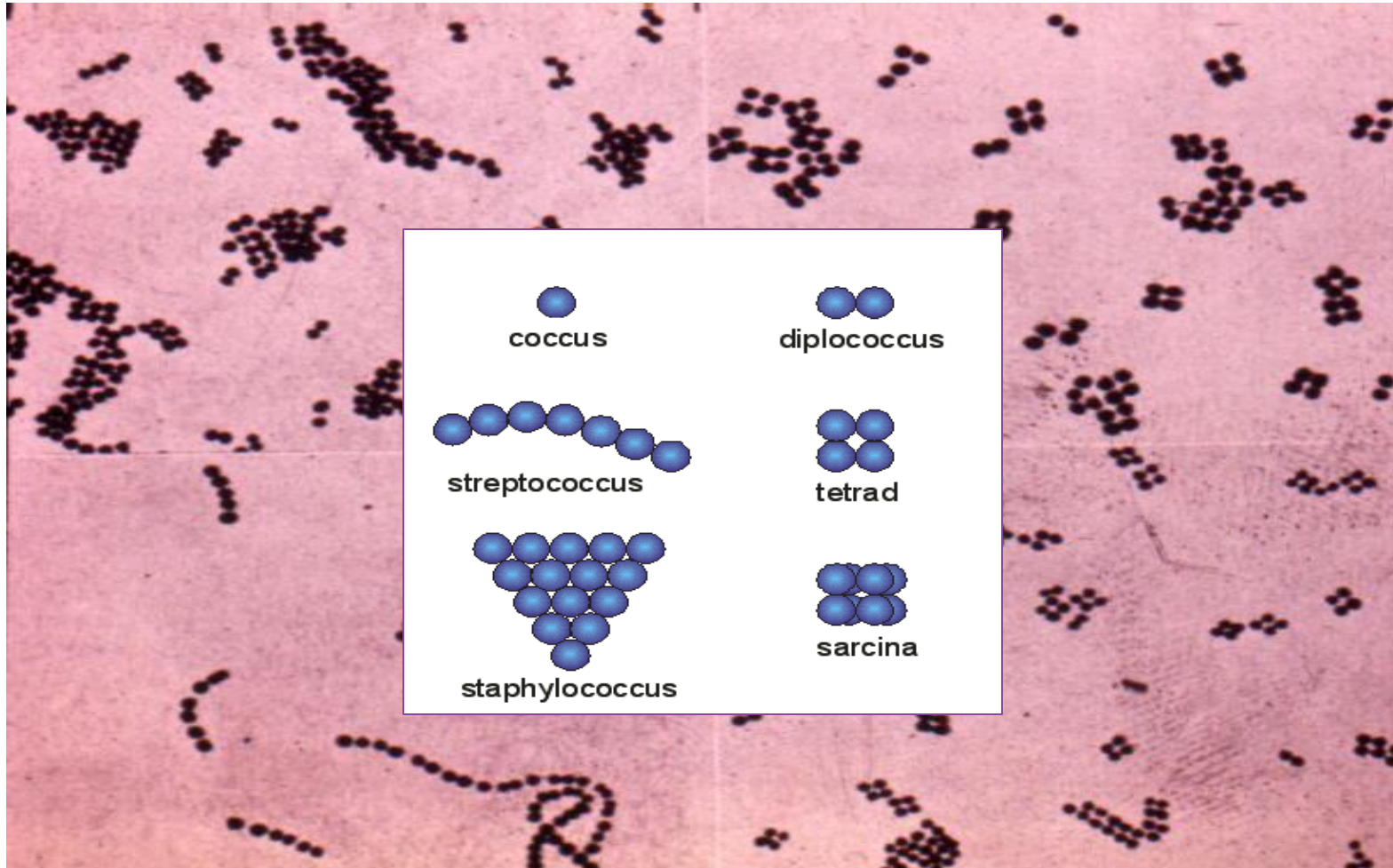
Gram-negative bacilli



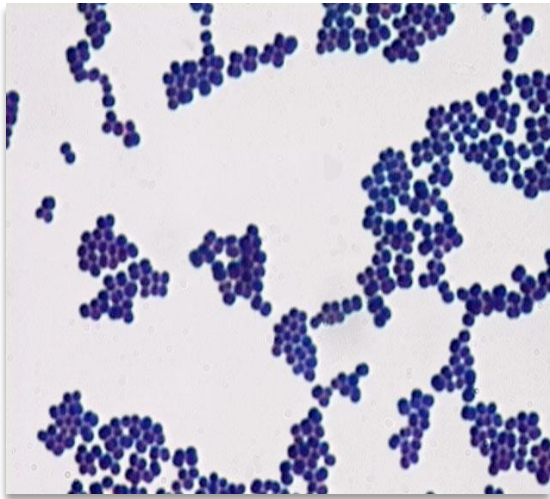
GRAM POSITIVE BACTERIA



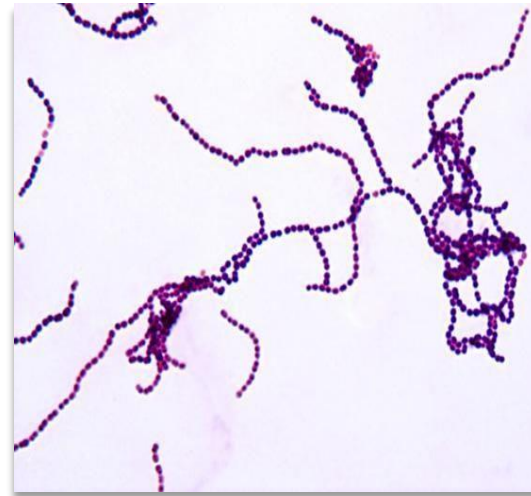
GRAM POSITIVE COCCI



GRAM POSITIVE COCCI



**Gram positive cocci
in clusters**
Staphylococcus



**Gram positive cocci
in chain**
Streptococcus

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

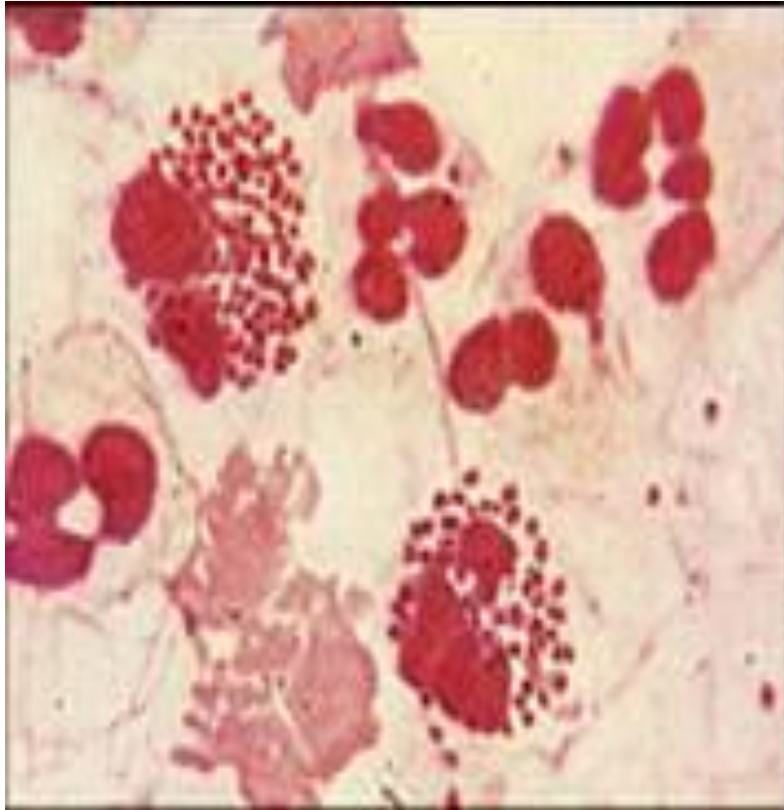
Oxidase -

Urease +
P. mirabilis
H. pylori
grows on
campy agar

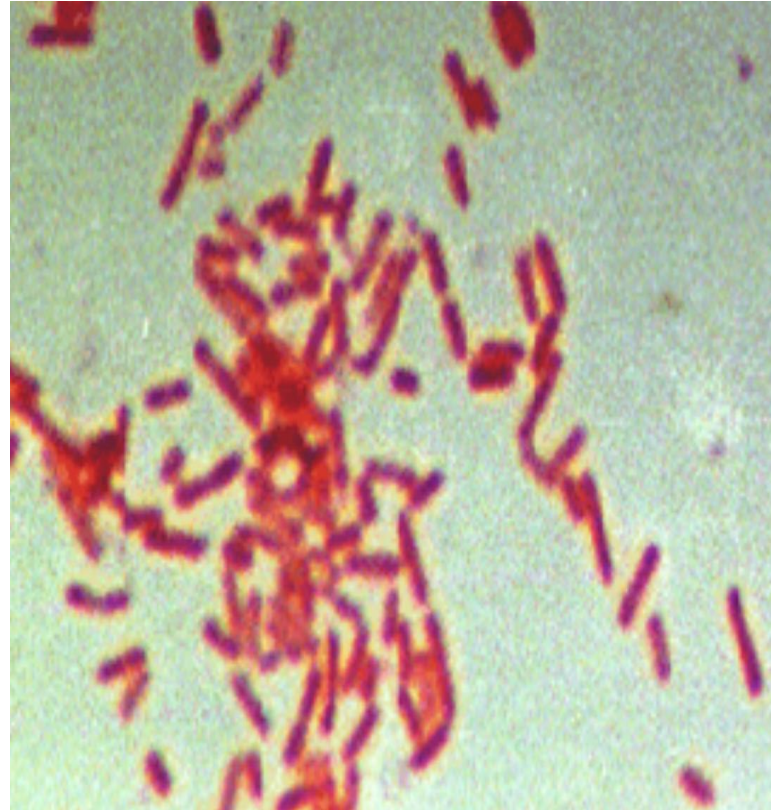
Urease -
Y. pestis, bipolar staining
Y. enterocolitica, motile at 25C, non-motile at 37C
C. jejuni, grows on campy agar
S. dysenteriae, non-motile
Salmonella spp. motile & produces H₂S

Strict anaerobe
B. fragilis

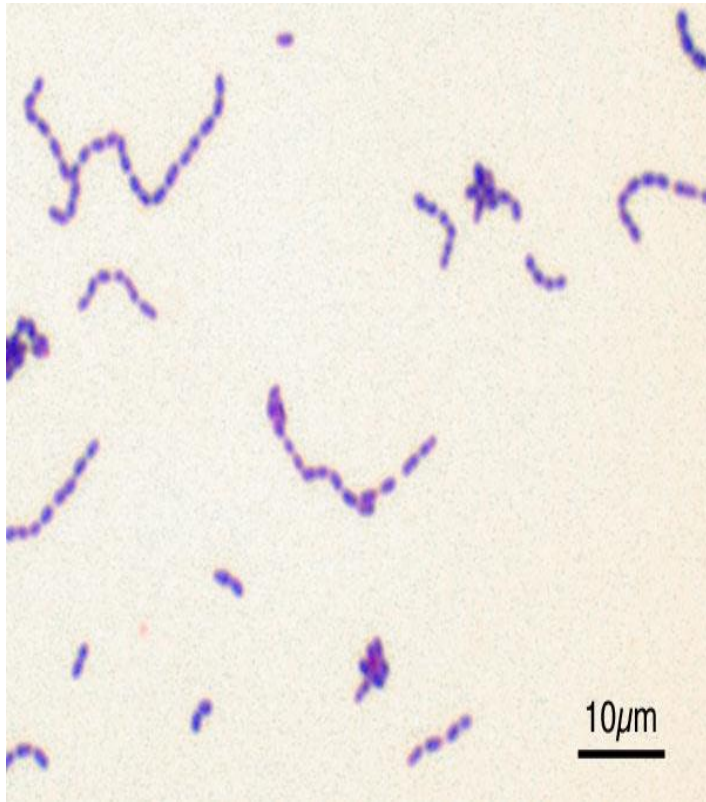
GRAM NEGATIVE BACTERIA



Gram negative cocci
(Diplococci)
e.g Neisseria



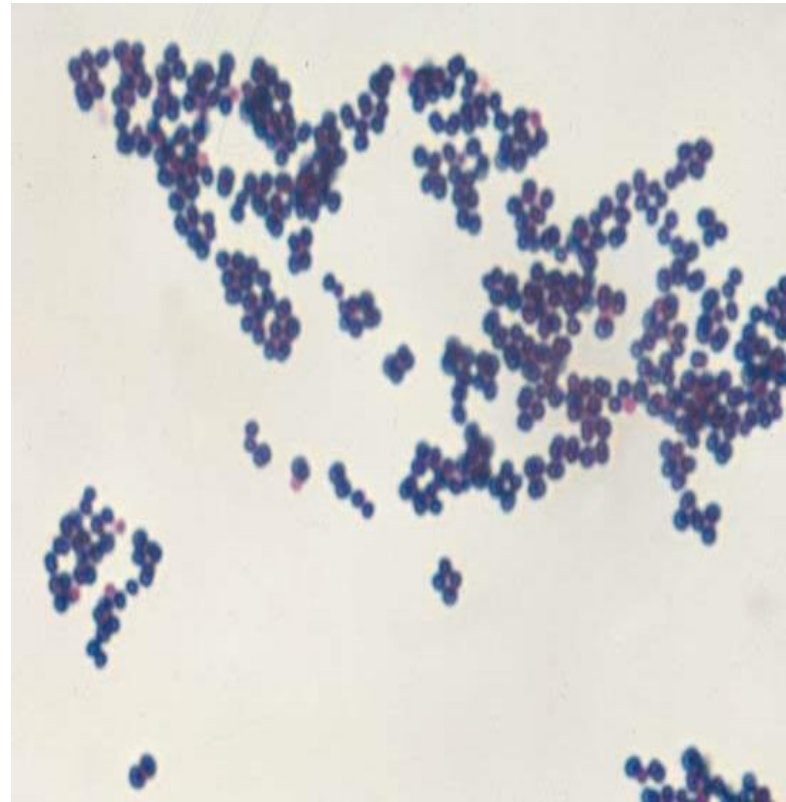
Gram negative bacilli
e.g E. coli
Salmonella



Gram positive cocci in chain
Streptococci

Penicillin
Cephalosporin

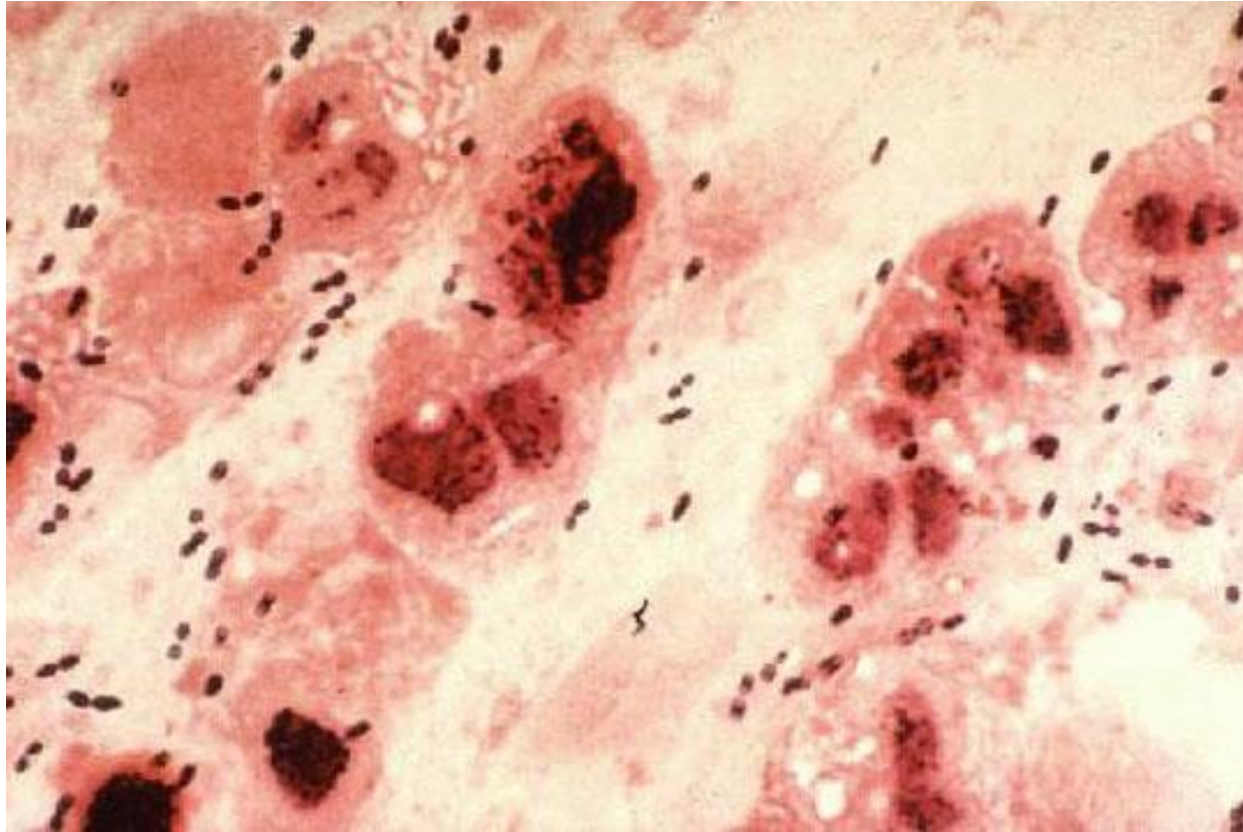
Rx



Gram positive cocci in clusters
Staphylococci

cloxacillin
Cephalosporin
if MRSA → vancomycin

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.



Gram-positive diplococci & pus cells
Streptococcus pneumoniae



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

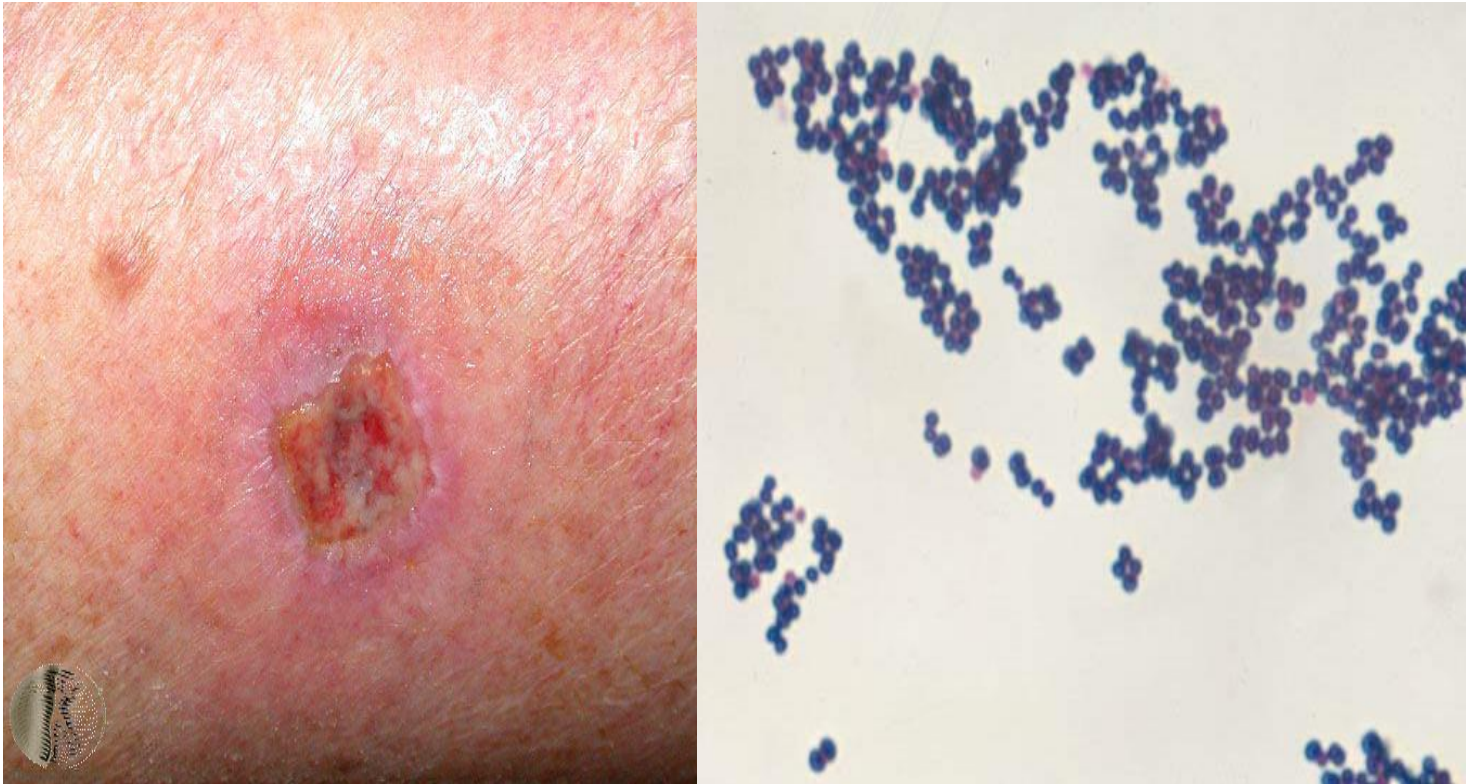
A: Describe the Gram stain

Gram positive

B: Describe the shape and arrangement of the bacteria

Cocci in chains

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



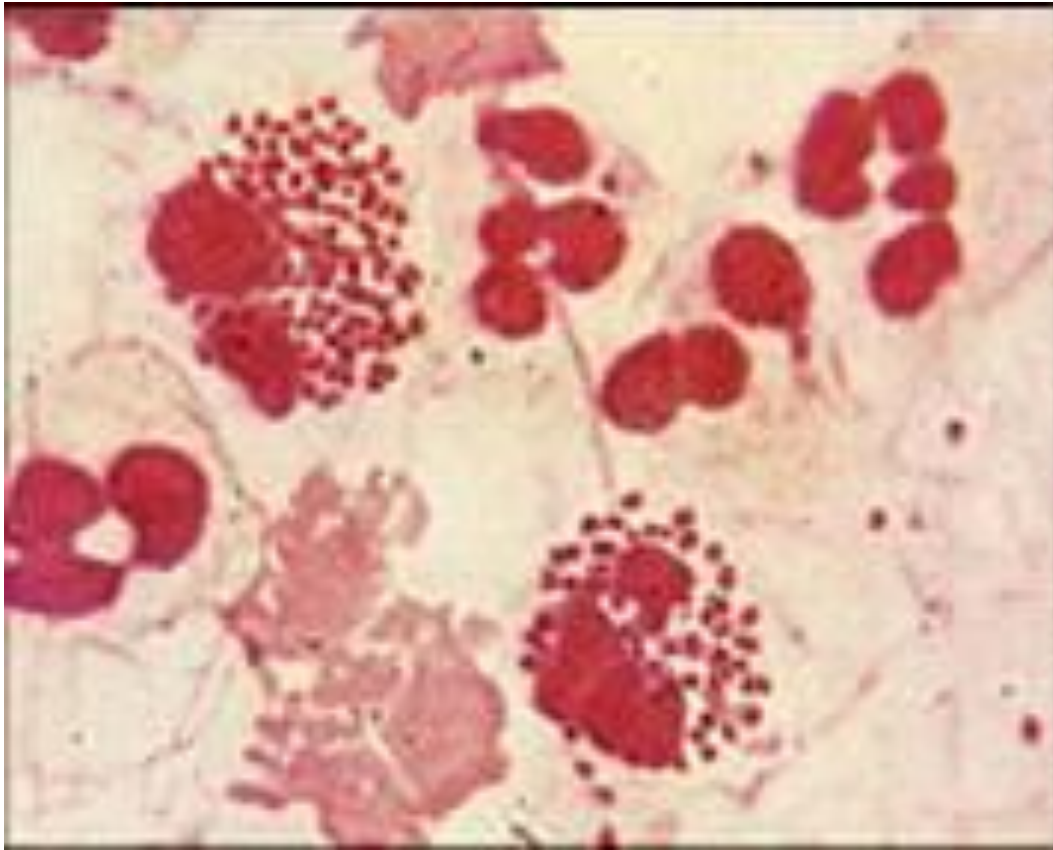
Describe what you see in the slide above.

Gram-positive cocci in clusters

What is the likely organism ?

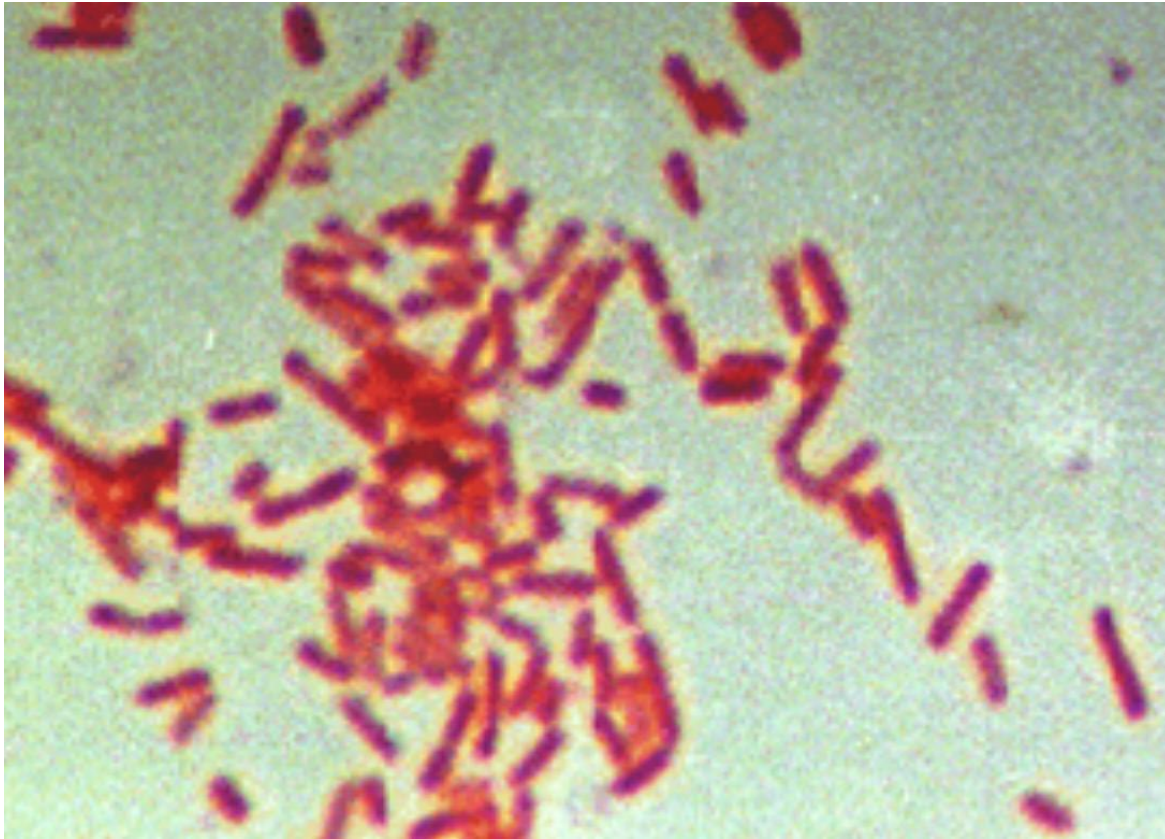
Staphylococcus aureus

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 Gram negative

Describe the shape of the bacteria cocci (diplococci)



Describe the Gram stain of this organism:

Gram negative

Describe its shape

bacilli (rods)

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.

BACTERIAL CULTURE MEDIA

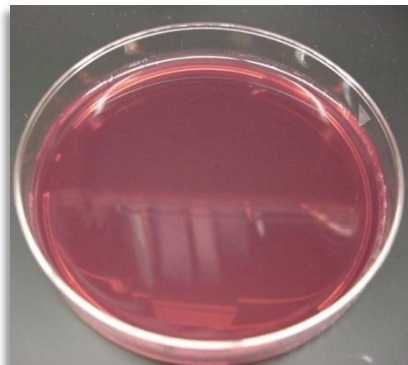
General culture medium
(Blood Agar)



Enriched medium
(Chocolate Agar)



Differential medium
(MacConkey Agar)

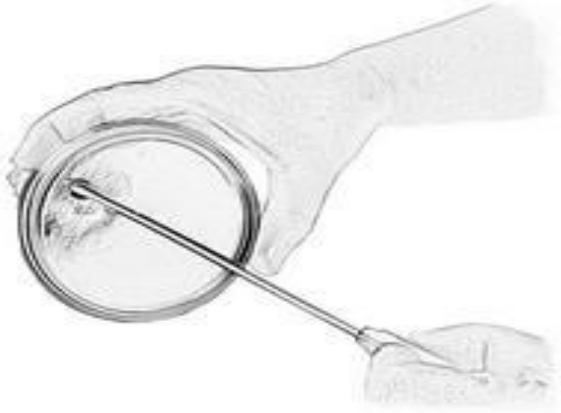


Selective medium
**(Thiosulphate citrate bile salt sucrose
TCBS)**



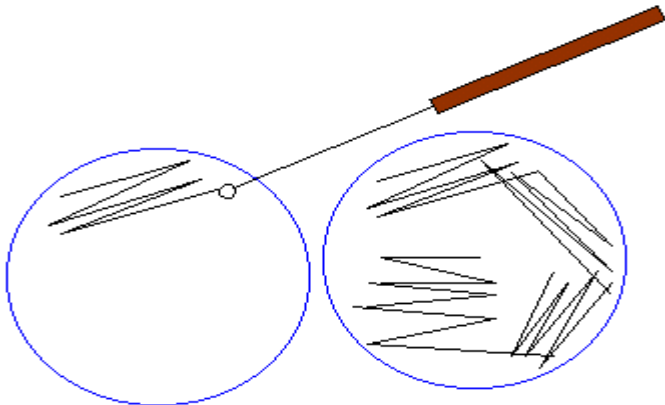
BACTERIA CULTURING

1-INOCULATION

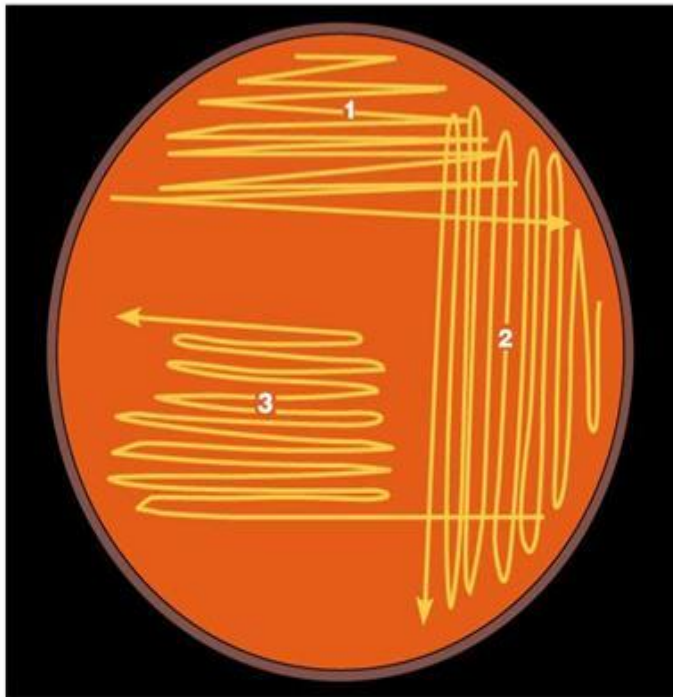


3-INCUBATION

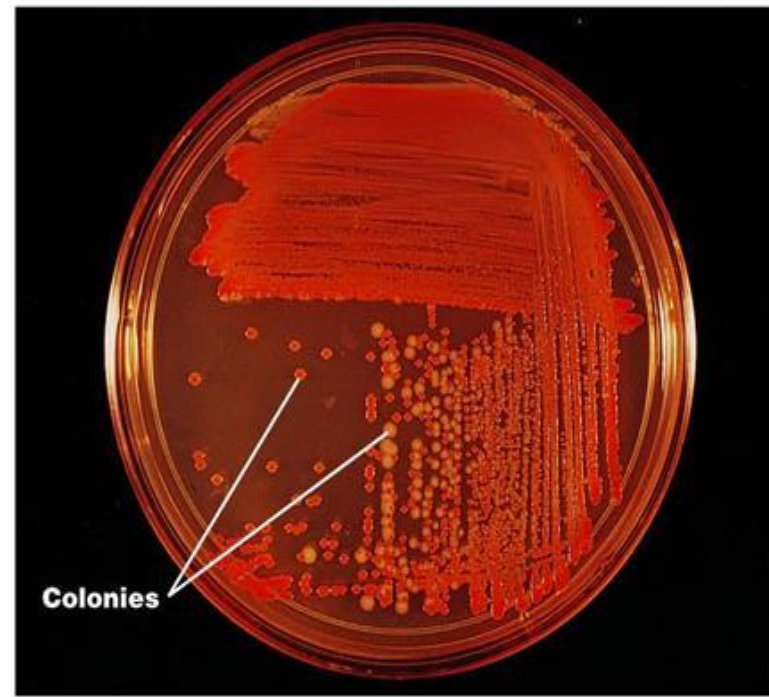
2-STREAKING



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.

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GRAM POSITIVE COCCI

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

Catalase



Staphylococcus (Clusters)



Streptococcus (pairs & chains)

Coagulase

Hemolysis




S. aureus
β hemolytic
mannitol
yellow





S. epidermidis
nonhemolytic (usually)
mannitol
white

(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

(*can also be Beta or Alpha hemolytic)

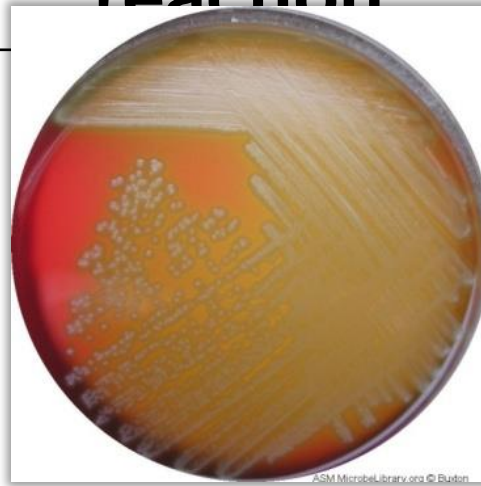


Identification of streptococci by hemolytic reaction



Colonies are surrounded by clear zone of hemolysis complete hemolysis

**Beta-hemolytic
Streptococcus colonies**
St. pyogenes



Colonies are surrounded by partial hemolysis with greenish color

**Alpha-hemolytic
Streptococcus colonies**
St. pneumoniae



No haemolysis

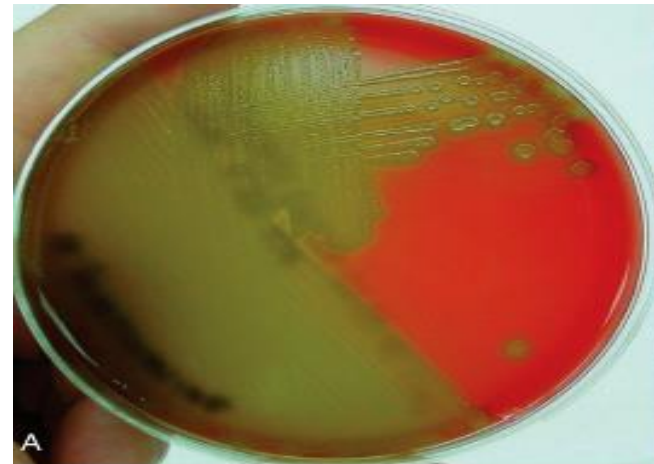
**Gamma-hemolytic
Streptococcus colonies**
Enterococcus faecalis

Identification of streptococci by hemolytic reaction

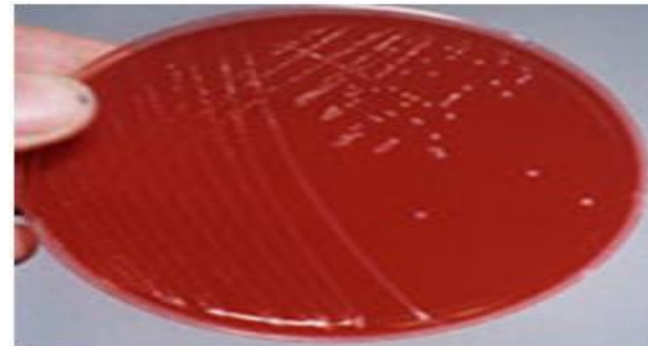
Beta-hemolytic *Streptococcus* colonies



Alpha-hemolytic *Streptococcus* colonies



Gamma-hemolytic *Streptococcus* colonies



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
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- 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. enterocolitica, motile at 25C, non-motile at 37C
C. jejuni, grows on campy agar
S. dysenteriae, non-motile
Salmonella spp. motile & produces H₂S

Strict anaerobe
B. fragilis

MacConkey's agar (DIFFERENTIAL MEDIUM)



MacConkey's agar



Lactose fermenting colonies

E. coli



non-lactose fermenting colonies

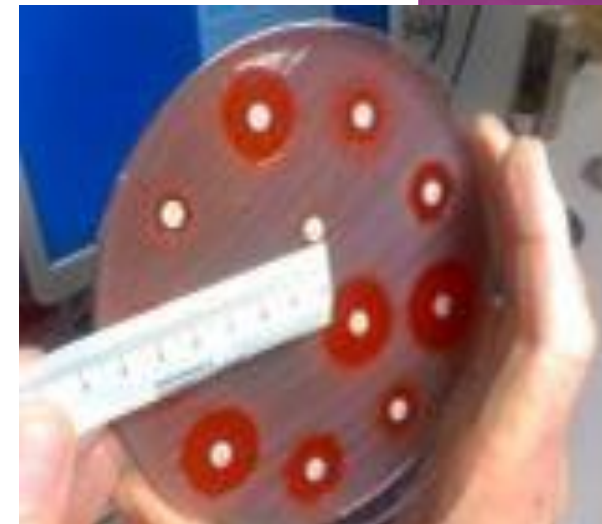
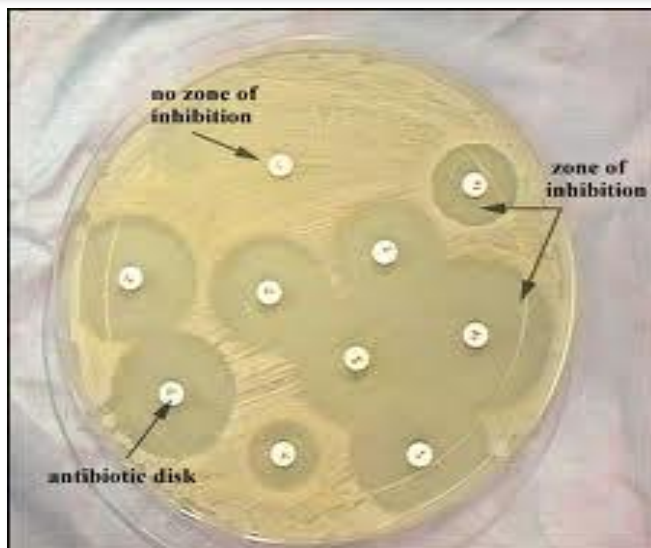
salmonella

Biochemical testings



To confirm the identity of bacteria.

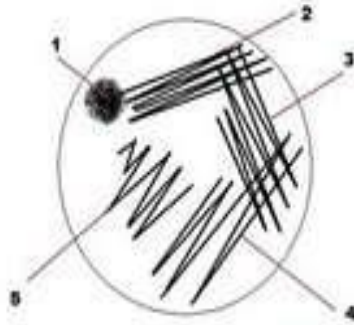
Antibiotic susceptibility testings



Automated instrument for identification and susceptibility testings

VITEK



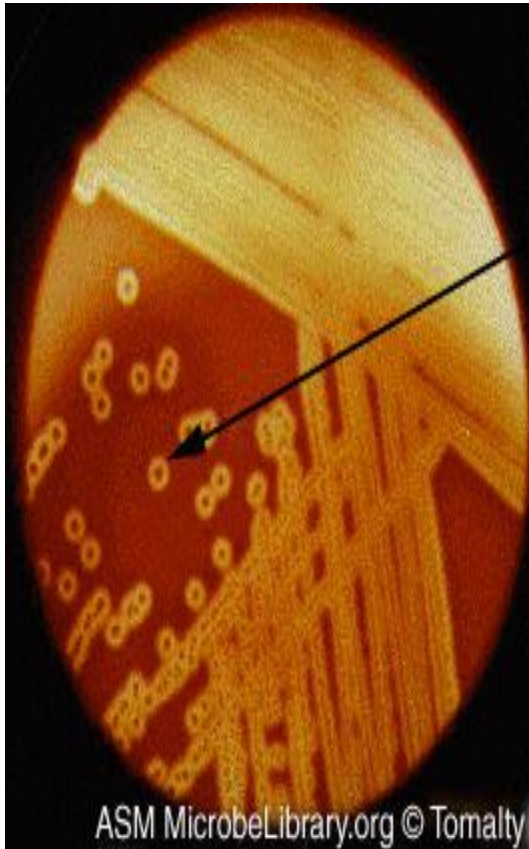


Blood agar

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



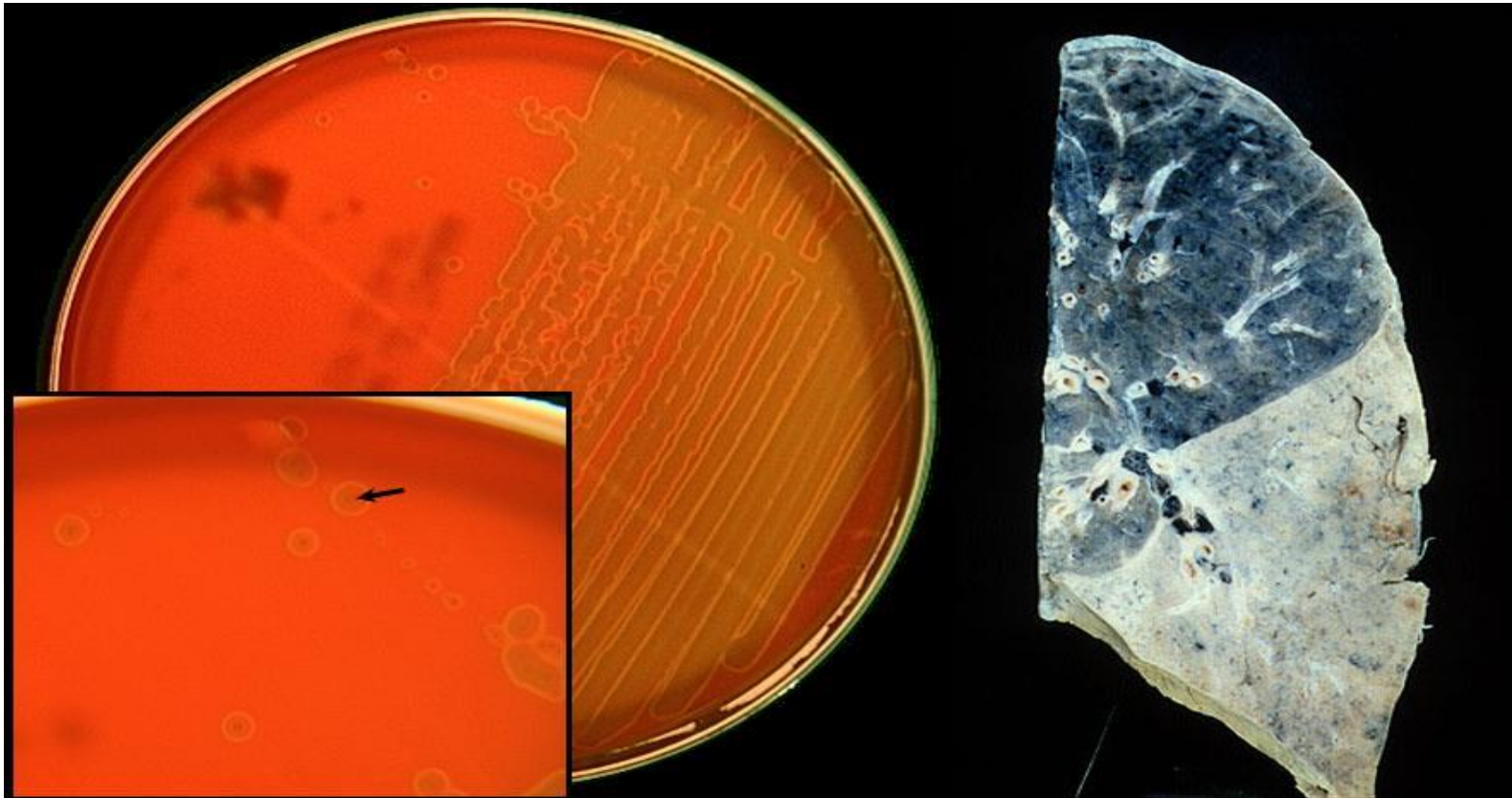
This is a blood agar growing beta hemolytic streptococci.



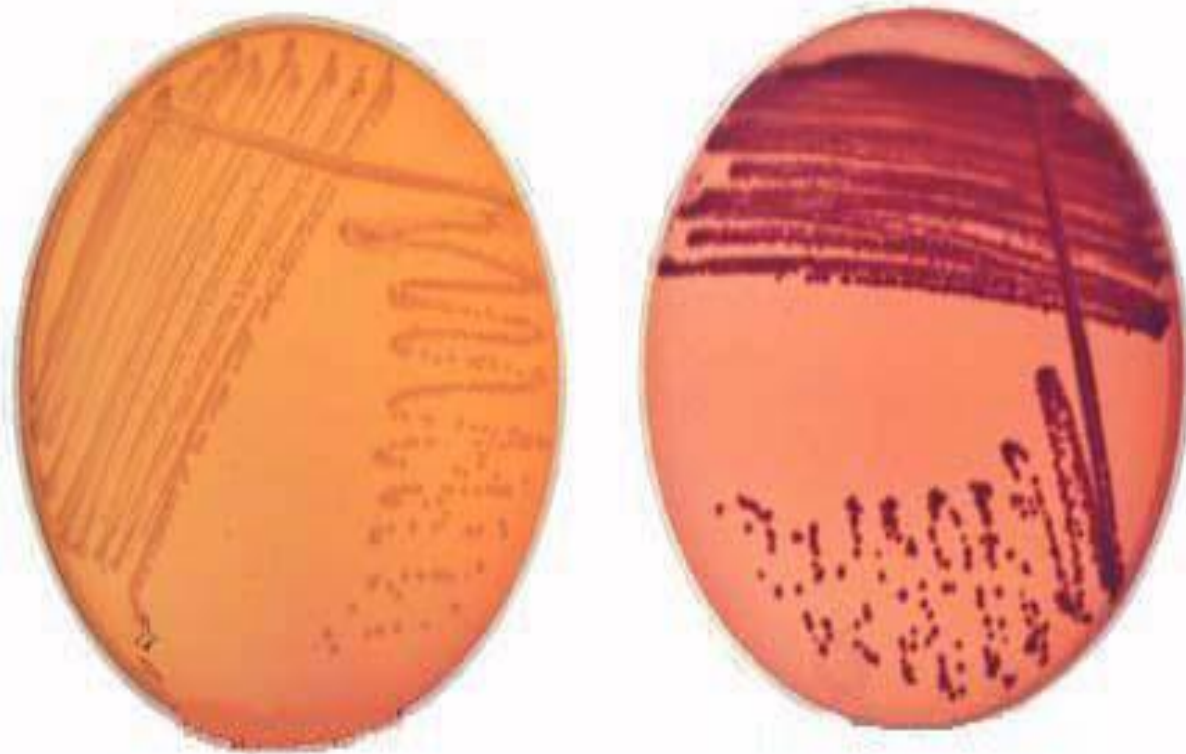
Note the clear zone of beta-hemolysis surrounding the *Streptococcus* colonies when grown on blood agar.



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

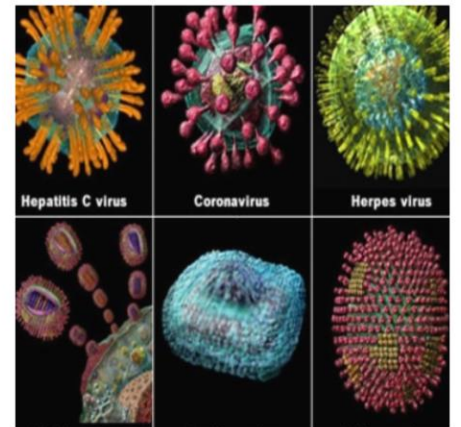


α hemolytic streptococci on blood agar

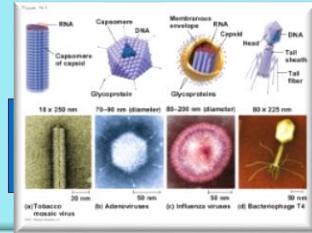


ASM MicrobeLibrary.org © Chamberlain

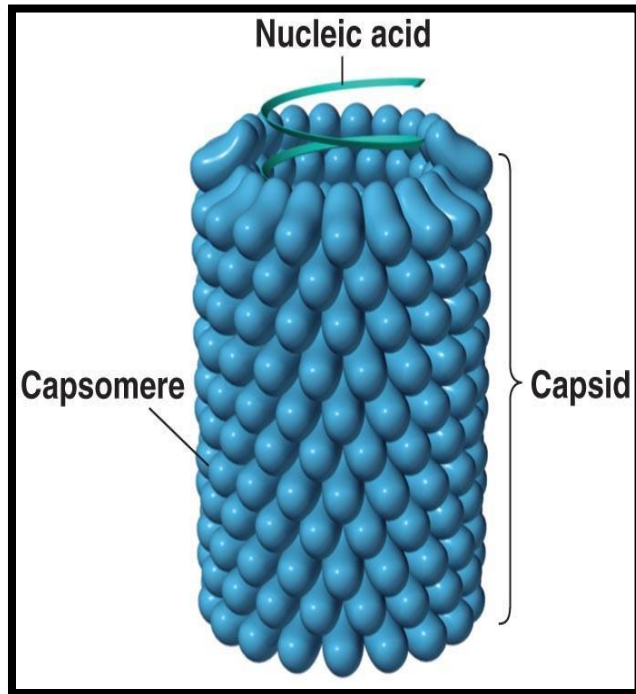
VIROLOGY



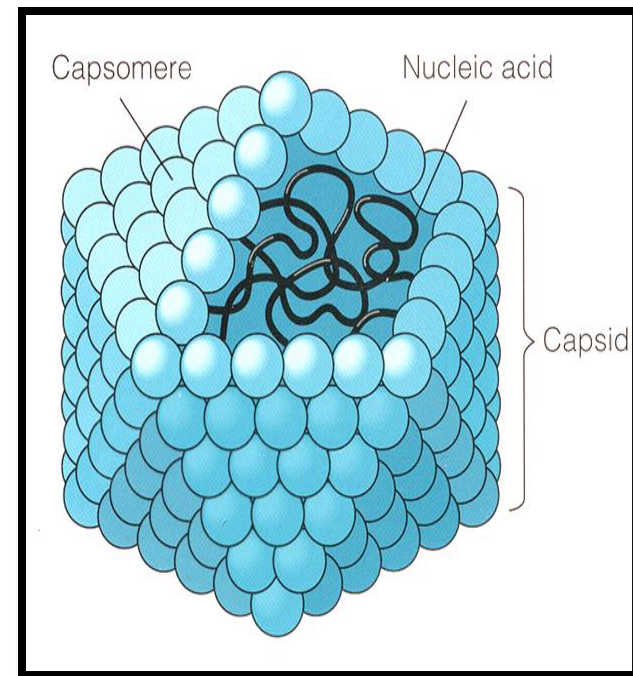
VIRAL STRUCTURE



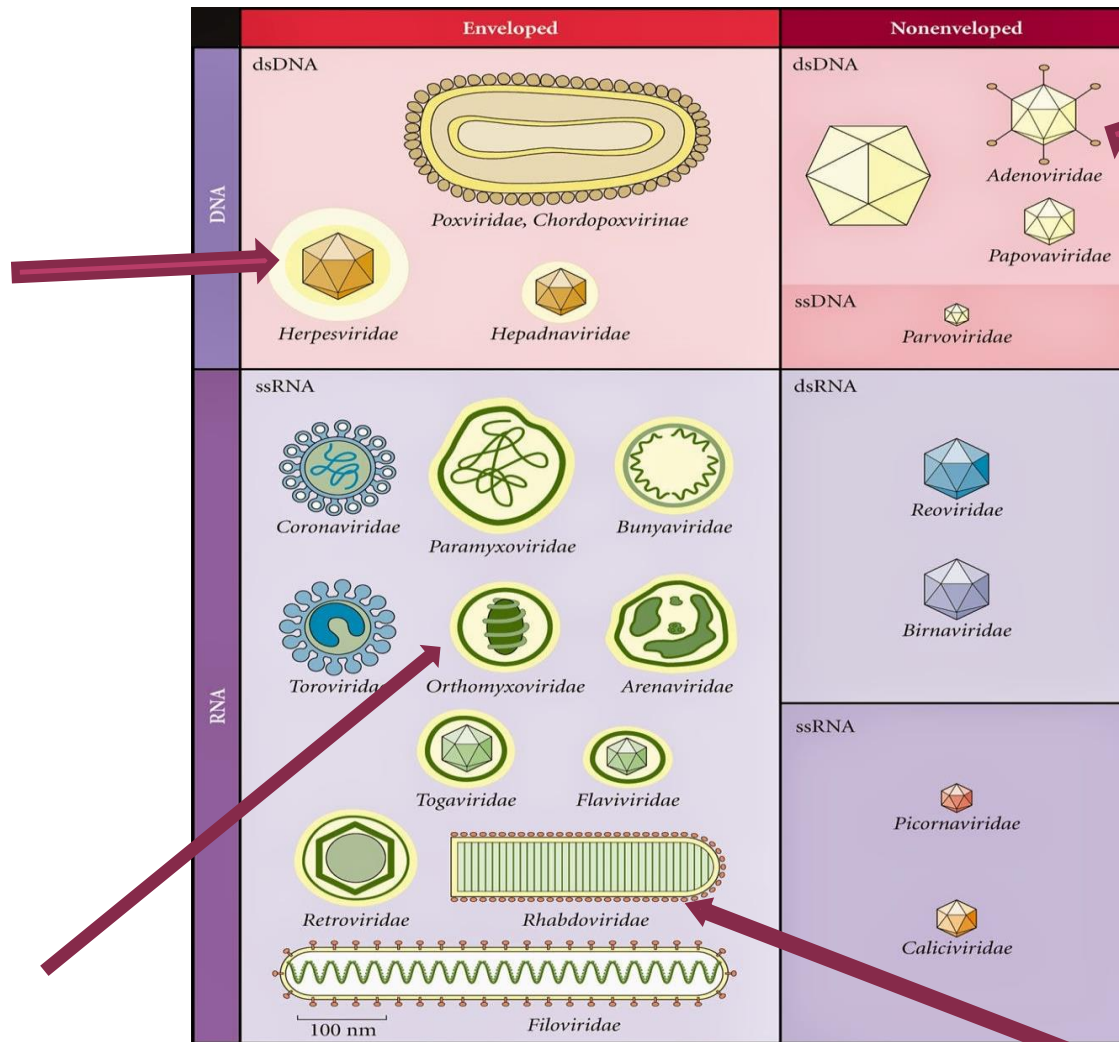
Helical Virus



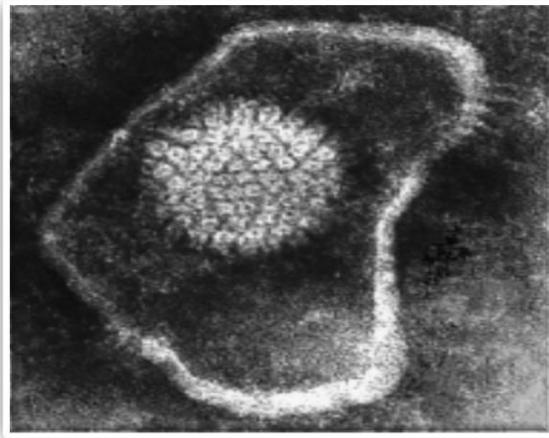
Icosahedral Virus



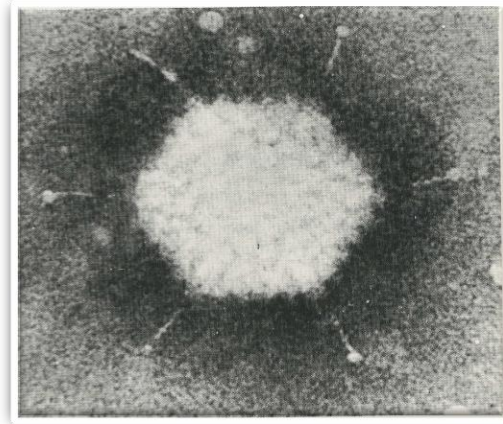
VIRAL CLASSIFICATION



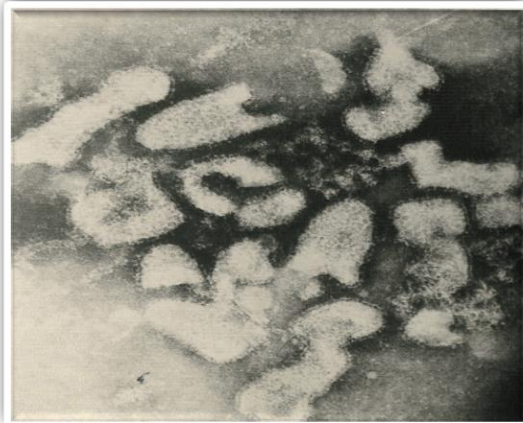
VIRAL ELECTRON MICROGRAPHS



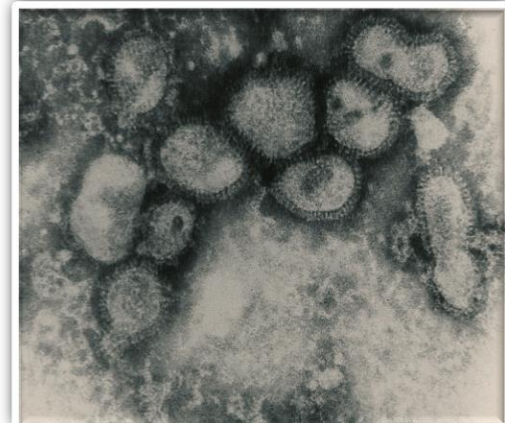
Herpes virus



Adenovirus

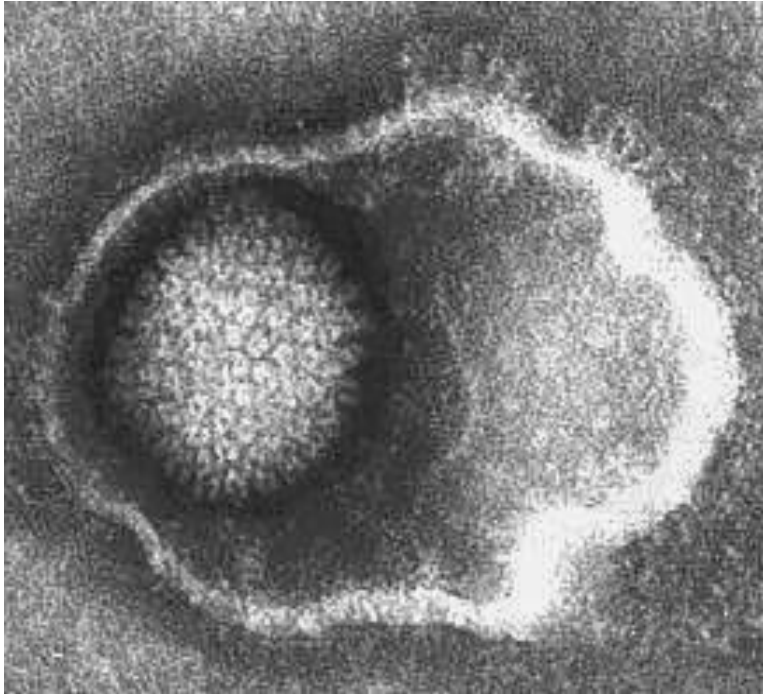


Rabies virus



Influenza Viruses

Herpes simplex virus -1 :
Herpesviridae



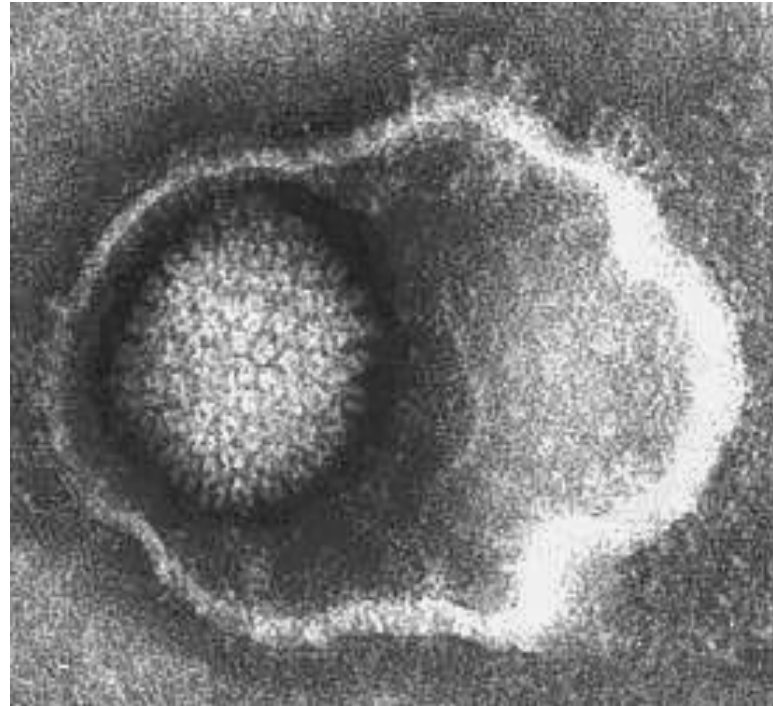
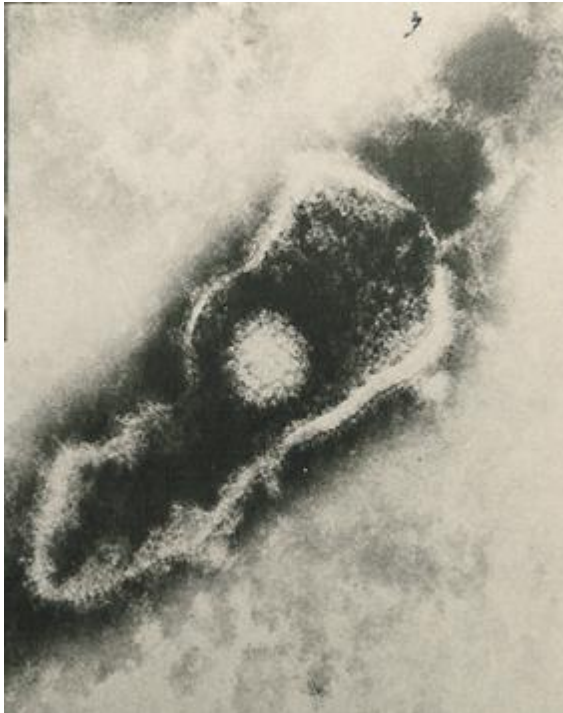
Enveloped virus

Icosahedral capsid

d.s DNA genome

Loose envelope

These are electron micrographs of a virus



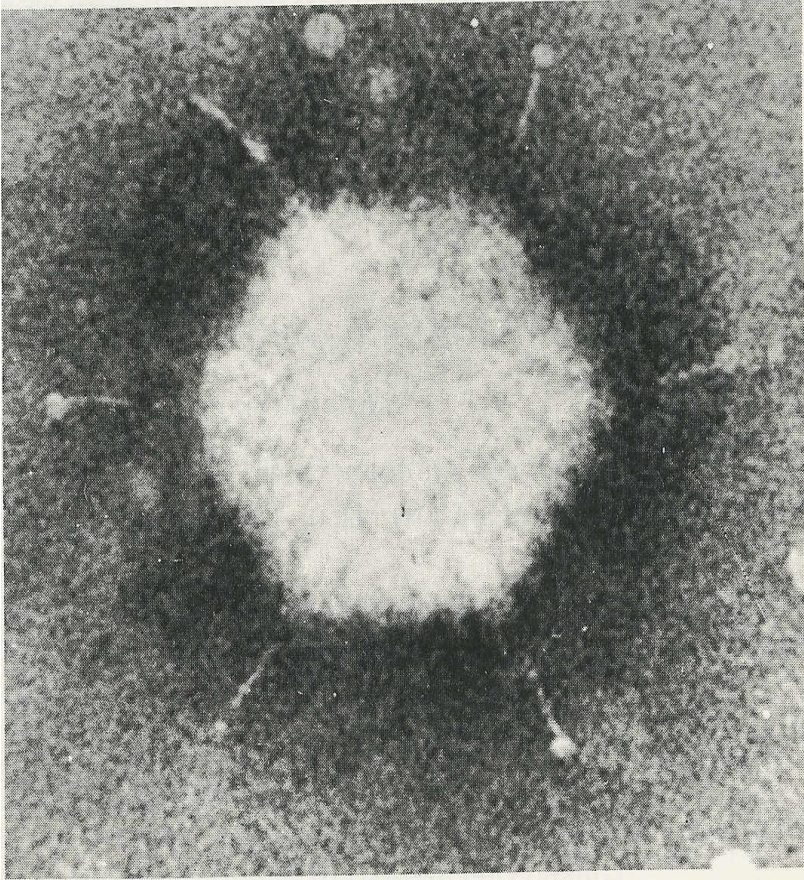
Q1: Name this virus

Herpes virus

Q2: Describe its structure.

Enveloped virus ,
Icosahedral capsid,
d.s DNA genome

Adenovirus : Adenoviridae



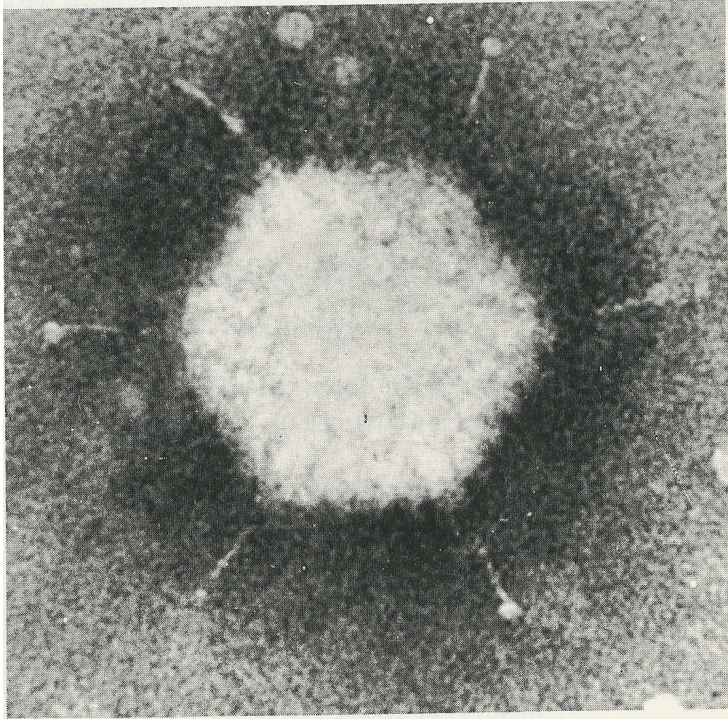
Nonenveloped virus

Icosahedral capsid

d.s DNA genome

Only V with fiber

This is an electron micrograph of a virus



Q1: Name this virus

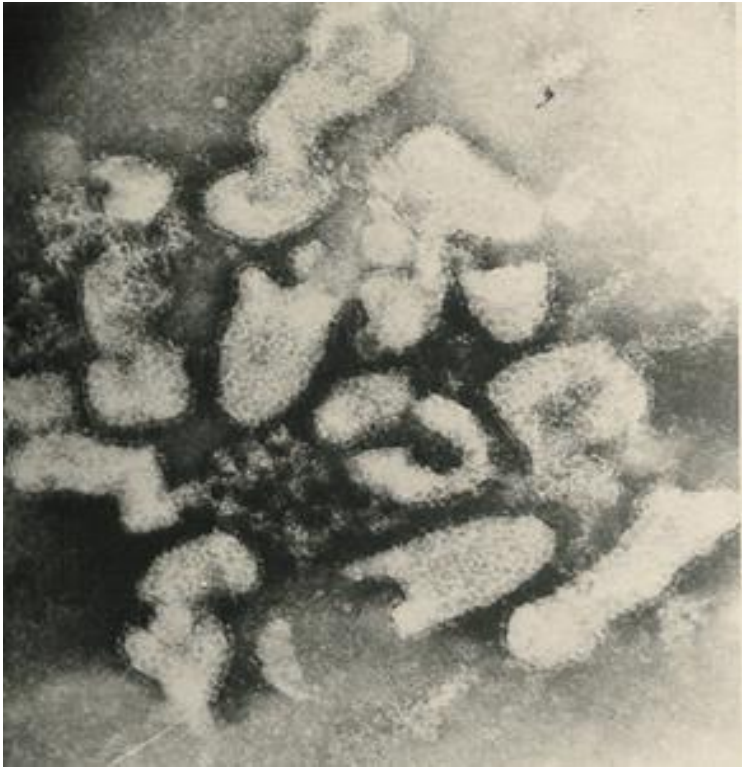
Adenovirus

Q2: Describe its structure.

Nonenveloped virus, with fiber

Icosahedral capsid & d.s DNA genome

Rabies virus: Rhabdoviridae



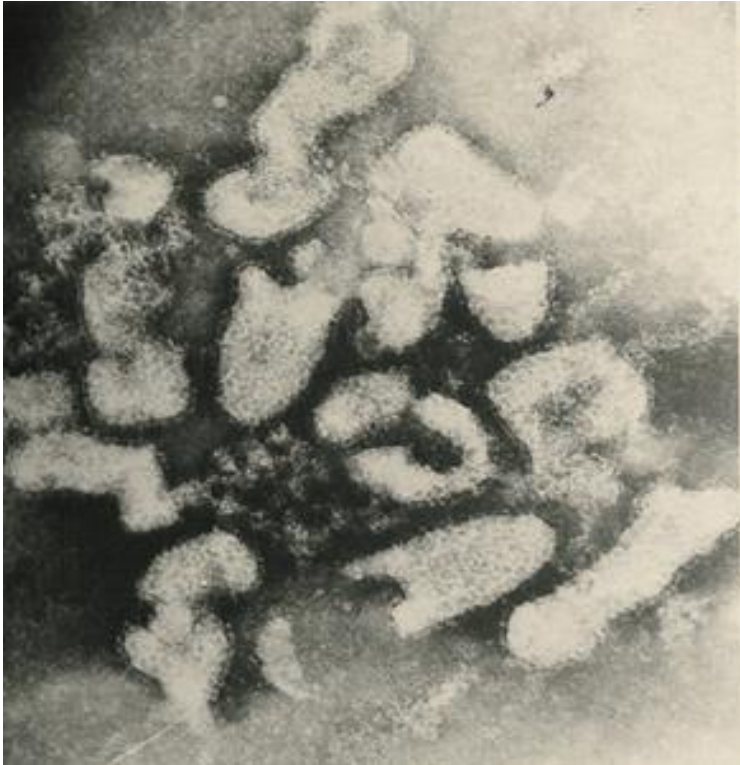
Enveloped virus

Helical capsid

s.s RNA genome

Bullet shape

This is an electron micrograph of a virus



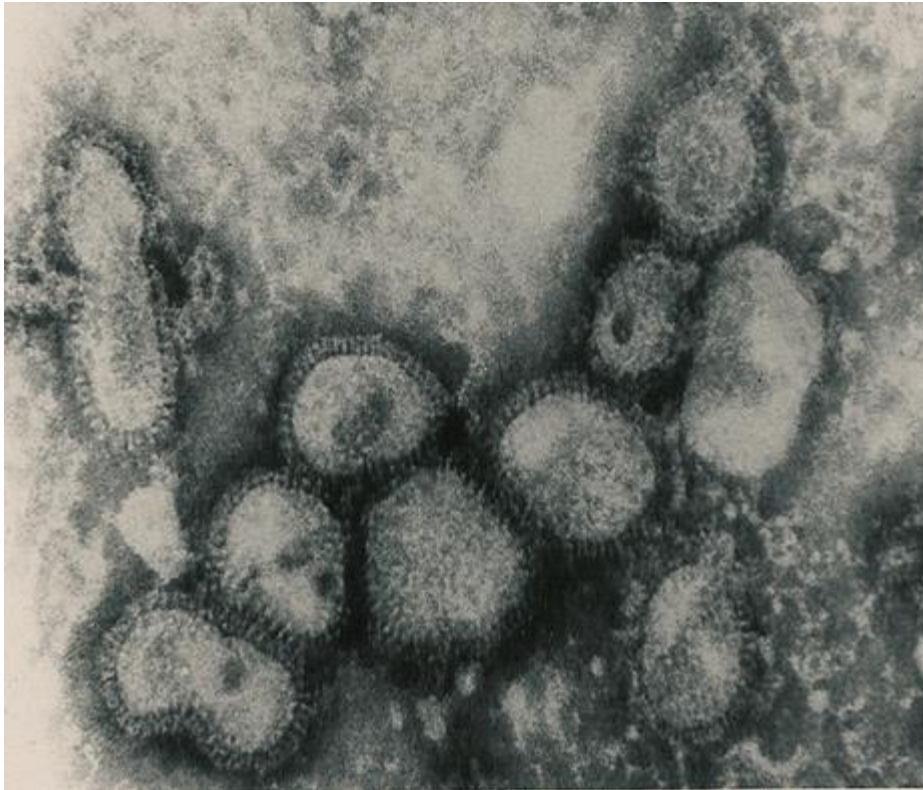
Q1: Name this virus

Rabies virus

Q2: Describe its structure.

*Enveloped virus , Helical capsid
& s.s RNA genome*

Influenza Viruses : Orthomyxoviridae



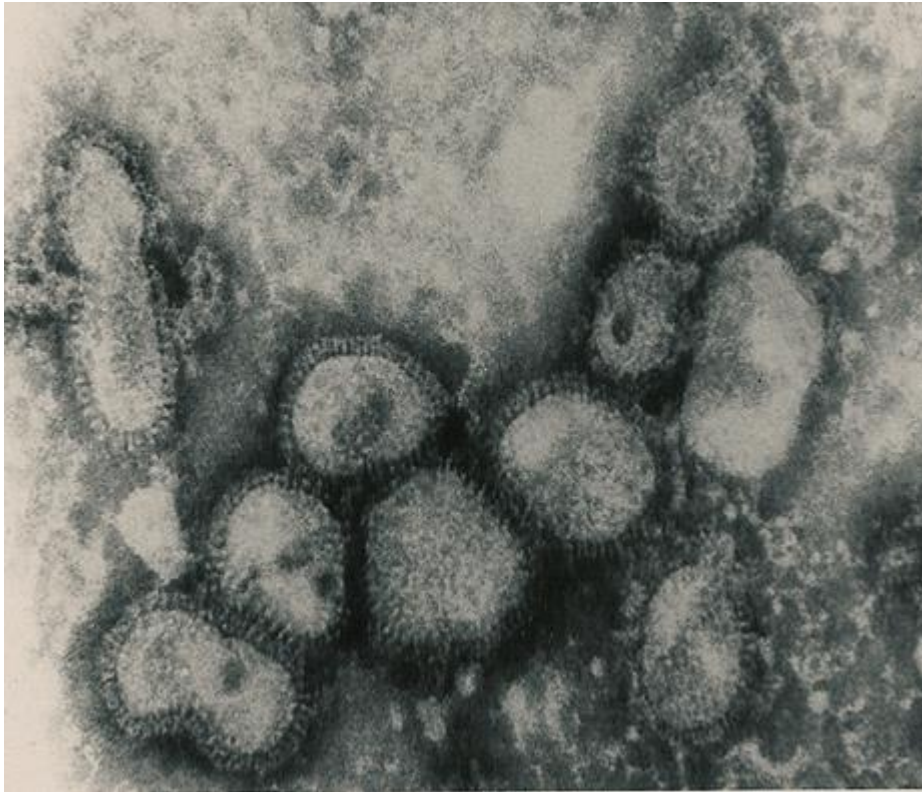
Enveloped V & spikes

Helical capsid

Segmented s.s RNA

Pleomorphic shape

This is an electron micrograph of a virus



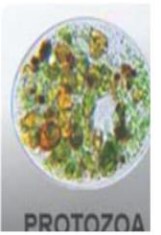
Q1: Name this virus

Influenza Viruses

Q2: Describe its structure

Enveloped Virus with spikes ,
Helical capsid ,Segmented s.s RNA

PARASITOLOGY



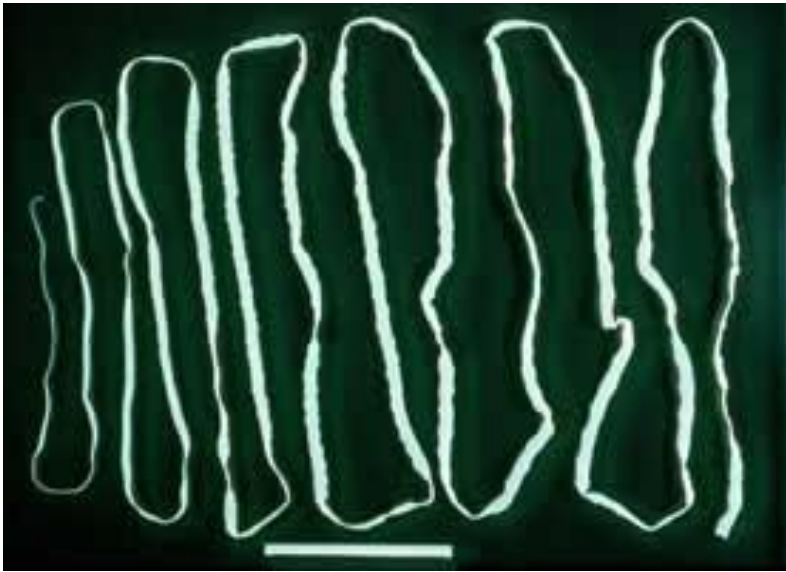
Classification of Parasites

Protozoa	Helminths
Unicellular Single cell for all function	Mulicellular Specialized cells
Amoebae: move by psudobodia. Flagellates: move by flagella. Ciliates : move by cilia Apicomplexa (sporozoa) Tissue parasites	Round worms (Nematodes) cylindrical, unsegmented Flat worms 1-Trematodes: leaf-like, unsegmented. 2-Cestodes: tape-like, segmented

Ascaris lumbricoides (roundworm)

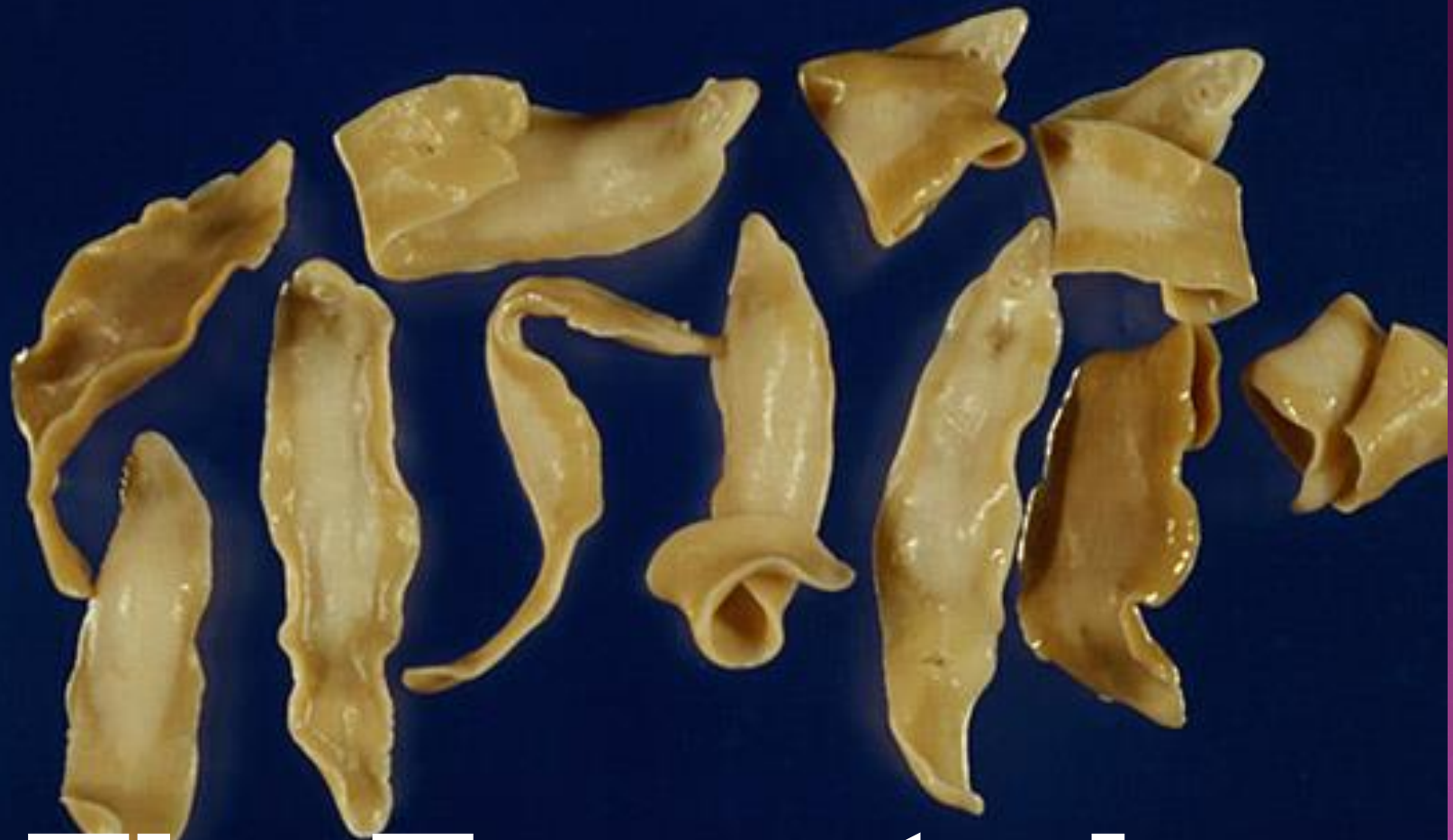


Ascaris adult



Taenia saginata

Cestodes



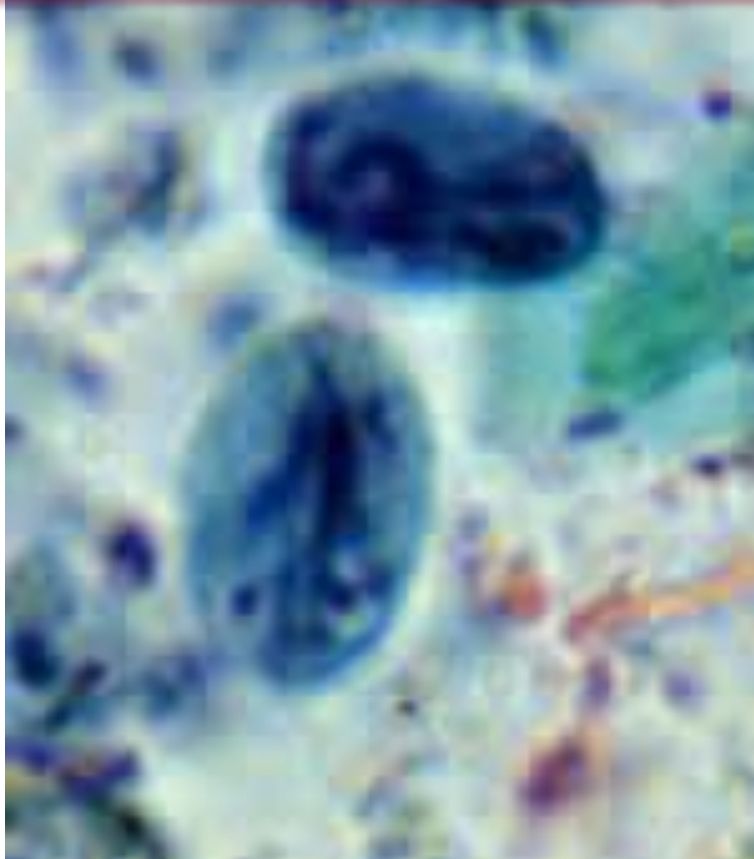
The Trematodes

Giardia lamblia trophozoite



Two nuclei,
each with central karyosome
Four pairs of flagella

Giardia lamblia cyst



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

ARTHROPODS OF MEDICAL IMPORTANCE

Class <i>Insecta</i> الحشرات	Class <i>Arachnida</i> العنكب	Class القشريات <i>Crustacea</i>
<ul style="list-style-type: none"> ● Muscid flies: housefly, Tsetse fly ● Myiasis-producing flies . ● Mosquitoes البعوض: <i>Anopheles, Aedes Culex</i> ● Sandfly ذباب الرمل (<i>Phlebotomus</i>) ● Black fly (<i>Simulium</i>) ● Fleas البراغيث ● Lice (<i>Pediculus, Phthirus</i>) القمل ● Bugs: <i>Cimex, Triatoma</i> البق ● Bees النحل 	<ul style="list-style-type: none"> ● Scorpions العقارب ● Spiders العنكب ● Ticks: القراد hard, soft ● Mites السوس -<i>Sarcoptes scabiei</i>, -dust mites 	<ul style="list-style-type: none"> ● Water flea (<i>Cyclops</i>)

LICE

Louse(singular) , Lice (pleural)

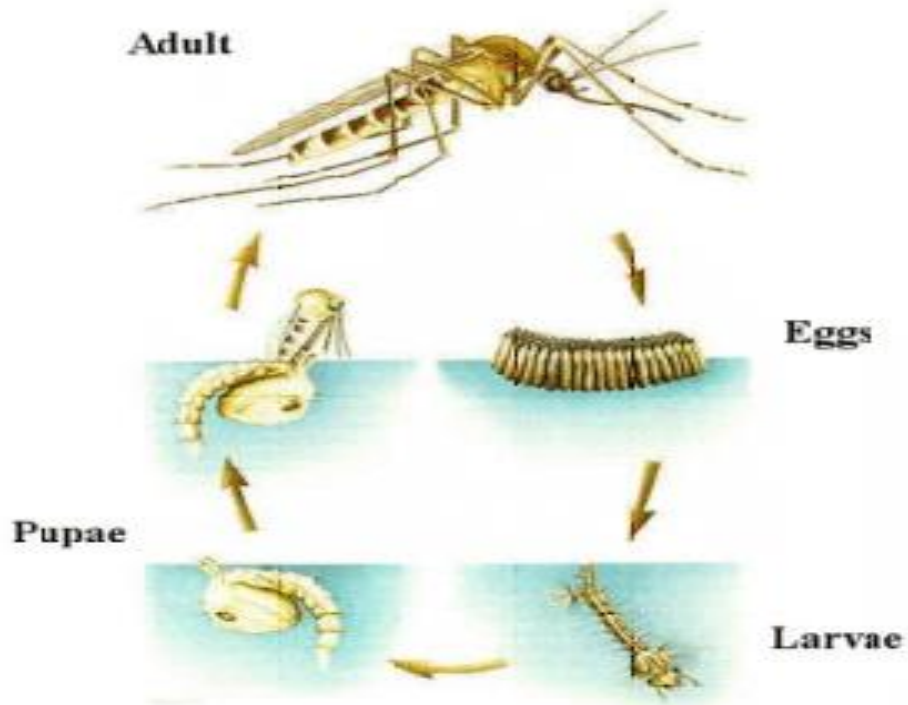
Pediculus humanus



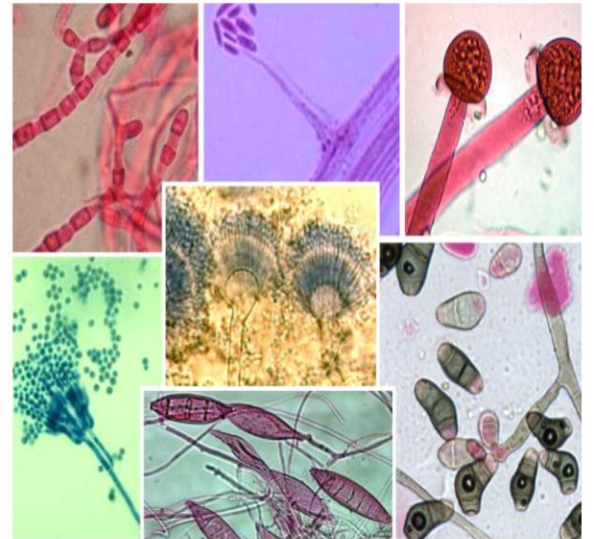
Phlebotomus (sand fly)



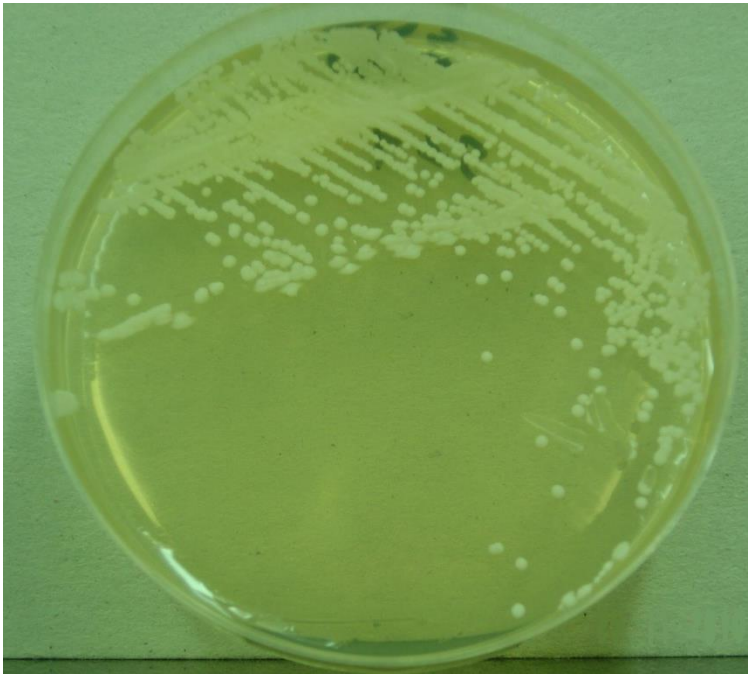
Mosquitoes :



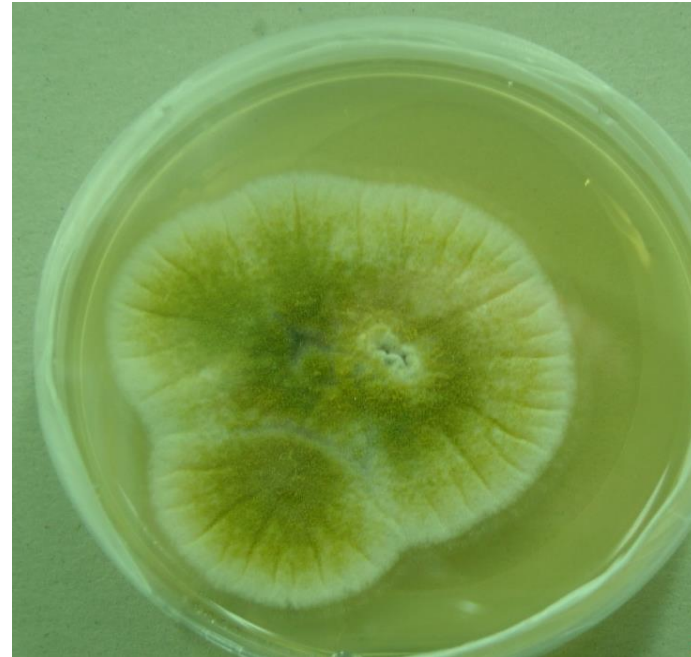
MYCOLOGY



Fungi can be divided to two types based on morphology



A



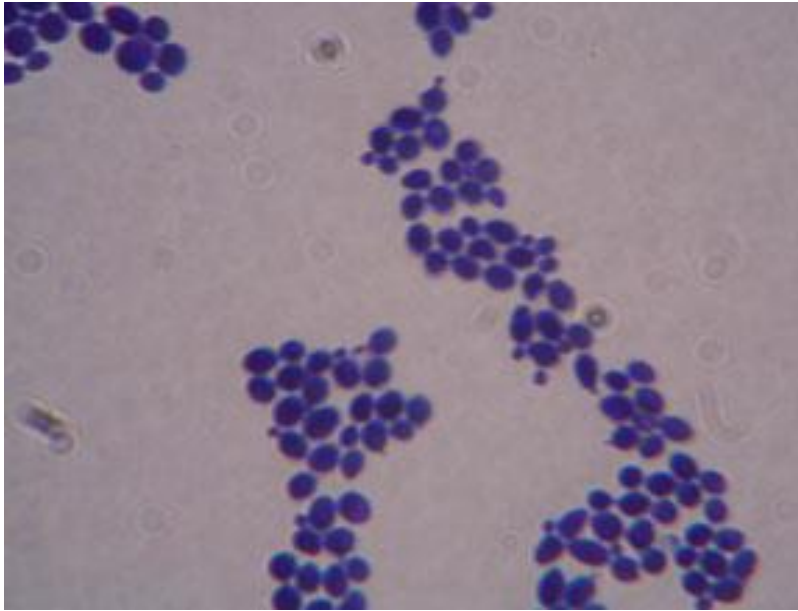
B

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

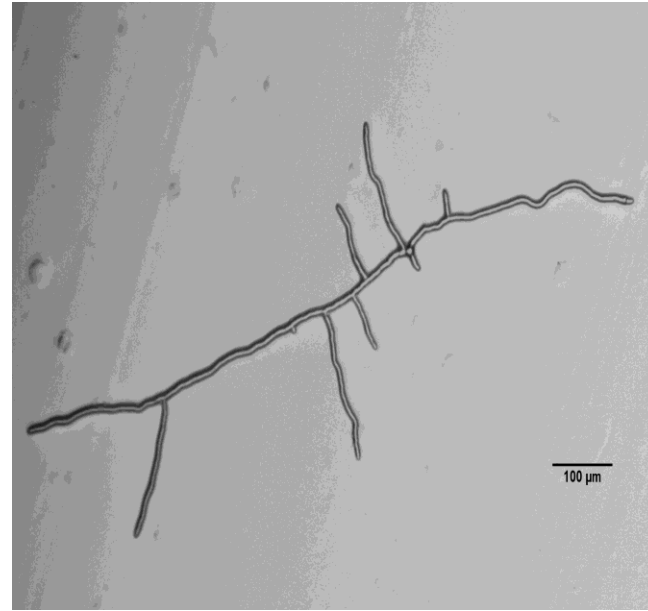
A: Yeast
e.g. *Candida*

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*

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

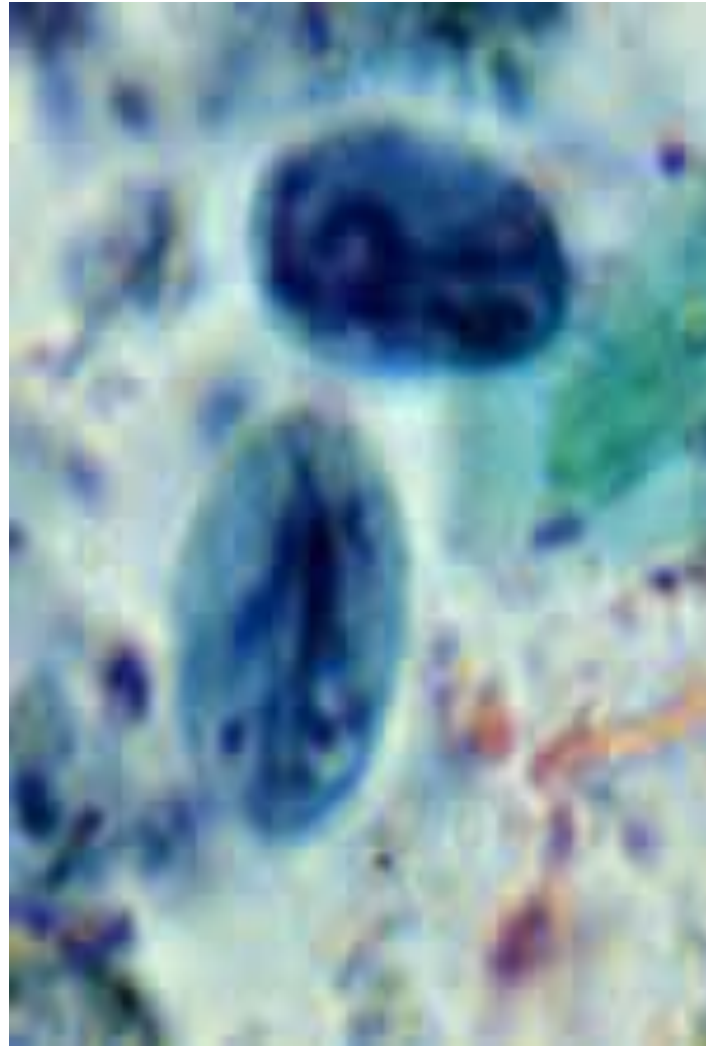
Following is the microphotograph of an organism found in stools

Name the Organism

Giardia lamblia

What is the Stage?

Cyst stage





END