1 GENERAL MICROBIOLOGY

Scientist

Louis Pasteur 00:01:15

- Pasteur Institute, Paris
- Pasteurisation
- Liquid media
- Father of microbiology
- Fermentation Principle
- Autoclave
- Disapproved theory of abiogenesis
- Germ cell theory
- Vaccines:
  - Cholera
  - Anthrax
  - Rabies

Robert Koch 00:04:31

- Father of modern microbiology
- Koch postulates (4+1):
  - Constant association of causative organisms with the disease (Mycobacterium TB causes TB).
  - Isolation in culture media possible
  - Culture growth inoculated in animals should produce the same lesion.
  - Re-isolation from the experimental animals is possible.
  - Whenever there is an antigen, the human should be able to produce antibodies in serum.
  - Exception from postulates:
    → L-Mycobacterium leprae (armadillo)
    → P-Treponema Pallidum
    → G-Gonococci
- Koch Bacilli (Tuberculosis)
- Cholera organism
- Solid culture media
- Aniline Dye colour
- Hanging drop motility- motility test.

Paul Ehrlich

- Ehrlichia organism
- Father of chemotherapy
- Toxin-antitoxin standardisation (Nobel prize)
- Acid-fast stain/ Ziehl Neelsen stain

Scientist	Found/Known as
Joseph Lister	• Father of antiseptic surgery • First used carbolic acid
Anton Von Leeuwenhoek	• Father of the light microscope (Unilocular) • The first thing he visualised under his microscope was Animal cules. • The Jansen duo gave us the compound microscope.
Ernst Ruska	Father of electron microscope
Edward Jenner	• First vaccine- smallpox ○ Prepared using cowpox
Karry B Mullis	PCR
Sanger	Sanger sequencing
H C Gram	Gram staining
Kleinberger	L forms (cell wall deficient)
Alexander Fleming	Penicillin
Barbara Mcclintock	Transposons (Jumping genes)

Nobel prizes

- For Hepatitis c virus- To Michael Hougton, Harvey J. Alter, Charles M. Rice
- CRISPR Cas9- Emmanuelle Charpentier and Jennifer A Doudna.

Stains 00:20:30

Fixation

- To ensure the sample stays on the slide
- Heat fixation
- Chemical fixation (methanol)

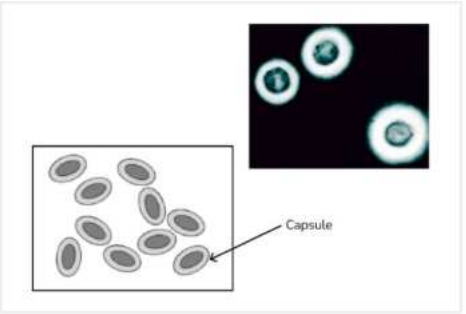
Simple stains (one colour)

- Methylene blue
- Basic fuchsin (red)

Negative stains (b/w)

- Staining the background, so the sample is highlighted.
- Cryptococcus- causes cryptococcal meningitis (CSF)
  - capsule that doesn't take up any colour

- India Ink
- Nigrosin



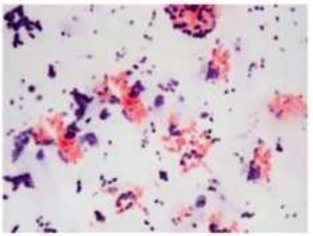
Impregnation Stains

- Deposit stain on the surface of the object to make it look thicker (for thin structures)
- Silver stains- black colour
- Thin structure
  - Flagella
  - Spirochetes (treponema- caused syphilis)
    - → Syphilis patient has Genital ulcers
    - → Fontana stain- fluid
    - → Levaditi's stain-tissue

Differential Stains

00:29:32

- **Gram stain (+/-):**

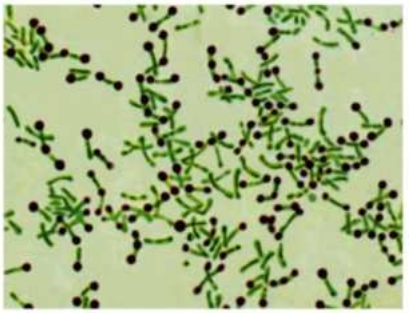


  - Gram positive- contain a lot of peptidoglycans, which retain the dye longer
  - Gram-negative - contains lipopolysaccharides (lipid), which dissolves in alcohol (decolourization).
  - Poorly gram staining:(gram stain doesn't work)
    mnemonic- MRCS
    - → **M**ycoplasma
    - → **R**ickettsia
    - → **C**hlamydia
    - → **S**pirochetes

Stains	Gram +ve	Gram –ve
Crystal/ Methyl/ Gentian Violet	Purple	Purple
Iodine (Mordant)	Makes Crystal violet penetrate	
Alcohol/ Acetone (decolouriser-important step)	Purple	Colourless
Safranin	Purple	Red/Pink

- **Acid-fast Stain:** 00:39:12
  - Carbol Fuschin (red)- organism colours
  - Heating (mordant)
  - Acid/ Acid-alcohol- Sulphuric acid (decolourized)
    - → Mycobacteria- 20% $H2SO_4$
      - TB - Alcohol (95% alcohol) and Acid fast (20% $H2SO_4$)
      - Atypical TB (M.avium)- Acid fast (20% H2So4)
      - Lepra- Acid Fast (5% H2SO4) - Fite Foracco stain .
    - → Nocardia, Legionella- 1% H2SO4
    - → Coccidian parasite family- COLD ZN stain (5% H2SO4)
      - Isopora, Cyclospora, Cryptosporidium
    - → Spores, Head of 0.25: 0.25-0.5% H2SO4
    - → Hooklets of Hydatid
    - → Eggs: Taenia Saginata
  - Methylene Blue/ Malachite green -Background colour
  - COLD ZN stain- Kinyoun Stain/Gabbet stain
    - → Instead of heating, increased concentration of phenol in carbon fuchsin.
    - → Used for the family of coccidean parasites

- **Albert ZN stain** 00:52:58

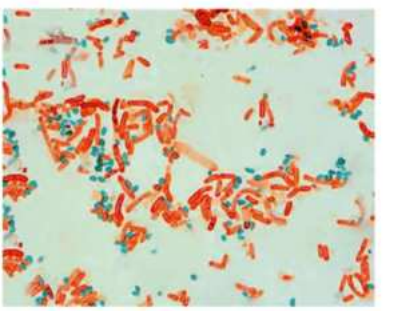


  - Albert solution 1:
    - → Malachite Green- organism
    - → Volutin granules-Toluidine blue (Metachromatic stain- 2 colours)
    - → Glacial Acetic acid
  - Albert solution 2: Iodine
  - Used for Volutin / Babes Ernt granules
    - → present in C-diphtheria
    - → located as 2 poles, it is also called bipolar Granules
    - → Metachromatic Granules-stained by Toluidine blue.
    - → Also seen in Spirillum, Gardnerella, Yersinia pestis, yeast, MTB
  - Stains for Volutin granules:
    - → Ponder's Stain
    - → Loeffler methylene Blue (best)
    - → Albert Stain
    - → Neisser stain

Flagella Stains 01:00:46

- Silver stains (impregnation method)
- Leifson and Ryu stain
  - Background - Methylene blue
  - Flagella - Basic fuschin Red
  - Mordant- Tannic acid

Spore Stain



- Spore- resting/ dormant form of bacteria.
- Schaeffer and Fulton stain/ Modified Ashby stain
  - Malachite green- spore
  - Heat- Dormant
  - Water- decolorizer
  - Safranin Red- organism

MCQs

Q. In Gram staining, the mordant is?
A. Tannic acid
B. Loeffer's mordant
C. Lugol's iodine
D. Bovine's fixative

Q. After the primary strain and mordant has been added but before the decolourizing agent has been used, gram-positive organisms are stained _____, and gram-negative organisms are stained _____.
A. Purple, Purple
B. Purple, colourless
C. Purple, pink
D. Pink, pink

Q. Metachromatic granules stained by all except?
A. Albert stain
B. Neisser
C. Ponder
D. Kinyoun

Q. Silver impregnation method of staining is used to demonstrate?

A. Mycobacteria

B. Spirochaetes

C. Both of the above

D. None of the above

Q. Which of the following is acid-fast with 20% $H2SO_4$?

A. M. avium

B. M. Leprae

C. Actinomyces

D. Nocardia

Q. In the ZN staining procedure, the secondary stain is?

A. Crystal violet

B. Safranin

C. Methylene blue

D. Alcohol

Q. Correct order of gram staining?

A. Carbol fuchsin-iodine-Acetone-methyl violet

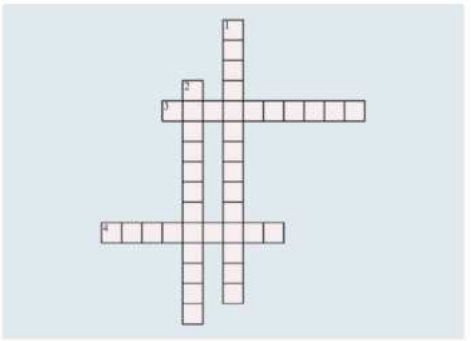
B. Crystal violet-iodine-Acetone-Safranin

C. Methyl violet-Acetone-iodine-Safranin

D. Crystal violet-Carbol fuchsin-Acetone-iodine

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Father of modern microbiology
4. Game positive- contain a lot of peptidoglycans, which retain the dye longer

Down

1. Silver stains (impregnation method)
2. Disapproved theory of abiogenesis

2 MICROSCOPES

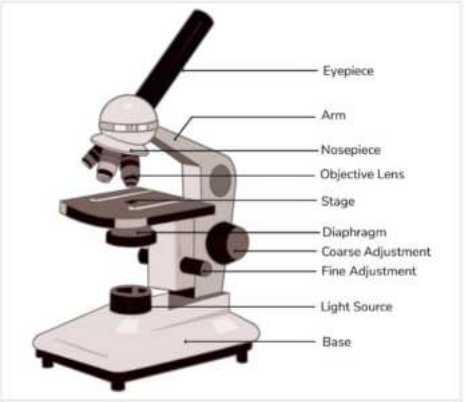
Features of Microscopes

00:00:26

- Uses lens to magnify
- Resolution - The ability to differentiate two points as separate
  - Human eye - 0.2 mm
  - L/M - 0.2 micron
  - Electron - 0.2-0.5nm
- Contrast - Dyes

Light Microscope

00:03:06



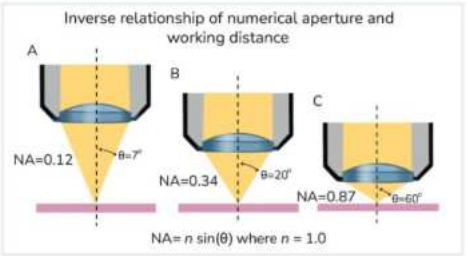
- Transmitted light is used for microscopes.
- Slides are kept on stage
- Light should go only to the slide; there is a condenser that has iris, which regulates the light.
- The condenser is placed Below the stage.

Components of Microscope

- Two knobs -
  - Big knob is the coarse adjuster
  - Small knob is for fine adjustment
- Two lenses-
  - EyePiece lens
    → Magnifies 10 times
  - Objective lens
    → Scanner lens - Magnifies for 4 times
    → Low Power - Magnifies 10 times
    → High Power - Magnifies for 40 times
    → Oil Immersion - Magnifies for 100 times
  - These lenses revolve around with the help of neck pieces.

Lens	Magnification	Eyepiece	Total Magnification	Numerical Apertures
Scanner	4x	10x	40	0.12
Low Power	10x	10x	100	0.22
High Power	40x	10x	400	0.65
Oil Immersion	100x	10x	1000	1.25

- Max magnification offered by light microscope is 1000x.
- As the lenses increase, numerical apertures also increase.

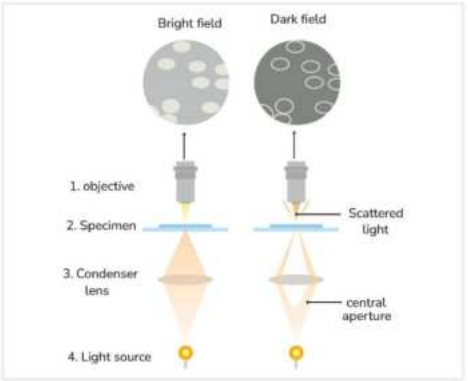


Q. What are Numerical Apertures?
Ans: There is one angle forming between lens and slide. Numerical aperture is the angle by which light falls on the slide and gets reflected.
Mathematically,
$NA = n(\sin\theta)$
where, n is the refractive index, $\sin\theta$ is half of the angle between the object and lens.

Dark Field Microscope

00:15:30

- Light is reflected
- Illuminates the objects
- Used for Thin structure
  - Flagella
  - Spirochetes - spiral structures

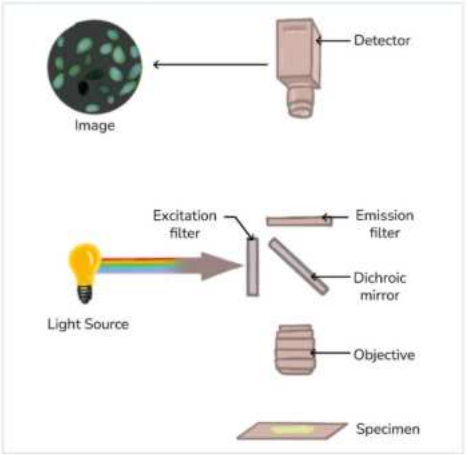


Interference Contrast Microscope

- Reveals cells organelles
- Measurements of chemical constituents of cells, such as:
  - Lipids
  - Protein
  - Nucleic acid

Fluorescence Microscope

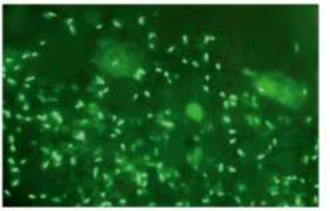
00:18:53



- Light source: Ultraviolet light
- Uses:
  - Cases of TB /Malaria / pathology of kidney biopsy
- Immunofluorescence + fluorescent dye

Different dyes

- For TB - Auramine and Rhodamine
  - For MTB - Auramine and Rhodamine is used
- For Malaria - Acridine Orange
- Fungus - Calcofluor White
- Flow Cytometry - FITC - Fluoro isothiocyanate



- Initially the light that come has shorter wavelength
- When the light falls on the specimen, it converts it into longer wavelength
- A dichroic mirror is used in a fluorescence microscope alongside a strong light source, an excitation filter, and an emission filter.

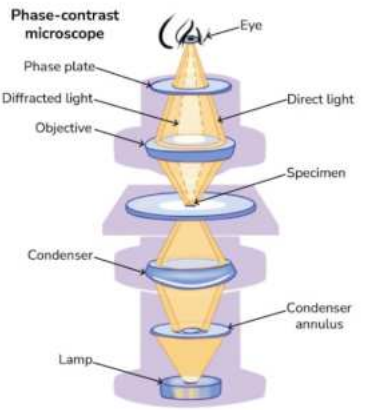
Autofluorescence

Specimen which has their own shine, kept under UV lights

- Spora Brothers
  - Cyclospora
  - Isospora
- Formalin
  - Skin biopsy for immunofluorescence in NS not formalin
- NADPH - Used in fluorescent spot test in G6PD deficiency
- Woodlamp: Ultraviolet light

Phase Contrast Microscope

00:29:38



- Annular diaphragm in front of the condenser - Added at the bottom
- Annular phase plate - Added at the top
- Differences in refractive indices, it will be shown in different phases
  - These will be converted into a change in the amplitude of light

Electron Microscope

00:32:17

- Invented by Ernst Ruska
- No light is used, a beam of an electron is used - electron is enclosed
- Medium is vacuum

Types of Electron Microscope

Scanning Electron Microscope	Features	Transmission Electron Microscope
Scattered Electrons	**Principle**	Transmitted electrons
3D	**Dimensional**	2D
More sample viewed in lesser time	**View and Time**	Less sample viewed in the same time
Surface details	**Details**	Internal Details

Differences between Electron light Microscope

Electron Microscope	Features	Light Microscope
2-2.5% Glutaraldehyde	**Fixation**	10% Neutral Buffered Formalin • Makes eye water
Embedded in resin	**Embedding**	Embedding in paraffin wax
Copper metal slides	**Slide**	Glass slides
Electron	**Source**	Transmitted light
Vacuum	**Medium**	Air
0.5 nm	**Resolution**	0.2 micron

MCQs

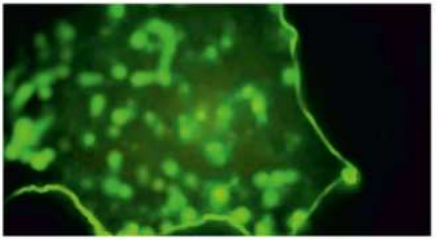
Q. Scanning Electron Microscope is used to reveal what?
A) Surface structure
B) Internal structure
C) Both of the above
D) None of the above

Q. Maximum magnification strength attained by a light microscope is?
A) 10x
B) 40x
C) 100x
D) 1000x

Q. In a light microscope, what function does a condenser serve?
A) Increase light intensity
B) Focus the light on sample
C) Focus the light on the eye
D) Reduced the glare

Q. A microscope that exposes specimens to UV LIGHT and forms an image with the emitted at a different wavelength is called a _____ microscope?
A) Phase-contrast
B) Dark-field
C) Scanning Electron
D) Fluorescent

Q. Which of the following structures are required in the microscope for taking this type of image?



A) Dark field condenser

B) Phase plate

C) Dichroic mirror

D) Cathode ray tube

Q. A microbiologist intern wanted to study cells and microorganisms. His senior advised him to use a light microscope. What is the arrangement from eye to light source in a light microscope?

A) Objective lens —- condenser —- eyepiece lens

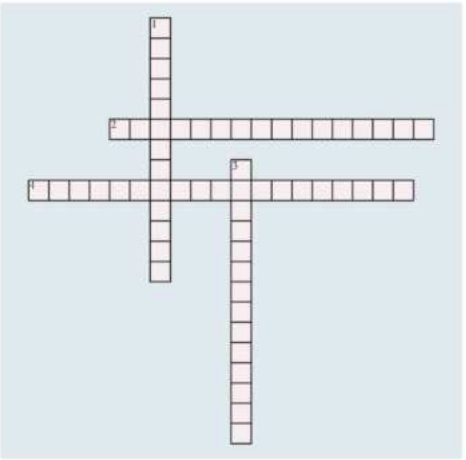
B) Condenser lens —- objective lens — eyepiece lens

C) Eyepiece lens — objective lens — Condenser

D) None of the above

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Specimen which has their own shine, kept under UV lights
4. Illuminates the objects

Down

1. Smallest lens - Magnifies for 4 times
3. is used in a fluorescence microscope alongside a strong light source, an excitation filter, and an emission filter.

3 BACTERIAL ANATOMY

Capsule and slime layer

00:00:26

- Bacteria has either slime layer or capsule
- Streptococcus salivarius-the only one to have both slime layer and capsule
- Capsule - tough and demarcated
- Slime layer - loose / undemarcated
- Virulence factor-prevents phagocytosis by preventing opsonization

Capsulated organisms

Mnemonic - Pretty NIce Capsule

- Streptococcus **P**neumonia
- Klebsiella **P**neumoniae
- Bordetella **P**ertussis
- Vibrio **P**arahaemolyticus
- Clostridium **P**erfringens
- Yersinia **P**estis -F1 peptide
- **N**eisseria meningococcus
- Haemophilus **I**nfluenzae
- **C**ryptococcus
- Staphylococcus **A**ureus- microcapsule
- Bacillus **A**nthracis

All capsules are made of polysaccharides

- Except:
  - Yersinia pestis - F1 peptide
  - Bacillus anthracis - polypeptide
- S. aureus has microcapsule
- S. pyogenes sometimes have capsule- made of hyaluronic acid

Demonstration of capsule

1. McFadyen's reaction- Bacillus anthracis
   - Add polychrome methylene blue stain
   - Gives purple color
2. Quellung reaction / Neufeld reaction
   - Example, pneumococcus
   - Bacteria capsule (Ag)and we add antisera (Ab) to it
   - There would be antigen antibody reaction → swelling of capsule

Modification of slime - biofilm

- Streptococcus mutans
  - In mouth - cause dental caries
- Staph. epidermidis-causes prosthetic heart valve endocarditis
- Also shown by Pseudomonas aeruginosa

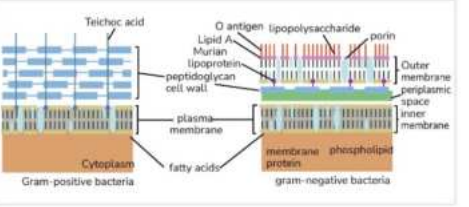
Biofilm advantages:

- Helps in adherence
- Anti- phagocytic
- Antibiotic resistance

Cell wall

00:09:10

Gram positive PPPT P-Positive P-Peptidoglycans P-Pentapeptide cross bridge T-Teichoic acid	Features	Gram negative
100 layer thick	Thickness	1-2 layer thickness
2-5 %	Lipid content	15-50%
Peptidoglycans Lipoproteins Teichoic acid	Composition	Lipopolysaccharide Lipoproteins Peptidoglycan
Absent	Outer membrane	present
Absent Porins-	Periplasmic space Porins	Periplasmic space + Porins +
Present	Teichoic acid	Absent
Pentapeptide cross bridge present	Pentapeptide cross brigde	Pentapeptide cross bridge absent
present	NAM-NAG	present



- Peptidoglycan layer:
  - Thick - gram positive
  - Thin - gram negative

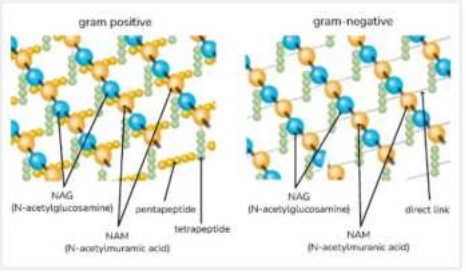
Teichoic acid-

- Present in gram positive organism
- Used for attachment

Lipopolysaccharide

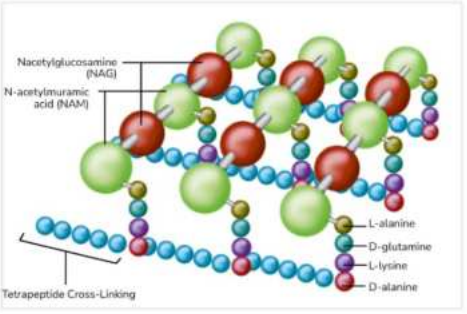
OPA - from outside to inside

- O - O antigen
  - Most variable part
  - E.g, salmonella typhi - widal test
    → We study O antigen and H antigen
- P - polysaccharide
- A - lipid - A
  - Have endotoxin
  - Present only in gram negative organism
  - Have lethal effect, pyrogeneticty



- **NAM - N-AcetylMuramic Acid**
  - 4 amino acids are attached to it
  - Different in gram negative and gram positive organisms
- **NAG - N - Acetyl Glucosamine**

Gram positive (Mnemonic-AGLA)	Gram negative (Mnemonic-AGMA)
Alanine	Alanine
Glutamine	Glutamine
Lysine	Meso diaminopimelic acid
Alanine	Alanine



- **Q. Identify the cell wall**
- AGLA in NAM
- Cross bridge - pentapeptide
- So, this is gram positive cell wall

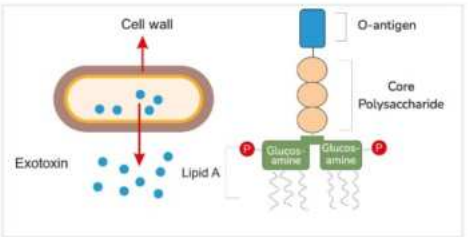
Demonstration of cell wall

Mnemonic-**R**egistered **M**edical **P**ractitioner Dr

- R - reaction of antibody
- M - mechanical rupture of cell, microdissection
- P - plasmolysis
- Dr- differential staining
  - Gram staining

Endotoxins vs exotoxins 00:21:35

Endotoxin	Exotoxin
Present in lipid - A In gram negative cell wall	Made of proteins
Gram negative bacteria	In both GNB and GPC
Heat stabile	Heat labile
Low antigenicity	High antigenicity
Cannot	Toxoid production is possible Can be used for vaccine production
Needed in large amounts to create effects	Small amounts can mediate effects
Produces constant effect	Variable effect
Release on lysis of cell	Released as secretions Except botulinum toxin - released on lysis of cell



- GNB - Lysis → release endotoxin
- Gram positive or gram negative - exotoxins are secreted

Exception:

- Only gram positive that shows endotoxin - LISTERIA
- Botulinum toxin-the exotoxin are released by lysis of the cell

Endotoxin

- Endotoxin binds to Toll like receptors (TLR) - 4

Demonstration

- **Limulus amebocyte lysate assay** - LALA
- Horseshoe crab - limulus
- Limulus amebocyte lysate mixed with clinical sample
- If there is gelling of lysate → endotoxin is present

Cell wall deficient forms - L form

- Identified in Lister institute - London
- Studied in Streptobacillus moniliformis
- Discovered by Kleinbeger

L form

- **Unstable L forms**
  - Lose their cell wall when treated with penicillin
  - Can revert back to their original form
  - **Protoplast**
    → Gram positive organisms
    → Whole cell wall lost
  - **Spheroplast**
    → Gram negative
    → Some part of cell wall remains
- **Stable L forms**
  - Mycoplasma
  - Permanently loss cell wall
  - Unable to revert to original form

Outer membrane

- In gram negative organisms

Cell membrane 00:31:26

- Dip in cell membrane - Mesosomes / Chondroids
  - Respiratory unit in bacteria
  - Seen in both gram positive and gram negative
    → But prominent in gram positive
  - It is an invagination of cell membrane

Ribosomes

- 70S - (50S + 30 S)

Nucleus

- Single circular
- Double stranded DNA

Inclusions

- Corynebacterium diphtheria - Volutin granules

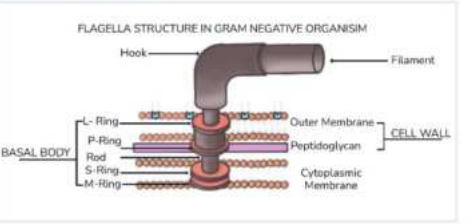
Flagella

- 3 - 20 micron in length
- 0.01 - 0.013 micron thick
- Locomotion
- Flagella antigen - H antigen
- **Widal test** for typhoid
  - O antigen - cell wall
  - H antigen - flagella

Endo flagella

- In spirochetes
  - Treponema
  - Borrelia
  - Leptospira
- Originates from periplasmic space
- In gram negative organism

Structure of flagella

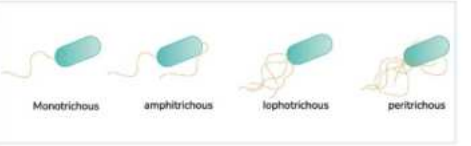


- 3 parts
  - Basal body
  - Hook
  - Filament

Flagella in gram positive and gram negative differs by Basal body -

- Gram negative - 4 rings
- Gram positive - 2 rings

Based on Location - flagella can be



- One flagella on side
- Vibrio
- Pseudomonas

- **Amphitrichous**
  - Flagella on either side
- **Lophotrichous**
  - Entire tuft of flagella on one side
  - Helicobacter
  - Campylobacter
- **Peritrichous**
  - Flagella all around the periphery
  - **PER**itrichous
  - **PR**- Proteus
  - **E**- E.coli
  - Listeria

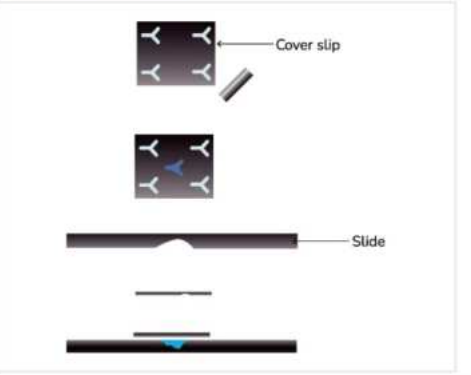
Types of motility

- **Tumbling**
  - Listeria
- **Darting**
  - Like shooting star
  - Vibrio
  - Campylobacter
- **Cork screw**
  - Looks like a tight screw - treponema
- **Twitching**
  - Seen with Eikenella
  - Trichomonas vaginalis
    → Cause Strawberry cervix
- **Falling leaf**
  - Giardia lamblia
- **Differential motility**
  - Motile at 22-25 ° C
  - Non motile at 37 ° C
  - Seen in Yersiniosis, Listeriosis

- **Swarming**

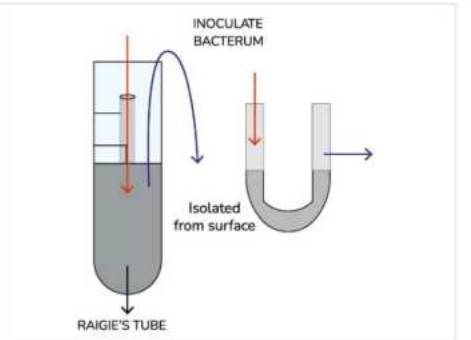
- Considered as growth pattern - in blood agar
- **Mnemonic-PVCS**
- P- Proteus
- V- Vibrio parahaemolyticus
  → Vibrio alginolyticus
- C- Clostridium tetani
  → Bacillus Cereus
- S- Serratia

Demonstration of motility



- **Hanging drop motility**
  - Organism on Cover slip
  - Put a slide on top of it
  - And then invert it
  - You will see something hanging from the cover slip
- **Semi-solid agar method**
  - Agar - normally used at 2 % concentration
  - At very less concentration-0.5 % - organism shows motility

- **Craige's tube**



- Tube inside another tube
- Bacteria inoculated in small tube
- And then isolate from the surface of the big tube

- **U tube**
  - Organism put on one end of U tube
  - And then isolated from other end

Flagella Stain

- Cannot be looked under light microscope -as this is very thin
- Dark Field Microscope is used
- Or use LEIFSON and RYU'S stain
- Mordant - Tannic acid

Fimbriae / pili

- Hair like
- Adhesion
- Antigenic
- Primary purpose - attachment
- If used for mating between male and female bacteria - Sex pili
- This process is called conjugation

Detection

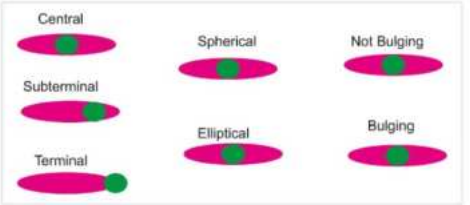
- **Hemagglutination**
  - E.coli,klebsiella,gonococci
  - Gonococcus - divided based on hemagglutination
- **Surface pellicle test**
  - Thin layer is produced on the surface of the broth

Exception:

- Flagella mainly used for locomotion
- Pili used for attachment
  - Type IV pili - in neisseria
  - Used for locomotion
  - Flagella independent motility

Spores 00:53:28

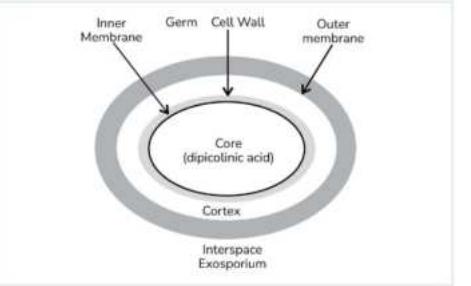
- Resting/dormant state
- Under unfavorable conditions, bacteria goes to dormant stage



Types of spores

- **Based on bulge**
  - Non bulging
    → Bacillus
  - Bulging
    → Clostridium
- **Based on location**
  - Sub terminal - most common location
  - Terminal
    → C. tetani
      - Round terminal spore
      - Drumstick appearance
    → C. tertium
      - Oval terminal spore
      - Tennis racket appearance - tertium
- Central spores
  - B. cereus
  - C. bifermentans

Parts of spore



- **Center - core**
  - Made of dipicolin ic acid
  - Makes it heat resistant

Outside the core

- **Cortex**
- **Coat**
  - Makes it chemical resistant
  - **Exosporium**

Innermost to outermost

- Core
- Cortex
- Coat
- Exosporium

Spore formation

Refer Image 3.1

- Bacteria was dividing
- But under unfavorable conditions it forms spores
- 7 stages

- Stage 1 - axial filament formation
- Stage 2 - divides into spore
- Stage 3 - engulf spore
- Matured in stage 4,5,6
- Released in stage 7

Stains

- ZN stain - 0.25%of H_2SO_4
- Modified Ashby Stain - Schaeffer and Fulton -used for spore staining
  - Green color
  - Malachite green
  - Safranin red
- Moeller stain

Uses of spores

- Used as controls
- Geobacillus Stearothermophilus
  - Control in autoclave
- Spores of bacillus subtilis / atrophaeus / clostridium tetani
  - Control in autoclave

Difference between prokaryote and eukaryote

Prokaryote	Feature	Eukaryote
Called nucleoid No proper nucleus	Nucleus	All present
Single circular dsDNA	Chromosome	Multiple linear
Plasmids	Extra chromosomal DNA	Mitochondrial DNA
Absent	Organelles	Present
NAM NAG	Cell wall	Absent
Sterols absent	Cell membrane	Sterols +
70 S	Ribosomes	80 S
Present Site of Respiration	Mesosomes	Absent

- Prokaryote lacking cell wall - mycoplasma
- Prokaryote having sterols in cell membrane - mycoplasma
- Prokaryote lacking muramic acid in cell wall - chlamydia

MCQs

Q. While examining a bacterial slide under the microscope, you notice that the organism lacks a cell wall. The cell deficient organism that could have been on the slide is

A. Chlamydia
B. Mycoplasma
C. Streptococcus
D. Anaerobes

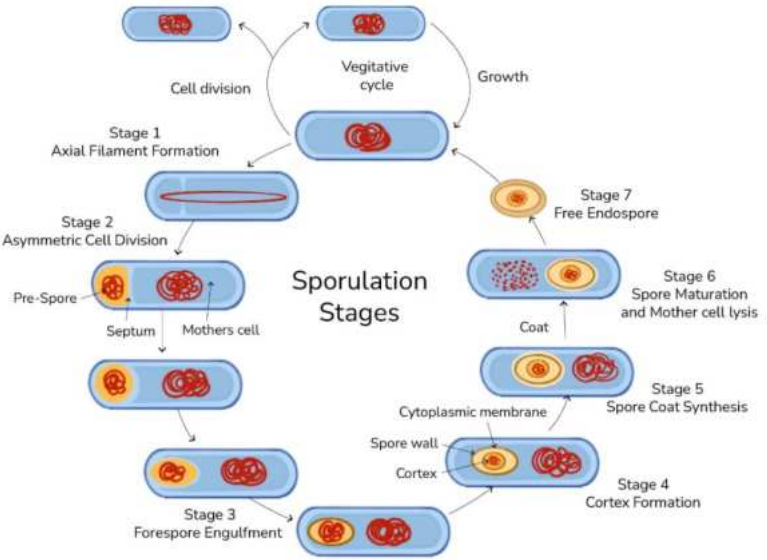
Q. A sputum sample was sent for testing in the microbiology laboratory. The gram staining showed the presence of Gram positive cocci which correlates with bacterial cell wall properties. Which of the following statements is false regarding the bacterial cell wall?

A. Cell wall of Gram positive bacteria is thicker than that of Gram negative bacteria
B. Teichoic acid is present in the cell wall of gram negative bacteria
C. Region II of LPS is core polysaccharide
D. LPS is responsible for antigen specificity of gram negative bacteria

Explanation:

- More peptidoglycans
- Teichoic acid - cell wall of gram positive
- Region II - polysaccharide

Image 3.1


Cell division
Vegitative cycle
Growth
Stage 1
Axial Filament Formation
Stage 2
Asymmetric Cell Division
Pre-Spore
Septum
Mothers cell
Sporulation Stages
Stage 3
Forespore Engulfment
Stage 4
Cortex Formation
Spore wall
Cortex
Cytoplasmic membrane
Stage 5
Spore Coat Synthesis
Coat
Stage 6
Spore Maturation
and Mother cell lysis
Stage 7
Free Endospore

4 BACTERIAL SHAPES AND PHYSIOLOGY

Bacterial Shapes (Cocci) 00:00:09

Shape	Name
Cocci in clusters (Grape like)	Staphylococcus Aureus
Cocci in chains	Streptococcus
Cocci in pairs (Diplococci)	Pneumococcus
Lens shaped (Diplococci)	Neisseria meningitidis
Kidney shaped (Diplococci)	Neisseria gonorrhoeae
Cocci in tetrads (Cluster in 4)	Micrococcus
Cocci in octate (Cluster of 8) as in 8)	Sarcina
Spectacle like appearance	Enterococcus

Bacterial Shapes (Bacilli) 00:03:29

Shape	Name
Bamboo stick appearance (B A as in BAmboo)	Bacillus Anthracis
Diplobacilli	Klebsiella Pneumoniae
Branching filamentous	Nocardia, Actinomyces
Curved GNB	Vibrio, bacters
Spiral	Spirochetes
Pleomorphic	Haemophilus Influenzae, Brucella

Bacterial Physiology 00:05:09

- Population doubling time
  - Example
    - → E.coli - 20 mins
    - → MTB - 20 hours
    - → M Leprae - 20 days
- **Total count:** Living and dead combined
- **Viable count:** Only living bacteria
- Definitely require Oxygen
  - Some require and some don't
- **Capnophilic:** Some that require CO2 is campylobacter
- **pH 6-8**
  - **Acidic pH:** Acidophiles
    - → Lacto: Lactobacillus
  - Alkaline pH: Alkaliphiles
    - → Vibrio (Causes vibrio cholera)
- Some needs light and some don't
- Temperature
  - **Psychrophile:** < 20° C
    - → **Psychrotrophs:** they grow in normal and lower temperatures (eg: yersinia, listeria)
    - → Grow better at low temperatures (4°C)
  - **Mesophiles:** 25°- 40°C
  - **Thermophile:** 55°-80°C
    - → Thermus aquaticus (Taq polymerase)

Q. Which are the organisms that require O2?

Ans.

- Obligate aerobes
- **Mnemonic**: Must Let Nagging Pets Breathe
  - MTB
  - Legionella
  - Nocardia
  - Pseudomonas
  - Bacillus
  - Brucella
  - Bordetella

Q. Which are the organisms that don't require O2?

- Obligate Anaerobes
- Choked By Air
  - Clostridium
  - Bacteroides
  - Actinomyces

Facultative anaerobes

- Staphylococcus
- Streptococcus
- Enterobacteriaceae
- Haemophilus
- Vibrio

Microaerophilic

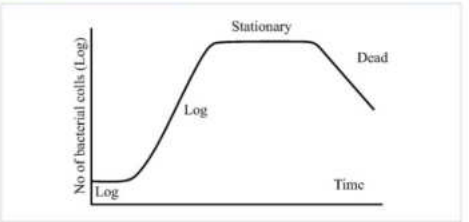
- 5% of oxygen needed
- The bacters
- M. Bovis

Quick Definitions

- **Bacterial Physiology**
  - **Autotrophs:** Can synthesize all compounds by using Co2
  - **Heterotrophs:** Cannot synthesize their metabolites, utilize CO2 and N2 from air

- **Lithotrophs:** Use inorganic compounds
- **Phototrophs:** Derive energy from from sunlight
- **Chemotrophs:** Derive energy from Chemical reactions

Bacterial Growth curve 00:16:03



Bacterial Growth curve

- **Phases**
  - Lag
  - Log
  - Stationary
  - Dead
- **Lag Phase**
  - No increase of number of bacteria
  - Cell adaptation
  - Increase in size, enzymes and metabolite

Q. When is the maximum size of the bacteria seen?
Ans. End of the lag phase

- **Log Phase**
  - Cell division
  - Smaller in size
  - Maximum metabolic activity
  - More susceptible to antibiotics
  - We get a uniform gram stain because all of them are living(viable)
- **Stationary Phase**
  - We see Some of the bacteria remain viable and some are dead
  - Bacterial growth cease due to depletion of nutrients
  - Viable bacteria=Non viable bacteria (Non uniform staining)
  - STAB
    → Sporulation
    → Exo Toxin production
    → Antibiotic production
    → Bacteriocin production
  - Antibiotic production happens in this phase
- Dead
  - Decline phase
  - Cell death
  - Involution forms

Q. A 65-year old blind man living in a rural area was admitted with a respiratory infection. There was no improvement despite treatment and the patient started showing septicaemia symptoms. Radio graphic images showed wide mediastinum. Bacillus anthracis along with some spores were isolated from blood culture. In which phase of bacterial growth spores are formed?

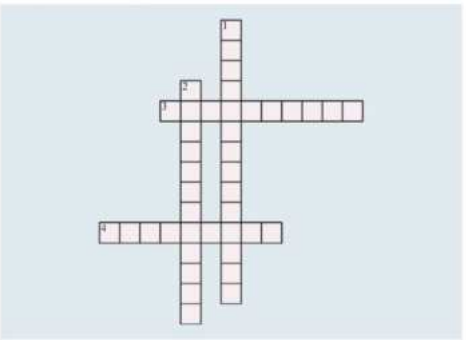
A. Decline phase
B. Lag phase
C. Stationary phase
D. Log phase

Q. A 9 year old boy was referred to the AIIMS NEW DELHI with a low-grade fever, cough and sore throat for 5 days. A swab was taken and inoculated into a suitable liquid culture medium and the growth of the bacterium was plotted. In a bacterial growth curve, in which phase is the maximum cell size obtained?

A. Beginning of lag phase
B. End of lag phase
C. Beginning of stationary phase
D. Log phase

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Father of modern microbiology
4. Game positive- contain a lot of peptidoglycans, which retain the dye longer

Down

1. Silver stains (impregnation method)
2. Disapproved theory of abiogenesis

5 BACTERIAL GENETICS

Basics

00:00:14

DNA	RNA
Deoxyribose sugar	Ribose sugar
4 nucleotides • 2 purines - AG • 2 pyrimidines - CT	4 nucleotides • 2 purines - AG • 2 pyrimidines - CU Uracil replaces thymine
Held together by H bonds	Held together by H bonds

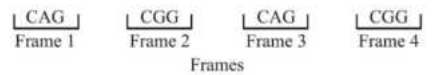
Mutations

- **Definition:** Permanent irreversible change in DNA
- **Types**
  - Point
  - Frame shift

Point Mutations

- **Transition**-Purine is replaced by purine/ pyrimidine is replaced by pyrimidine (same family)
- **Transversion**-Purine replaced by pyrimidine (opposite family)
- **3 types:**
  - **Silence**
    → No effect
  - **Missense**
    → **Ex:** Sickle cell anemia (HbA changed to HbS)
    → Sense has changed
  - **Nonsense**
    → **Ex:** Beta thalassemia (Hb production is stopped)
    → No sense at all
    → Results in formation of stop codons (UAA, UAG, UGA)

Frame Shift



- Frame = triplet
- **Deletion**

  - Deletion of nucleotide
- **Addition**
  - If any nucleotide is added in the sequence, frame is shifted

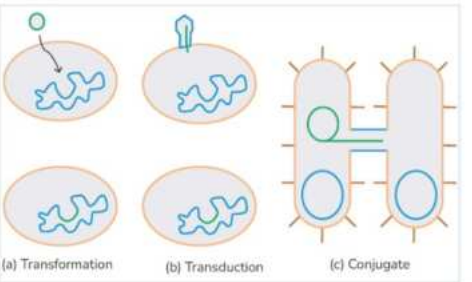
Addition of nucleotide

  - **Ex:** Thalassemia

Gene Transfer

00:07:20

- **3 methods**
  - **Transduction:** Bacteriophage is involved
  - **Conjugation:** Connection formed
  - **Transformation:** Direct entry to DNA

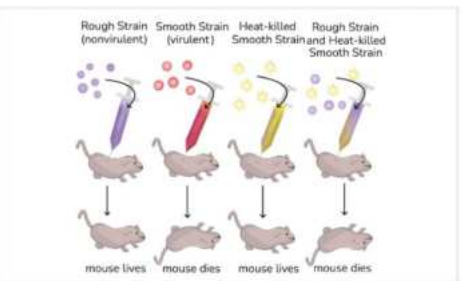


1. Transformation

- **Identified:** Griffith
- **Rediscovered:** Avery, McLeod, McCarty
- Transfer of free DNA
- Uptake of soluble DNA fragments by bacteria
  - Through cell wall
  - During log phase (division)
  - Required competence factor

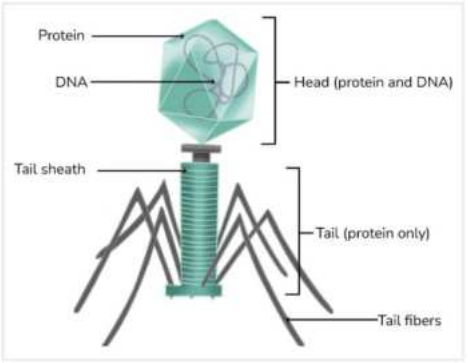
Griffith Experiment

- **Bacteria:** S.pneumoniae (capsulated+virulent+ smooth strain)



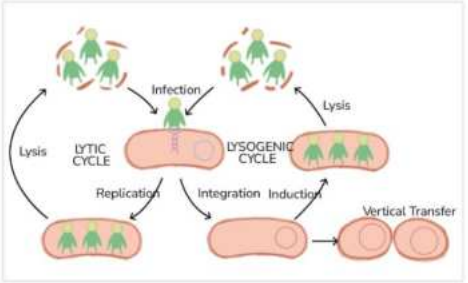
- **Unencapsulated strain:** Mouse lives
- **Capsule + Heat (Heat killed capsulated strain):** Mouse lives
- **No capsule + Heat killed capsulated strain:** Mouse dies
  - **Reason:** Virulence is transferred

2. Transduction



- Tadpole shape/ circular/ filamentous
- This is a DNA virus (mediator)
- First demonstrated in salmonella
- MC mode of drug resistance in S.aureus

Bacteriophage Cycle

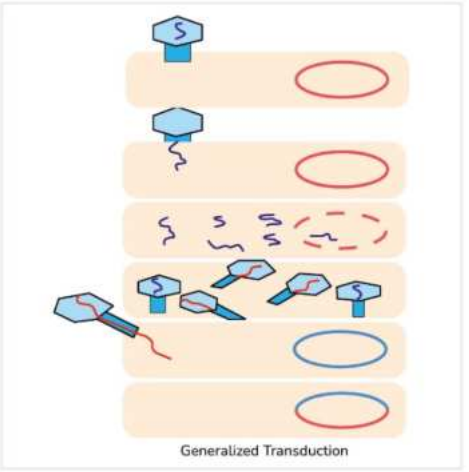


- **Two cycles**
  - **Lytic:** Lysis of infected bacteria
  - **Lysogenic:** Combine with bacteria

Types of Bacteriophages

- **Virulent phage**
  - Dangerous
  - Follow only lytic cycle
  - **Ex:** T_1 and T_2 phage of E.coli
  - Less dangerous
  - Follow both lytic and lysogenic
  - **Ex:** Lambda phage of E.coli

Generalized Transduction



Bacteriophage injects DNA into Donor bacteria

↓

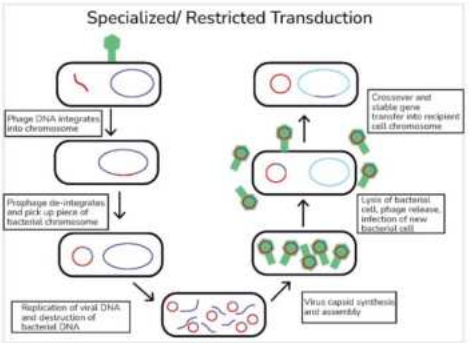
New bacteriophages are formed (Mispackaging is seen - took donor DNA)

↓

New bacteriophages give donor DNA to Recipient bacteria

* Lytic cycle is followed

Specialized/ Restricted Transduction



↓

Viral DNA + Donor DNA (fusion - Prophage)

↓

Separation of DNA (Induction) by UV light

↓

But some Donor DNA is left

↓

New bacteriophages with donor + self DNA are formed

↓

New bacteriophages give donor DNA to Recipient bacteria

Bacteriophage Cycle

- **Lytic cycle:** Bacterial cell breaking
- **Lysogenic:** Fusion is seen
  - **Vertical Transfer**
    → Fusion may get into vertical transfer (no separation)
    → The offsprings will have new properties

Generalized vs Specialized Transduction

Generalized	Feature	Specialized
Lytic	**Cycle**	Lysogenic
Mis-packaging	**Occurs due to**	Defective excision during induction of prophage
Virulent or temperate phages	**Mediated by**	Only temperate phage

Important Information

- **Virulent:** Lytic cycle
- **Temperate:** Both

Bacterial Toxins Coded by Lysogenic Conversion

- **A - A** and C toxins of S.pyogenes
- **B** - C and D toxins of **B**otulinum
- **C - C**holera
- **D - D**iphtheria
- **E - E**nterohemorrhagic **E**.coli

3. Conjugation

- **Demonstrated first by:** Lederberg and Tatum
- **Bacteria:** E.coli K12 strain
- MC mode of MDR using plasmids is conjugation

Important Information

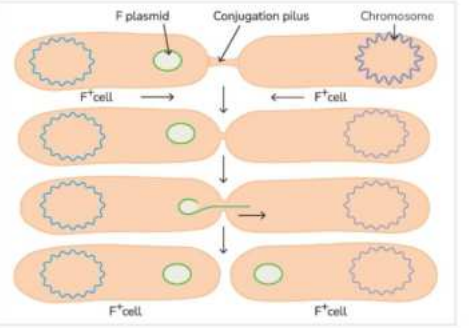
- MC mode of MDR **except:**
  - S.aureus - Transduction
  - Strep.Pneumoniae - Transformation
  - All others - Conjugation

- **Plasmid:** Extrachromosomal circular dsDNA
- **Episome:** Plasmids may combine with cell DNA
- **2 Types** (based on transfer)
  - **Conjugated:** Both horizontal (bacteria-bacteria) and vertical transfer (bacteria-offsprings)
  - **Non-conjugated:** Only vertical (bacteria-offsprings)
- **4 types** (based on what they carries)
  - **Virulence:** Code for virulence factors like toxins
  - **Fertility:** Code for fertility factor (male or female)
  - **Resistance:** Code for resistance
  - **Col plasmids:** Codes for bacteriocin

F-Plasmid

- **F:** Fertility factor
  - **Male** - Present (F^+)
  - **Female** - Absent (F^-)

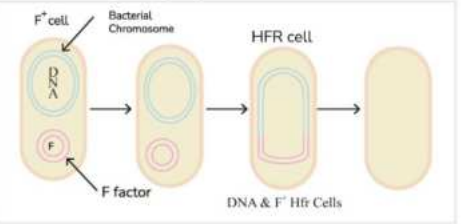
F^+ with F^-



- Conjugation tube formed between F^+ and F^-
- Other name: Bridge
- Formed by sex pili
- Fertility factor is copied
- Both has fertility factors (both males)

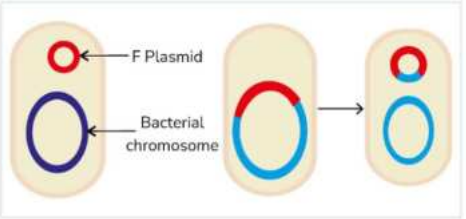
Hfr with F^-

- Cell DNA combined with F^+ = Hfr
- **Hfr cells**: High Frequency Recombinant cells



- Conjugation tube formed
- Tube broken mid way
- Leads to incomplete transfer
- F receives only small amounts of donor DNA and donor plasmid
- Hfr with $F^- = rF^-$

F' (Sex Duction)



- Hfr splits to donor plasmid and donor DNA
- But donor plasmid carries some donor DNA with it
- Thus called F prime cell (F')
- When the F' conjugates with F^-
- F will get a copy of F' plasmid
- Thus, $F' \times F^- = F'$ and F'

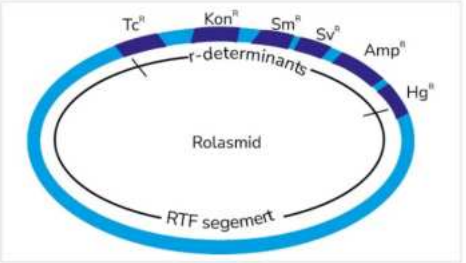
Summary

- F^- (plasmid)
- F^- (no plasmid)
- Hfr (DNA + plasmid)
- F' (plasmid separated along with DNA)
- $F^- \times F^- = F^+ F^+$
- $Hfr \times F^- = F^-$
- $F' \times F^- = F' F'$
- Trick: Male dominance (patriarchal society)

Transferable Drug Resistance

00:42:26

- **Resistance** = Resistance Transfer Factor (RTF) + Resistance determinants ®
- RTF can carry many r at same time



- More dangerous
- Difficult to treat

Mutational Drug Resistance

00:43:58

- Due to single mutation
- Easier to treat
- **Ex:** Rifampicin resistance in TB is due to rpoB gene

Restriction Endonucleases

00:44:48

- Produced by bacteria for defense against foreign bacteria
- Every RE needs a co-factor Mg^{2+}
- There are 4 types of RE
- Type 2 is used for
  - Genetic engineering
  - Drugs
  - Tests
- Restriction site is identified by RE, which is very short
- Known as the Palindromic sequence (Left to Right and Right to Left are same)

Important Information

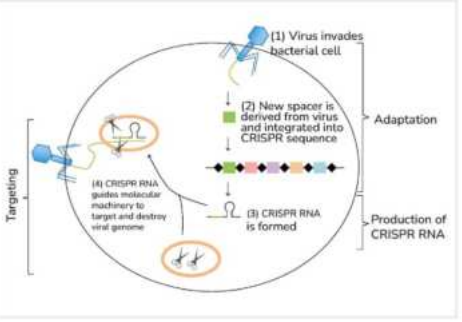
- Bacteria protects its own genome from RE by Methylation

CRISPR-Cas9

00:49:00

Trick

- **Cas9** - Scissors
- **C** - Clustered
- **R** - Regularly
- **I** - Interspersed (spaces)
- **S** - Short
- **P** - Palindromic
- **R** - Repeats



- Virus is killed and is saved in memory (interspersed - spaces)
- Cas9 will kill the virus

Important Information

- CRISPR-Cas9 is bacteria's defense system

MCQ

Q. Transductions is:

A. Bacterial-medial viral recombination

B. Viral-mediated viral recombination

C. Viral-mediated bacterial recombination

D. Bacterial-mediated bacterial recombination

Q. The cell in which F factor carries along with it some chromosomal genes are known as:

A. F⁻ cell

B. F⁺ cell

C. F' cell

D. F" cell

Q. F factor integrates with bacterial chromosome to form:

A. Hfr

B. RTF'r

C. F

D. RTF

Q. An 86-year-old male who is a known case of DM × 3 years presented to ER with a complaint of a non-healing foot ulcer. The patient also complained that he is been taking different antibiotics for a long time for his foot ulcer but the medications had no results. The doctor suspected drug resistance. Which of the following statements is false regarding drug resistance?

A. In mutational drug resistance one drug resistance at a time is seen

B. Virulence is decreased in transferable resistance

C. Drug combination can prevent Mutational drug resistance

D. High degree of resistance is seen in transferable drug resistance

Q. The restriction endonuclease is a defence mechanism in the bacterial system against foreign DNA such as viruses. But how is it able to protect its own DNA?

A. By methylation of bacterial DNA

B. By methylation of foreign DNA

C. By phosphorylation of bacterial DNA

D. By phosphorylation of foreign DNA

Q. Function of CRISPR is?

A. Bacterial genome editing to protect against viral infections

B. Bacterial genome editing to protect against human immunity

C. Mechanism for viral immunity

D. Genetic reactivation

6 CULTURE MEDIA

- **History**
  - Liquid culture media
    → By Sir Louis Pasteur
    → Urine, meat broth
  - Solid media
    → Robert Koch
    → Potato

Agar

- **Origin:** Seaweeds (red algae of gelidium and gracilaria).
- **Composition:** Polysaccharide
- Nutritive value: NONE
- Temperature:
  - Melts 98°C.
  - Solidify 42°C.
- Concentration:
  - SOLID: 2%
  - Semi solid: 0.5%
  - Firm 5-6%

- Whenever we want to inhibit the Swarming motility, it will inhibit the motility.
- Peptone
- Agar
- Water
- Electrolytes
  - All 4 will remain constant.
- Meat extract/ Yeast extract
- Serum/Blood

Simple Media / Basal Medium / Basic Medium

- Only Non-Fastidious organisms will grow.
- Peptone Water
  - Peptone 1%
  - NaCl 0.5%
  - Water

- Nutrient Broth
  - Peptone 1%
  - NaCl 0.5%
  - Water
  - 1% Meat extract
- **Nutrient Agar:**
  - Peptone 1%
  - NaCl 0.5%
  - Water
  - 1% Meat extract
  - 2% Agar

Enriched Media

- Used to grow Fastidious organisms.
- Egg/Blood/Serum is added to provide extra nutrition.
- **Blood Agar**:
  - Autoclaved nutrient agar cooled to 50 °C.
  - 5-10% sterile sheep RBCs

  - Made rich by adding blood.
  - This is a differential media.
- **Chocolate agar**
  - Autoclaved nutrient agar cooled to 70 °C.
  - 5-10% sterile sheep RBCs
  - More nutritious
  - Releases many factors.
    → V and X factors

- **Loeffler's serum slope**
  - Corynebacterium diphtheria
    - → Klebs Loffler's media
    - → Enriched media
    - → 8 hours (8 letters in Loffler and enriched)

    - → Inspissation is done.
- **Lowenstein Jensen medium:** Tuberculosis
  - Composition is like an English breakfast.
    - → Coagulated hen's egg
    - → Mineral salt solution
    - → Asparagine
    - → Malachite green

  - Inhibits other media.
- Liquid media that is selective in nature

Important Information

- Whenever there is word media it is solid.
- Whenever there is the word broth it is liquid.
  - Enriched media: Adding something extra.
  - Enrichment broth: Liquid media with selective media characteristics.
  - Selective media: Adding something which doesn't let other organisms grow.

Enrichment Broth

- Alkaline peptone water.
  - Vibrio
- Selenite F broth
- Tetrathionate broth
  - Salmonella
  - Shigella

Selective Media

- **MacConkey Agar**
  - Made of (MNEMONIC- PLANT)
    - → **P**eptone
    - → **L**actose
    - → **A**gar
    - → **N**eutral Red
      - Fermentation of lactose is pink.
      - If not, it is pale.
      - It's an indicator.

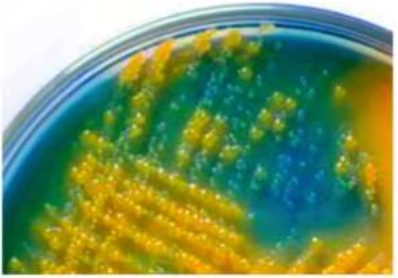
    - → **T**AUROCHOLATE (Bile salt) (selective)
  - SELECTIVE medium
  - DIFFERENTIAL medium
  - INDICATOR medium
- **Mannitol Salt agar**
  - Selective Media (only lets S.aureus grow).
    - → Mannitol fermentation
    - → Yellow color
  - Differential Media

- Indicator Media- Phenol Red

Staphylococcus aureus

Staphylococcus epidermidis

- **CLED**
  - Cysteine lactose electrolyte deficient.
    → Differential media.
    → Selective media
    → For lactose fermentation yellow colonies.



  - Indicator-bromothymol blue
  - Better than MacConkey- less inhibitory (non-selective)
- **Thiosulfate citrate bile salt sucrose**
  - TCBS
  - Vibrio
  - Bile salt makes it selective.

Vibrio cholerae on TCBS Agar

Vibrio parahaemolytics on TCBS Agar

  - Indicator: Bromothymol blue.
  - Differential media.

	Indicator	Color
Mannitol Salt agar S.aureus	Phenol red	MF (yellow)
TCBS V.cholera	Bromothymol blue	Sucrose fermentation SF (yellow)
CLED	Bromothymol blue	Lactose fermentation Yellow
McConkey	Neutral red	Lactose fermentation Pink

- Only McConkey the fermentation is pink.
- CVBA (crystal violet Blood agar)
  - Streptococcus pyrogens

- Modified Thayer Martin Medium
  - Neisseria M
  - Neisseria G
- Cetrimide Agar
  - Pseudomonas

- PLET (polymyxin Lysozyme EDTA thallous acetate).
  - Bacillus Anthracis
- MYPA (Mannitol egg yolk phenol red polymyxin) and PEMBA.
  - B.Cereus

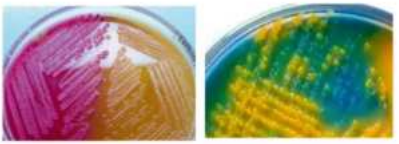
- DCA (deoxycholate citrate agar)
- XLD (Xylose lysine deoxycholate)
  - Salmonella
  - Shigella

Transport media: Sach's Buffered Glycerol Saline

- **Potassium Tellurite Agar**
  - Black color
  - C. diphtheriae
    → Selective media: 48hrs

Differential media

00:30:49



Transport Media (maintain the vitality of bacteria).

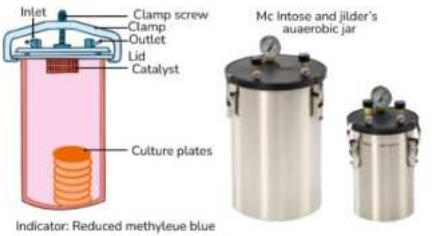
- Stuart's medium
- Amies medium
  - Neisseria
- VR medium
- Cary Blair medium
  - Vibrio
- Pike's medium
  - Streptococcus pyogenes
- Sach's Buffered Glycerol Saline
  - Salmonella and shigella sisters

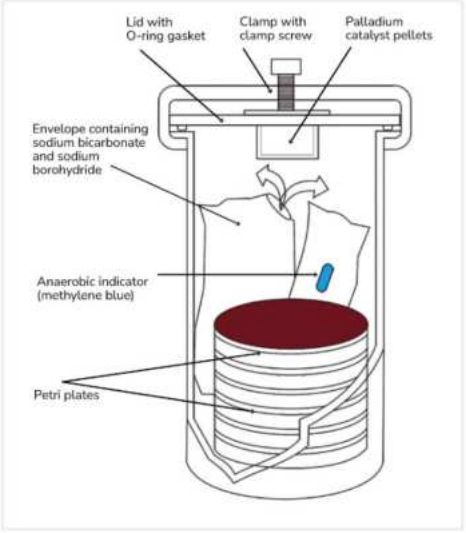
Anaerobic Media

- **Clostridium**
- RCMB (Robertson's cooked meat broth).
- **Proteolytic**
  - Turns meat black.
  - Clostridium tetani.
- **SACCHAROLYTIC**
  - Turns pink.
  - Clostridium perfringens
    → Per Sac Pink

Anaerobic methods

McIntosh and Fildes jar





- On the culture plate a packet is kept
- Methylene blue is placed too.
- Methylene blue will look blue till O2 is present.
- It will become colorless if O2 is removed.

Latest Gaspak

- Takes lots of manual effort.

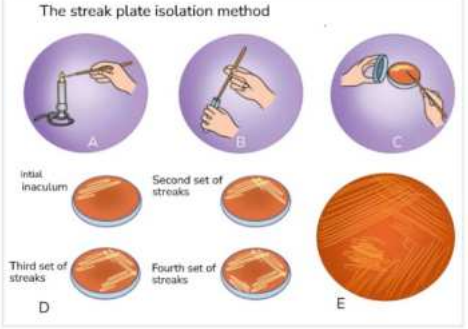
Anaerobic methods

- Anoxomat.
- Glove Box/Anaerobic chamber.
- PRAS (Pre-Reduced anaerobic system).
- **GASPAK (method of choice for anaerobiosis).**

Culture Technologies

00:41:45

Streak Culture



- We take a nichrome loop and sterilize it.
- Dip in sample
- Start streaking at 90 degrees.
- We can obtain Pure colonies.

Stroke Culture

- If done in a test tube: Sterilize, pickup sample and stroke in Agar slope/slant.
- **Uses:** Provide a pure growth for the bacterium of slide agglutination and other diagnostic tests

Stroke Culture
- Stroke culture is made in tubes containing agar slope/ slant.

- Uses
- Provide a pure growth of bacterium for slide agglutination and other diagnostic tests.

Lawn culture

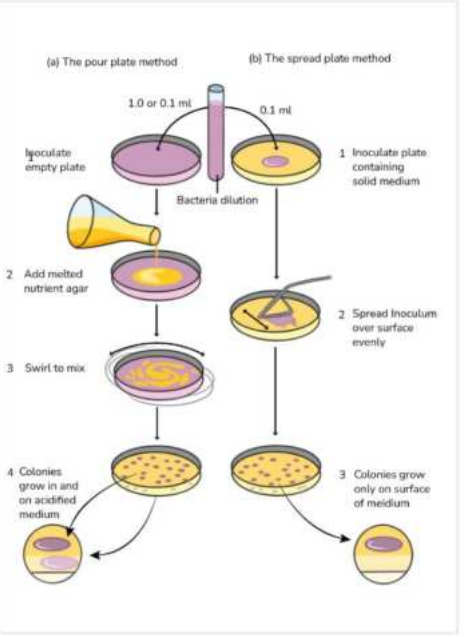
- Lawn cultures are obtained by flooding the plate's surface with the bacterium's suspension.
- AKA carpet culture.
- **Mainly used for:**
  - Antibiotic sensitivity testing.

  - Take the sample directly and it put on a culture plate.

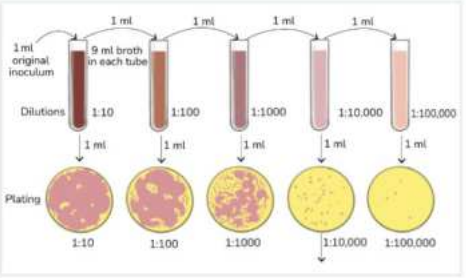
Stab Culture

- It is performed by a straight wire, charged with culture material, by puncturing deep inside the agar.

- Gelatin liquefaction
  - Inverted Fir tree: shown by B. anthrax.
  - Fir tree: C. tetati
  - Napiform/turnip: V.cholera
- It is used to:
  - Demonstrate gelatin liquefaction.



- **Pour plate method.**



1. Add bacteria first.
2. Then add agar.
   - Quantification of bacteria

- **Spread plate method.**
  1. You have agar.
  2. Then add patients' samples (Bacteria).

Antibiotic Sensitivity Testing

00:51:00

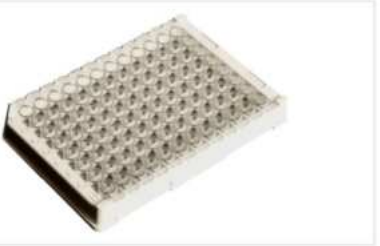
- Minimal inhibitory concentration MIC.
  - lowest concentration of the antibiotic that inhibits the growth of the pathogen in the medium.
- Minimal bactericidal concentration MBC.
  - lowest concentration of antibiotic that kills the bacteria in a medium.

Methods

- Dilution method
- Disc diffusion method
- E-Test (combo of first two methods)
- Automated methods

Dilution method

- **Inoculum prepared.**
- Add E. coli to sterile peptone water and incubate.
- After growth measures the turbidity using a spectrophotometer/ Mc Farlands standard media
- 0.5 Mc Farlands = 1.5 x 108 CFU/ml is the standard.
  - There are 1.5 x 108 colony-forming units.
- **Micro broth**
  - Done with 96 well plate.
  - Saves medium and time used.
- **Macro broth**
  - Done in test tubes.
  - Requires time.

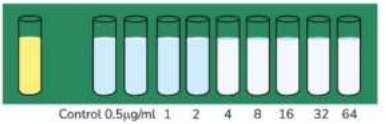


- Growth
- At 4µg/ml, bacterial growth is inhibited. For the tested bacterial sample, this is the MIC of the antibiotic. This is compared with the breakpoint concentration laid down by CLSI and determines if the strain is sensitive or resistant.

Procedure :

Control has no antibiotic

1 ml of standard inoculumn is added to each test tube and incubated overnight



Serial dilution of antibiotic after overnight incubation with turbidity

Observation : First 3 test tubes are turbid indicating microbial

Disc Diffusion Method or
Kirby Bauer Disc Diffusion Method

Lawn culture

↓

Incubate overnight.

↓

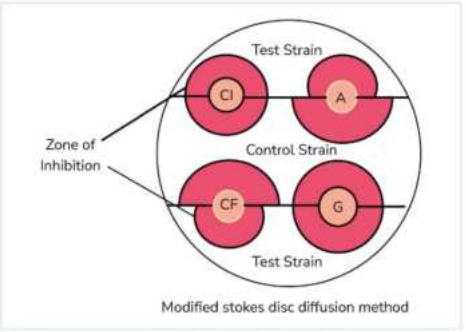
Dispense antibiotics discs 25mm distance.

↓

Overnight incubate at 37°C

- Agar used: CAMHA.
  - Cation adjusted Mueller Hinton agar.
- Sample spread out.
- If the zone of inhibition is seen the bacteria are antibiotic sensitive.
- Can measure the zone of inhibition.
- Disadvantage: Cannot quantify (Minimum inhibition concentration).
- Zone of inhibition present- Sensitive.
- Zone of inhibition absent- Resistant.

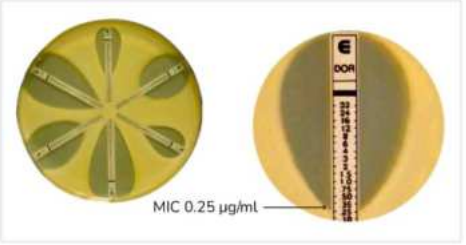
Stoke's Disc Diffusion Method



Modified stokes disc diffusion method

- Control tells validity.
- Can Compare (By control strain).

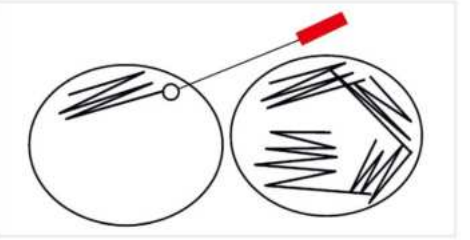
Epsilometer Test



- Aka E-test
- We can see a zone of inhibition.
- We can see concentration marked.
- We can see MIC.

Q. Not true about agars?
A. Source of nutrition
B. long chain polysaccharide
C. Melts at 95-98°C
D. Solidifies around 40°C

Q. A patient suffering from cough, sore throat, fever, and chills visited the morning session of the OPD. His sample was sent for culture. The lab technician performed the culture method shown in the figure below. Identify the method.
A. Streak culture
B. Lawn culture
C. Stroke culture
D. Pour plate method.



Q. A patient with complaints of a burning sensation when urinating and lower abdominal pain presented to the outpatient department. She was on antibiotics for a few days but there was no improvement. A urine sample was collected and sent for antibiotic sensitivity testing in the laboratory. What is the most commonly used Disc diffusion method in antibiotic sensitivity testing?
A. Kirby Bauer method
B. E test method
C. MIC method
D. Stokes method

Q. A 35-year-old male resident presented with a fever and cough. He was well until 3 days earlier when he suffered the onset of nasal stuffiness, mild sore throat, and a cough productive of small amounts of clear sputum. The physician sent him for a sputum culture and the microbiologist used blood agar for culturing it. What type of media is blood agar?
A. Enriched media
B. Indicator media
C. Enrichment media
D. Selective media

Q. A 40-year-old female presented to the OPD with a burning sensation while urination, pelvic pain, and an increase in urge and frequency while urination. Antibiotics given showed no results. So, the sample was collected and sent for an antibiotic sensitivity test. The inoculum prepared should match which of the following Mc farland standard?

A. Mcfarland standard 0.5
B. Mcfarland standard 1
C. Mc farland standard 2
D. Mc farland standard 3

Q. A 22-year-old female presents with complaints of frequency and some urgency associated with painful micturition. The doctor suspects UTI & sends her urine sample to the laboratory for testing. The microbiologist decides to grow the culture on MacConkey agar. Which of the following is a constituent of MacConkey agar?

A. Lactose
B. Bile salt
C. Neutral Red
D. All of the above

Q. Antibiotic sensitivity testing is carried out to determine the appropriate antibiotics to be used for a particular strain isolated from clinical specimens. The most commonly used medium for this is:

A. CLED agar
B. Nutrient Agar
C. Mueller-Hinton agar
D. Salt milk agar

7 STERILIZATION AND DISINFECTION

Definitions

- **Sterilization**: Removal of pathogens including spores.
- **Disinfection**: Removal of pathogens but not spores.
- **Asepsis**: To make the body surface/skin/ wounds free of microorganisms.

Sterilization Methods 00:01:00

- **2 types**
  - **Physical methods**
    - → Heat
    - → Filtration
    - → Radiation
  - **Chemicals methods**

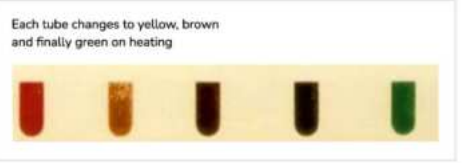
Heat	
Dry heat	**Moist heat**
• Direct contact with flames, or sunlight ○ Candle ○ Flame burners ○ Sunlight ○ Hot air oven ○ Incinerator	
• Mechanism (C O D E) ○ Charring ○ Oxidative damage ○ Denaturation of proteins ○ Elevation of Electrolytes	• Mechanism ○ Coagulation and denaturation of proteins ○ Moist heat is considered better. → Steam is created and acts as latent heat. → Example boiled water. The steam will settle on whatever object we are going to sterilize

Hot Air Oven

- Temperature and duration
  - 160 ° for 2 hr
  - 170 ° for 1 hr
  - 180 ° 30 mins
- **Uses**
  - Glassware
  - Cotton swabs
  - Wax,oils,dusting powders,gel
  - Greasy paraffin wax
  - Store metallic sharps or nonmetallic sharps.
- There are certain controls to check whether the machine is working or not.

Methods for Sterilisation Control

- **For Dry Heat**
  - **Physical:** Temperature monitoring by thermocouples
  - **Chemical:** Brownie's tube (green spot).
    - → Brownie's tube is kept inside hot air oven along with sharps and dusting powder
    - → If we get a green spot, then sterilization is complete.



  - **Biological:** Clostridium tetani or Bacillus subtilis / atrophaeus

Incineration

- All hospital waste goes there.
- There are 2 chambers.
  - 700° - 800°
  - 1000° - 1100°

- Used for
  - Hospital waste.
  - Human anatomical waste.
  - Animal carcasses.
  - Soiled bedding, dressings
  - Expired cytotoxic drugs.

Moist Heat

Mechanism

- Coagulation and denaturation of proteins
- Moist heat is considered better

↓

Steam

↓

Now act as "Latent Heat"

↓

Steam will settle on surface

Below 100°C	At 100° C	Above 100°C
• Inspissation • Pasteurization	Boiling	Autoclave
	Tyndallization	
Vaccine water bath		

Pasteurisation

- Milk is pasteurized.
- Holder Method
  - 63°C for 30 minutes
- Flash Method
  - Heat at 72°C for 20 secs and then rapidly cool at 13°C
  - Flash method is better as it kills Coxiella burnetii
  - Ultra-high-temperature method
    - → Done in foreign countries.
    - → 149°C 1-2 secs

Test for Efficacy of Pasteurization

- Phosphatase test
  - Milk has an enzyme called phosphatase.
  - If pasteurization is the done enzymes will get denatured.
  - On adding the substrate there is no color change.
- Coliform test
  - If milk is pasteurized there is no growth on Maconkey agar

Inspissation

- 80°C x 20-30 mins x 3 consecutive days.
- Used for
  - LJ media for (TB)
  - LSS for (C.Diph)

Vaccine water bath

- Water bath
- 60°C x 60min
- Bacterial vaccines are heat inactivated.

At 100°C

- Boiling: At 100°C for 15 min
- Does not kill spores.
- They came up with Tyndallization
  - 100°C x 20min x 3 days.
- T20
- Used in every media that includes gelatin and sugar.
- TCBS, XLD, DCA, SELENITE F BROTH.

Above 100°C

- Autoclave
  - 121°C x 15 min x 15 psi(pound sq. inch)
- If you want to kill prions
  - 134°C x 1.5 hrs

- Geobacillus StearoThermophilus

- **USES**
  - Instruments
  - Aprons
  - Sutures except Catgut sutures.
  - Media except for LJ, and LSS.
    → Because LJ and LSS are in <100°C
  - Sputum

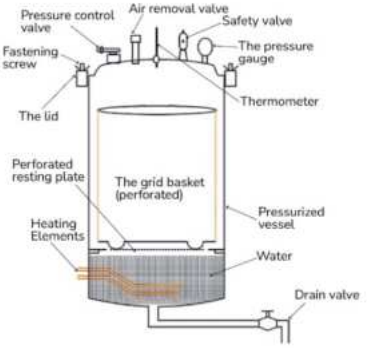
Pressure cooker type

Common laboratory autoclave

Horizontal autoclave

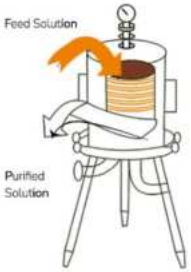
Large automatic hospital autoclave

Vertical Autoclave



- Pore size- 0.22μ
- Types of filters
  - Depth filters
    → Candle filters: Water purifier

→ Seitz filters
- There are asbestos sheets for filtration.
- Not used at all



- Glass filter
  - Membrane filters
    - Cellulose acetate, cellulose nitrate

Uses

- Heat-sensitive substances
  - Vaccines
  - Antibiotics
  - Serum
  - Toxins
  - Sugar solutions
- **HEPA:** High-Efficiency Particulate Air filter
  - 99.97% efficient
- **ULPA:** Ultra Low particulate Air filter
  - 99.99% efficient
- **Control**
  - Brevundimonas diminuta
  - Serratia

Sterilization Methods: Radiation 00:29:26

- Control
  - Bacillus Pumilus
    → P = R
- Types
  - Ionizing radiation
  - Non-Ionizing radiation

Ionizing Radiation

- Cold radiation
  - Does not increase the temp of the surface.
- High penetrating power
- Sporicidal
  - X rays
  - Gamma rays
  - Cosmic rays

Non-Ionising Radiation (hot radiation)

- Low penetrating power
- Bactericidal (not sporicidal)
  - UV rays
  - Infrared rays
    → OT Fumination.

- Blue rays are UV rays.
- Microbiologist works.

Q. Identify?

A. Autoclave
B. Incineration
C. Seitz Filter
D. Water Bath

Q. Control for filtration?
A. Bacillus pumilus
B. B.diminuta
C. Bacillus globigii
D. Clostridium tetani

Q. The incorrect combination of temperature is mentioned for?
A. Tyndallization-100 degrees: 20 mins
B. Inspissation-80 degree: 30 mins
C. Holder method of pasteurization: 72 degree
D. Autoclave-121 degree: 15 mins

Q. Incineration is done for
A. Human body parts
B. Syringe
C. Body fluids
D. Gloves

Q. Grease, dusting powder, and paraffin are sterilized by?
A. Gamma radiation
B. Sunlight
C. Dry heat sterilization
D. Autoclave

Q. Method of choice for sterilization of liquid paraffin?
A. Flaming
B. Moist heat
C. Autoclave
D. Hot air oven

Q. Pasteurisation of milk is done at?
A. 63 degrees for 30 minutes
B. 63 degrees for 20 minutes
C. 72 degrees for 30 minutes
D. 72 degrees for 20 minutes

Q. Cold sterilization is done by?
A. Steam
B. ionizing radiation
C. Infra-red
D. UV

Chemical Methods 00:37:03

Alcohols

- Ethyl alcohols 70%: Spirit
- Isopropyl alcohol: 70-80%
  - Stethoscope and thermometer

Aldehydes

- Formaldehyde
  - Mechanism - cross-linking and alkylating the molecules.
  - Gas converted to liquid.
  - Gas- OT fumigation.
  - Formalin - All human specimens stored.
  - Duckering - killing bacillus anthracis spores from animal wool.
    → Process where animal wool is treated with formaldehyde.

Glutaraldehyde

- Mechanism - cross-linking and alkylating the molecules.
- Glutaraldehyde
  - CIDEX -bronchoscope, laryngoscope
    → Arthroscope and Urethroscope are done by plasma sterilization.
  - Activated by alkalinisation- active for 14 days.
- Ortho-phthalaldehyde (opa-cidex)
  - 0.55% solution
  - Used for Cystoscopes,etc
  - No activation needed and more stable
  - o More bactericidal activity.

Important Information

- **Formaldehyde:** 10% NBF Fixative for Light microscope
- **Glutaraldehyde** 2.5%: Fixative for Electron microscope

Phenol

- **Scientist:** Joseph Lister.
- MOA-protein precipitation and cell membrane damage Sharp instruments.
  - Phenol (5%).
  - Cresol (1-3%).
  - Lysol (2-5%)
  - Modified phenol.
    → Chlorhexidine + Cetrimide = Savlon
    → Chloroxylenol = Dettol

Halogens

- MOA-oxidizing agents
- Iodine
  - Skin antiseptic
  - Betadine (povidone iodine)
  - Tincture iodine (2% iodine) for skin antiseptic
  - Povidone iodine-Betadine (IODOPHORE)
    → I2 absorbed on the neutral carrier (poly vinyl pyrrolidone)
    → Has residual action.
- Chlorine
- 1% sodium hypochlorite- depends on how much blood spills.
  - <10ml spill 1:100.
  - >10ml spill 1:10.

Blood Spill Management

- Block off the area to be disinfected.
  - Use a caution board.
- Wear a pair of non-sterile gloves.
  - Has PPE ready
- Use tongs or a pan and brush to sweep up as much of the broken glass (or container) as possible. Do not pick up pieces with your hands.
- Discard the broken glass in a sharp container.
- Use disposable or towels to absorb as much of the body fluids as possible.
- Saturate the area again with sodium hypochlorite (1:10 dilution)
  - for minimum 20 mins
- Rinse off the tongs, brush, and pan, under running water and place to dry
- Remove gloves and discard them.
- Wash hands carefully with soap and water, and dry thoroughly with single-use towels
- Record the incident in the incident book if a specimen was lost, or persons were exposed to blood and body fluids.

Oxidizing Agents 00:50:53

- H_2O_2
  - Strong oxidizer, free radical injury
    → 3-6% most effective concentration
    → 10% needed for spores.
  - Peracetic acid
    → <1% is effective spores.

Plasma Sterilization or Sterrad

- **Plasma**
  - Gaseous state containing ions and electrons.

- UV photons used.
- Vacuum
- Low temperature
- H2O2 chemical present

- **Advantage**
  - No toxic residues
  - Short cycle time - 75 mins
- **Use**
  - Surgical instruments
    → Arthroscopes, urethroscopes
- **Control**
  - Bacillus Stearothermophilus (autoclave, Plasma sterilization).

Gas Sterilization

- Ethylene oxide ETO
- **MOA**
  - Microbicidal
  - Sporicidial
- **Side effects**
  - Highly inflammable.
  - Highly irritant.
  - Highly explosive.
    → Mixture of inert gasses
- **Factors affecting ETO**
  - Concentration
  - Humidity
  - Temperature
  - Entire cycle might take 18-24 hours.
- **Used**
  - Cardiopulmonary machines.
  - Pre-packed syringes
- **Control**
  - Bacillus Globigi.

Heavy Metal Salts

- **Ophthalmia neonatorum:**
  - Crede's method 1% $AgNO_3$.
- Silver sulfadiazine: for burns
- Antisera vaccines: Mercury(Thiomersal)

Surface Acting Agents

- MOA-Lower the surface tension.
- Anionic
  - Detergent-like action.
  - Weak antimicrobial activity.
  - Ex-SOAP
- Cationic
  - Quaternary ammonium compounds
  - More effective against GP than GN bacteria
  - Eg-CETRIMIDE (savlon)
- Amphoteric (TEGO compounds)
  → Two Tego Teeth
  - Anionic plus cationic.
  - Detergent + antimicrobial activity.
  - Used as an antiseptic in dental procedures.

Dyes

- Aniline dyes
- More active against GP.
- Not active against MTB.
  - Crystal Violet
  - Gentian violet
  - Malachite violet
    → Culture media for TB is LG media.
    → Malachite green
    → Will damage gram-positive organisms.
    → Allows TB to grow.
- Acridine dyes
  - More active against GP
  - Can act in presence of organic waste
    → Acriflavine
    → Proflavine

Testing of Disinfectant

01:03:00

- 4 tests

1. Phenol coefficient test / Rideal Walker Test

- $$\frac{\text{Highest dilution of disinfectant needed to kill Salmonella typhi broth}}{\text{Highest dilution of phenol needed to kill Salmonella typhi broth}}$$

- Phenol can kill salmonella typhi.
- If the result is > 1 The test is passed
- **Disadvantage:** We don't know the efficacy with organic matter

2. Modified Rideal walker test/ Chick Martin Test.
   - Disinfectant present in organic matter.
3. Kalsey Sykes test/ capacity test.
- Capacity of a disinfectant to be used in increasing Microbiological Load.

4. Kalsey Maurer test/ in use test.
- Test for contamination of disinfectant
- Reality testing.

Decreasing Order of Resistance

- Prions:
  - Spread via neuro surgeries.
  - Treat 1 normal NaOH
  - 121°C 30 mins or 134°C x 1.5 HR.
  - Routine sterilization
- Cryptosporidium oocyst.
- Bacterial spores.
- Mycobacteria.

- Small non-enveloped virus.
- Trophozoites.
- Gram-negative bacteria.
- Fungi.
- Large non-enveloped viruses.
- Gram positive bacteria.
- Enveloped virus (easiest to kill).

Spaulding Classification

Critical Device

- Penetrate / enter sterile sites.
- HLD/Chemical sterilant

Semi Critical Device

- Come in contact with mucus membranes HLD.

Non-Critical Device

- Come in contact with skin.
- Intermediate /LLD

Non-Critical Surfaces / Medical equipments

- Computers, desks, tables
- LLD is used

Refer Table 7.1

Refer Table 7.2

Sporicidal Agents

- E - Ethylene oxide
- F-Formaldehyde
- G-Glutaraldehyde
- H-H2O2, Hot air oven, 1% Hypochlorite
- A-Autoclave
- P-Per Acetic acid
- P-Plasma sterilization
- P-o-phthalic acid
- T-Tyndallization

capacity of a disinfectant?

A. Rideal walker test
B. Chick martin test
C. Kelsey sykes test
D. Kelsey Maurer test

Q. Glutaraldehyde is used for all of the following EXCEPT?

A. Bronchoscope
B. Thermometer
C. Proctoscope
D. Endoscopic tubes

Q. Control for ethylene oxide (chemical) disinfection is?

A. Bacillus pumilis
B. Bacillus stearothermophilus
C. Bacillus globigii
D. Clostridium tetani

Q. ALL are true about plasma sterilization EXCEPT?

A. Produces less toxic products than Eto
B. Uses bacillus stearothermophilus as control.
C. Can be utilized for sterilization of products which are heat sensitive.
D. Chamber used for carrying out plasma sterilization uses ambient air as are medium.

Germicide	Level of disinfectant	Bacteria and Env virus	Fungi	Unenveloped virus	MTB	Spore	Inactivated by organic matter
Glutaraldehyde	High/CS	+	+	+	+	+	-
Formaldehyde	High/CS	+	+	+	+	+	-
H2O2	High/CS	+	+	+	+	+	+/-
Chlorine	High	+	+	+	+	+/-	+
Iso propyl Alcohol	Intermediate	+	+	+/-	+	-	+/-

Table 7.2

Germicide	Level of disinfectant	Bacteria and Env virus	Fungi	Unenveloped Virus	MTB	Spore	Inactivated by organic matter
Phenol	Intermed	+	+	+/-	+	-	-
Chlorhexidine	Low	+	+/-	+/-	+/-	-	+
Iodophore	Intermediate	+	+	+	+/-	-	+
Quaternary ammonium compound	Low	+	+/-	-	-	-	+

Q. Sporulation occurs in this phase of bacterial growth curve (FMGE June 2019)

A. Stationary phase
B. Lag phase
C. Log phase
D. Decline phase

Q. STERRAD is a gas plasma sterilizer used in operation theatres for disinfecting OT articles. Which of the following is the active agent used in it? (AIIMS Nov 2018)

A. H2O2
B. N2O
C. Ozone
D. E10

Q. Glutaraldehyde is used for all the following except? (FMGE Nov 2017)

A. Bronchoscope
B. Thermometer
C. Proctoscope
D. Endoscopic tube

Q. Which of following high level disinfectant? (FMGE Dec 2020)

A. Alcohol
B. Phenol
C. Lysol
D. Glutaraldehyde

Q. Blood on OT floor is cleaned by? (AIIMS Nov 2017)

A. Phenol
B. Alcohol based components
C. Chlorine based components
D. Quaternary ammounium compounds

Q. Disinfectant used to disinfect blood splits in hospital floors? (FMGE Aug 2020)

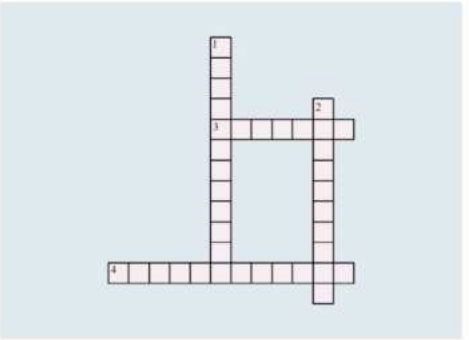
A. Phenol
B. Quaternary ammonium compound
C. Alcohol
D. 1% sodium hypochlorite

Q. Which is used as disinfectant but not as sanitizer? (INICET May 2022)

A. H202
B. Bleaching powder
C. Penicillin
D. Paraben

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Make the body surface/skin/ wounds free of microorganisms

4. All hospital waste go there

Down

1. Cross-linking and alkylating the molecules

2. There are asbestos sheets for filtration

8 STAPHYLOCOCCACEAE

- All cocci are gram positive.
- mnemonic VeNoM
  - **V**eillonella spp.
  - **N**eisseria spp.
  - **M**oraxella spp.
- All bacilli are gram negative.

Classification of gram positive cocci 00:01:12

a) Micrococcacae family.
- Staphylococcus.
- Micrococcus.

b) Streptococcaceae family.
- Streptococcus.
- Pneumococcus.
- Enterococcus.

Micrococcacae family 00:02:22

Difference between Staphylococcus and Micrococcus 00:02:27

Features	Staphylococcus	Micrococcus
Shape	Bunch of grapes.	Tetrad.
Glucose utilization	Fermentative pattern.	Oxidative pattern.
Oxidase	Negative.	Positive.
Catalase	Positive.	Positive.
Bacitracin	Resistant	Sensitive.

Note:-
- Hugh and Leifson medium used to assess glucose utilization.
- Bromothymol blue is the indicator.

Staphylococcus aureus 00:05:17

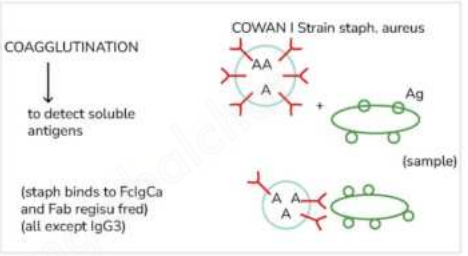
- Bunch of grapes appearance.
- Non-motile.
- Non-sporing.
- Catalase positive.
- Oxidase negative.
- Produce golden pigment

Virulence factors 00:06.13

Cell wall
- Peptidoglycan.
- Teichoic acid - for attachment.

Cell surface
- Protein A.
  - Anti-complementary.
  - Anti-phagocytic.
  - Responsible for co-agglutination
- Clumping factor.

Co-agglutination test 00:07:41



- Used to detect soluble antigens.
- COWAN I strain is used for Staphylococcus aureus.
- Protein A binds to Fc segment of IgG.
- Fab region left free.
- Antigen can get attached and form clumps.

Toxins
- Hemolysins.
  - Alpha-hemolysins.
    - → Inactivated at 70 degrees and activated again at 100 degrees.
    - → Lyse sheep and human red blood cells.
  - Beta-hemolysins or sphingomyelinase.
    - → Hot-cold phenomenon i.e., starts working at 37°C and completes working at 4°C.
    - → Lyse sheep red blood cells only that contain high content of sphingomyelin.
  - Gamma-hemolysins.
    - → Lyse sheep and human red blood cells.
    - → Synergohymenotropic toxin.
  - Delta-hemolysins.
    - → Lyse sheep and human red blood cells.
- Panton-Valentine (PV) toxin.
  - Secreted by Methicillin-Resistant Staph. aureus (MRSA).
  - Synergohymenotropic toxin with gamma-hemolysin.
- Epidermolytic/Exfoliative toxin.
  - Staphylococcal scalded skin syndrome (SSSS).
    - → Site of action for toxin is Stratum granulosum (desmoglein 1).

- Ritter's disease i.e., SSSS in children.
- Toxic Epidermal Necrolysis (TEN).

- Enterotoxin.
  - A-I, not F, R-T, V.
  - Enterotoxin A is the most common.
  - Heat stable.
  - Food poisoning.
  - Vagomimetic action leading to emesis.

Differential diagnosis of food poisoning 00:16:15

- Incubation period of food poisoning is 1-6 hours i.e. vomiting occurs 1-6 hours after food consumption.
- Food poisoning may be due to:
  - Staphylococcus aureus Milk, meat.
  - Bacillus cereus Chinese fried rice.

	S. aureus	B. cereus
Food consumed	Milk. Meat (poultry).	Chinese fried rice.

- Enterotoxin F or TSST.
  - Toxic shock syndrome toxin.
  - Associated with long-term tampon usage.
  - Superantigen.

Criteria for toxic shock syndrome 00:18:59

- Fever > 102°F.
- Hypotension with systolic blood pressure < 90mmHg.
- Maculopapular rash.
- Involvement of any 3 of the following organs:
  - GIT -> nausea, vomiting.
  - Kidney -> serum creatinine levels 2 times the norm.
  - Liver -> SGOT and SGPT level 2 times the norm.
  - CNS -> confusion.
  - Muscle -> creatine phosphokinase (CPK) level 2 times than the normal.
  - Mucous membranes -> hyperemia.
  - Coagulopathy -> platelet count < 100,000.

Enzymes 00:21:05

- Thermonuclease.
- DNAase.
- Phosphatase.
- Catalase.
- Coagulase.

Clinical Features 00:21:50

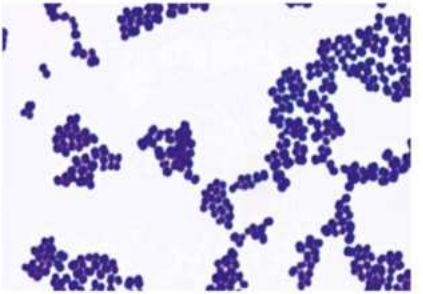
- Skin and soft tissue infections
  - Abscess
  - Cellulitis
  - Furuncles.
  - Botryomycosis.

- Osteomyelitis.
- Food poisoning.
- Toxic shock syndrome.
- Toxic epidermal necrolysis.
- Pneumonia
  - Hospital acquired
  - Ventilator acquired
- Pneumatocele.
- Acute infective endocarditis.
- Infective arthritis.
- Necrotizing fasciitis.
- Sepsis.
- Staphylococcus aureus is the most common cause of
  - Osteomyelitis.
  - Hospital-applied pneumonia.
  - Ventilator-applied pneumonia.
  - Acute infective endocarditis.
  - Native valve endocarditis.

Diagnosis 00:25:30

1. Microscopy.

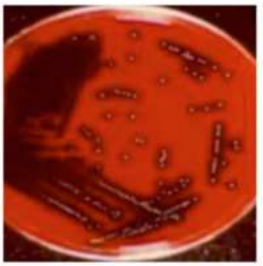
- Gram positive
- Purple branch of grapes.



2. Culture.

- Nutrient agar.
  - Golden color in nutrient agar.
  - Non-diffusible β-carotene staphyloxanthin pigment produced at 22°C.

- Blood agar.
  - Pin-head colonies with narrow zone beta-hemolysis.



3. Selective media.

- S. aureus can survive in NaCl 7-10%.
- Mannitol salt agar (MSA).
- Ludlam's agar.
- Salt milk agar.

Mannitol salt agar

- Salt presence makes it a selective medium.
- Mannitol presence helps in determining whether bacteria's mannitol fermentation is positive or negative, making it a differential medium.
- Phenol red is the indicator.
- Mannitol fermentation in S. aureus causes color change to yellow.

4. Biochemical tests

- Catalase positive.
- Coagulase positive.

Catalase test 00:31:53

Culture + H_2O_2 + catalase --> H_2O + O_2 --> Bubbles

Catalase positive bacteria

- Staphylococcus aureus
- Serratia spp
- Pseudomonas spp
- Escherichia coli.
- Enterobacteria.
- Bacillus anthracis.
- Neisseria gonorrhoeae.
- Neisseria meningitidis.
- Mycobacterium tuberculosis.
- Micrococcus spp.

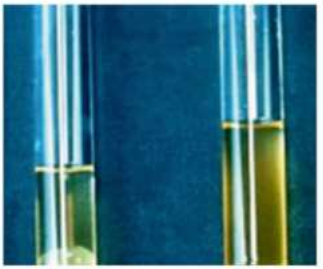
Catalase positive fungi

- Aspergillus spp.
- Candida spp.
- Cryptococcus spp.

Coagulase test 00:36:05

Feature	Slide coagulase	Tube coagulase
AKA	Bound coagulase.	Free coagulase.
Positive in	S. aureus. S. intermedius. S. hyicus. S. lugdunensis.	S. aureus. S. intermedius. S. hyicus. S. schleiferi.

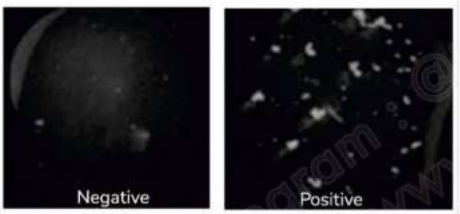
Tube coagulase test



Positive Negative

- Rabbit/Human plasma + Culture broth (containing coagulase).
- Plasma contains coagulase reacting factors (CRF).
- CRF-coagulase complex formed, activating fibrinogen and clotting of plasma.

Slide coagulase test/Clumping factor



- Coagulase bound to the cell wall.
- Reacts directly with fibrinogen in plasma leading to precipitation on the cell wall and clumps of cocci.

5. Phage typing 00:40:37

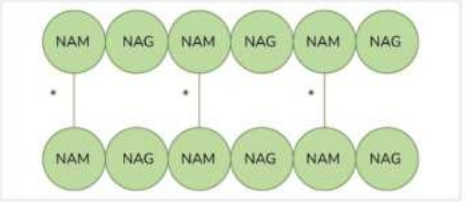
- Makes use of bacteriophages.
- Phage 80/81A associated with Staphylococcus infections that lead to outbreak.
- Phage III associated with MRSA.

6. Genotypic typing

- Pulse field gel electrophoresis (PFGE).

Treatment 00:42:27

- Penicillin/β-lactams are the drug of choice.
  - Mechanism of action: Bind to penicillin binding proteins (PBPs) 1, 2, 3, 4 that are also known as transpeptidases and break their bonds.



- Resistance: β-lactamase production regulated by BLA gene and plasmids.
  - Transfer of resistances mediated by transduction > conjugation.
- Methicillin.
  - Given in penicillin resistance.
  - Resistance by chromosomal-mediated MecA gene leading to MRSA with PBP altered to PBP2a that has a lower affinity for methicillin.

MRSA 00:46:55

Diagnosis of MRSA

- Best method is the dilution test.
- Minimum inhibitory concentration-minimum amount of antibiotic required to kill bacteria.
- MIC for cefoxitin ≥ 8 μg/ml indicates resistance.
- MIC for oxacillin ≥ 4 μg/ml indicates resistance.
- Cefoxitin disc diffusion agar/oxacillin.
  - Cefoxitin is a better determinant than oxacillin.
- Latex agglutination test for PBP2a.
- ELISA for MecA.
- PCR for MecA.

MRSA Spread: 00:49:20

- Most common form of spread is via the hands of healthcare workers.
- Precaution should be taken to perform rigorous hand washing.
- Elimination involves:
  - Administration of ointment mupirocin for nasal carriers.
  - Chlorhexidine washes for other sites of the body.

Treatment of MRSA

- Vancomycin
- Daptomycin.

VISA and VRSA 00:51:07

VISA	VRSA
Vancomycin-intermediate Staph. aureus.	Vancomycin-resistant Staph. aureus.
MIC for vancomycin is 4-8 μg/ml.	MIC for vancomycin is ≥ 16 μg/ml.
Altered cell wall synthesis and structure.	• D-ala-D-ala conversion of D-ala-D-lactate/serine. • VAN-A gene.

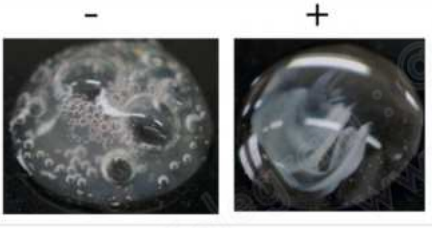
Feature	Staphylococcus Epidermidis	Staphylococcus Saprophyticus
Incidence	More common.	Less common.
Causes	Prosthetic heart valve endocarditis.	Urinary tract infections (UTIs).
Novobiocin	Sensitive.	Resistant.
Biofilm formation	Present.	Absent

- Biofilm is made up of a polysaccharide matrix that protects the bacteria against antibiotics.

MCQs

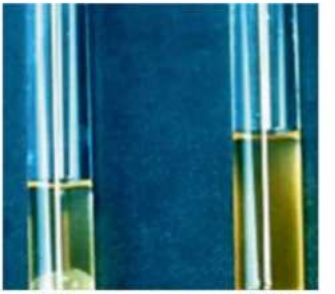
Q: Which of the following is incorrectly matched below?
A. Staphylococcus→Fermentative pattern on glucose utilization.
B. Micrococcus → Fermentative pattern on glucose utilization.
C. Staphylococcus → Oxidase negative.
D. Micrococcus → Oxidase positive.
Answer: B. Micrococcus → Fermentative pattern on glucose utilization.

Q: Which of the following organisms do not show the test below?



A. Staphylococcus.
B. Streptococcus.
C. Mycobacterium.
D. Pseudomonas.
Answer: B. Streptococcus.

shown below?



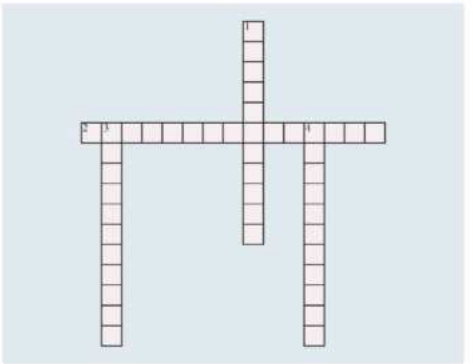
A. Staphylococcus aureus.
B. Staphylococcus Schleiferi.
C. Staphylococcus lugdunensis.
D. Staphylococcus Hyicus.
Answer: C. Staphylococcus lugdunensis.

Q: Indicator used in MSA?
A. Malachite green.
B. Bromothymol blue.
C. Phenol red.
D. Azure.
Answer: C. Phenol red.

Q: Mannitol salt agar is a?
A. Selective media.
B. Differential medium.
C. Both.
D. None.
Answer: C. Both.

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Pulse field gel electrophoresis (PFGE)

Down

1. Given in penicillin resistance
3. Heat stable
4. Makes use of bacteriophages

9 STREPTOCOCCACEAE

Classification

00:00:08

- Based on hemolysis.

Alpha-hemolysis	Beta-hemolysis	Gamma-hemolysis
Partial hemolysis.	Complete hemolysis.	No hemolysis.
Green color.	Yellow color.	No color change.
• Streptococcus viridans. • Streptococcus pneumoniae.	Lancefield classification.	Enterococcus.

Hemolysis of Streptococci-Types and Examples

Beta Hemolysis | Alpha Hemolysis | Gamma Hemolysis

Lancefield classification of beta-hemolysis streptococci

00:01:39

- Based on the presence or absence of the C antigen.
- There are 20 groups (A-V, without I and J).
- Example of group A include Streptococcus pyogenes.
- Example of group B include Streptococcus agalactaie.

Group A beta-hemolysis streptococci

- Can further be divided into two types:
  - Griffith typing.
  - Emm typing.
- Griffith typing is based on the M protein.
- Emm typing is based on M gene.

Important Information

- Lancefield classification is determined using the ring precipitation test/reaction.
- Modern laboratories use latex agglutination test to determine Lancefield classification.

Streptococcus Pyogenes

00:04:51

- Group A β hemolytic streptococci.

Virulence Factors

1. Capsule

- Non-antigenic.
- Made up of Hyaluronic acid.
  - Promotes cross reaction with synovial tissue.
  - Breaks connective tissue.

2. Cell wall

- C antigen.
- Outer membrane proteins M, T and R.
- M protein forms the basis for Griffith typing.
- M protein resembles glycoproteins in the human body i.e., molecular mimicry seen in rheumatic heart disease.

3. Enzymes and toxins

- Streptokinase causes fibrinolysis.
- Hyaluronidase –breaks down connective tissue.
- DNAase A, B, C, D-protects against neutrophil extracellular traps (NETs).
  - DNAase B is the most important.
- Hemolysin/Streptolysin.
- Streptococcoal pyrogenic exotoxins (SPEs) A, B, C.
- Serum opacity factor (SOF).

Important Information

Therapeutic use of streptokinase-

- Streptokinase extracted from S. equisimilis used as a fibrinolytic agent in cardiac patients.
- S. equisimilis is a group C beta-hemolysis streptococcus.

Streptolysin (SL)

00:09:44

Feature	SL-O	SL-S
O2 and heat	Labile.	Stable.
Serum	Stable.	Soluble.
Property	Antigenic.	Non-antigenic.
Function	• Lethal. • Cardiotoxic. • Leucocidal.	Only leucocidal.
Hemolysis	Deeper.	On surface.

- Anti-streptolysin O antibody titres used to determine presence of antigenic SL-O.

SPEs

00:13:16

- SPE A and C are bacteriophage-mediated.
- SPE B is chromosomal mediated.

- Conditions associated with SPEs include:
  - Scarlet fever.
  - Toxic shock syndrome (TSS).
  - Necrotizing fasciitis.
- TSS can also be caused by toxic shock syndrome toxin (TSST) of Staphylococcus aureus.

Suppurative clinical features 00:14:40

- Necrotizing fasciitis.
- Impetigo.
- Pneumonia.
- Pharyngitis.
- Lymphangitis.
- Erysipelas.
- Streptococcal toxic shock syndrome.
- Scarlet fever.

Pharyngitis 00:15:52

- White exudate on tonsillar area and soft palate.
- Presents with:
  - Pain.
  - Tender cervical lymph nodes.
  - No cough.
- Treatment with penicillin.

Difference between erysipelas and cellulitis 00:16:27

Erysipelas	Cellulitis
Raised.	Not raised.
Well-demarcated.	Diffuse with no clear demarcation.
Painful.	Painful.
Red lesion.	Pink lesions.
Superficial skin is affected	Deeper dermis is affected.

Scarlet fever 00:18:25

- Sore throat.
- Circumoral pallor.
- Antecubital fossa demonstrate pastia lines.
- Sandpaper rash.
- Lymphadenopathy.
- Erythrogenic toxin (SPEs).
- Strawberry tongue.

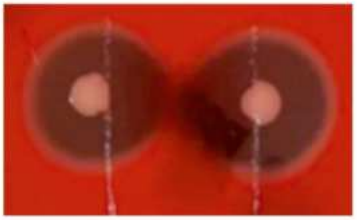
Non-suppurative clinical features 00:20:05

- Post-streptococcal glomerulonephritis (PSGN).
- Rheumatic heart disease (RHD).
- Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS).
  - Patients present with OCD.

Diagnostic criteria 00:21:21

- Stain-
  - Gram positive cocci in chains.
  - Purple appearance.

- Transport medium - Pike's media
- Blood agar findings:
  - Pin-point colonies with wide zone hemolysis.



- Selective media include:
  - PNF.
  - Crystal Violet blood agar (CVBA).
  - Liquid media-shows granular turbidity.
- Biochemical tests:
  - Catalase negative.
  - Oxidase negative.
  - PYR test positive.
  - Bacitracin sensitive.

Treatment 00:25:03

- Penicillin.
- Erythromycin in case of resistance.

Streptococcus Agalactiae 00:25:15

- Group B beta-hemolytic streptococci

Epidemiology 00:25:26

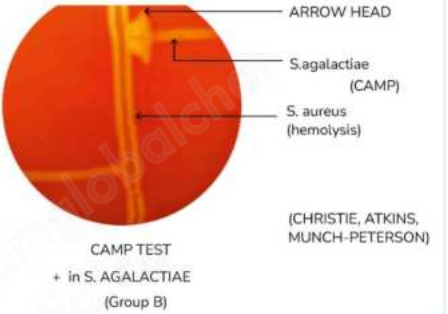
1. Affects neonates (<1 week).
- Neonates affected by serotypes I, III and V.
- Presents with:
  - Meningitis.
  - Pneumonia.
  - Septicaemia.

2. Affects older children (>1 week to 3 months).
- Older children affected by serotype III.
- Presents with:
  - Pneumonia.
  - Endocarditis.

Laboratory Diagnosis 00:26:21

- CAMP test positive.
- Hippurate hydrolysis positive.
- Bacitracin resistant.
- Red/orange pigment on Islam media.

CAMP test 00:28:08



- Christie, Atkins, Munch, Peterson test.
- Blood agar shows two lines:
  - Longitudinal line representing Staphylococcus aureus haemolysis.
  - Horizontal Line due to Streptococcus agalactiae haemolysis.
- Arrowhead location-area of enhanced haemolysis at the meeting point of the two lines.

Treatment 00:29:59

- DOC- Beta Lactams

Alpha-Hemolytic Streptococci 00:29:45

Features	Streptococcus viridans	Streptococcus pneumoniae
Arrangement	Chains.	Lanceolate.
Bile solubility	Insoluble.	Soluble.
Optochin	Resistant.	Sensitive.
Inulin fermentation	Not seen.	Seen.

Streptococcus viridans 00:33:15

- Group of organisms i.e.
  - S. mitis.

- S. sanguis.
- S. Salivarius.

- S. Mutans → Dental caries.
- S. sanguis → Late prosthetic heart valve endocarditis.
- S. Salivarius unique features include capsule and slime layer with the longest chains.

Streptococcus pneumoniae 00:34:40

Virulence factors

- Capsular polysaccharide.
  - Antigenic.
  - >90 serotypes
- Pneumolysin.
- Autolysin.
- Hemolysin/Streptolysin.

Tests to demonstrate antigenic capsule in *S. pneumoniae*

- There are>90 serotypes of S. pneumoniae that can be distinguished using the Quelling reaction.
  - Addition of antisera results in an antigen-antibody reaction, leading to swelling of the capsule.
- Other tests to demonstrate the antigenic capsule include PCR, ELISA and latex agglutination test.

MNEMONIC- CCLuBS 00:36:42

- **C**lostridium tetani.
- **C**lostridium perfringens.
- **L**isteria.
- **B**acillus cereus.
- **S**treptococcus pneumoniae.

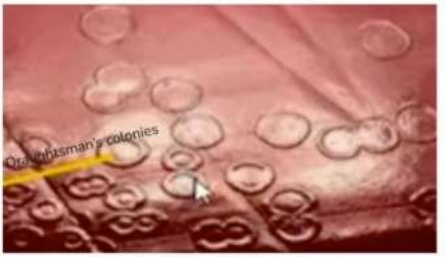
Clinical Features 00:37:34

- Meningitis
  - Affects all age groups.
  - Most common cause of meningitis.
- Otitis media.
- Sinusitis.
- Community-acquired pneumonia (CAP).

Laboratory Diagnosis 00:38:11

- Microscopy:

  - Lens lanceolate shape
  - Capsule is seen.
- Culture:



Depressed Centre with an elevated rim

  - Carrom coin or Draughts-Mann colonies
    → Depressed centre with elevated rim.
    → The depressed centre develops due to autolysin presence (an amidase enzyme).

Treatment 00:39:29

- Beta-lactams e.g., penicillin.
- 3rd generation cephalosporins in case of resistance.
  - Resistance involves alteration of penicillin binding proteins (PBPs).
  - Most commonly transferred by transformation in Streptococcus.
  - In contrast, altered PBPs are transferred in Staphylococcus aureus by transduction and conjugation.

Prevention 00:40:29

- Vaccination.

1. PNEUMOVAX.
   - 23 valent PPV polysaccharide vaccine.
   - Not given below 2 years.

2. PREVNAR.
   - 13 valent vaccine.
   - Administered below 2 years.
   - 3 doses of PREVNAR given at 2 months, 4 months and 6 months consecutively.
   - Booster shot given at 12-15 months.

Gamma-Hemolytic Streptococci 00:42:06

- Can be classified as
  - Enterococci
  - Group D non-enterococcus bacteria.

MNEMONIC ENTEROCOCCUS 00:43:05

- Spectacle-shaped gram-positive cocci.
- Non-motile.
- Non-sporing.
- Survives in NaCl 6.5%.
- Heat tolerant.
- Survives in 40% bile.
- PYR test positive.

Significance of resistance to 40% bile

- Culture media- Bile Aesculin Azide Agar.
- Enterococci causes aesculin lysis.
- Results in black color.

Epidemiology 00:46:32

- Enterococcus faecalis – most common.
- Enterococcus faecium.

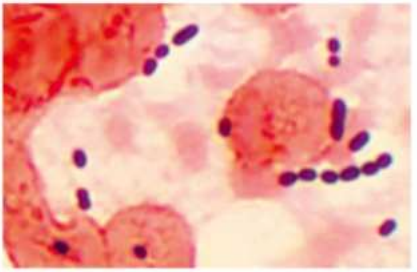
Clinical Features 00:46:42

- Catheter-associated UTI (CAUTI).
- Septicemia.

Diagnosis 00:45:44

- Blood agar-no hemolysis

- Microscopy- spectacle shaped cocci seen



- BEA- Bile aesculin Azide agar

Treatment 00:47:10

- Penicillin.
- Vancomycin.
  - Given in response to penicillin resistance.
- Linezolid.
  - Given in response to vancomycin resistance.

Vancomycin-resistant enterococcus

- Change of D-ala-D-ala to D-ala-D-serine/D-lactate.

Difference between Enterococcus and Group D non enterococcal streptococci

	Enterococcus	Group D non-enterococcal streptococci
BEA	Black color.	Black color.
6.5% NaCl	Survives.	No survival.
PYR	Positive.	Negative.
Examples	E. faecalis.	Streptococcus Gallolyticus.

Important Information

- S. Gallolyticus implicated in:
  - Colorectal cancer.
  - Polyps.
  - Sepsis.
  - Endocarditis.

MCQs

Q: All of the options are characteristic of Streptococcus pneumoniae except?

A. Optochin sensitivity.
B. Bile solubility.
C. Inulin fermentation.
D. Catalase positive.

Answer: D. Catalase positive.

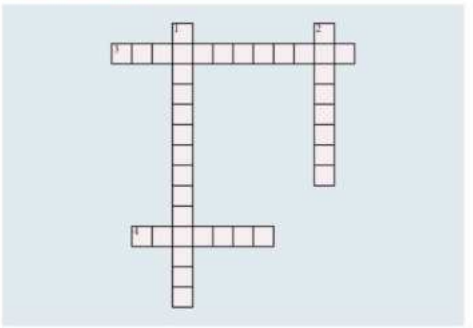
Q: An 8 year old boy is brought to the emergency room with a 3-day history of fever of 102°F and abdominal pain. He also complains of pain in his right knee and right elbow. He was seen four weeks ago because of a sore throat and a rash. Amoxicillin was prescribed but the boy's mother did not fill the prescription. On physical exam, his temperature is 101.7°F, HR 96, and BP 100/60. Cardiac exam reveals a pansystolic murmur heard best at the apex. His right elbow is tender on extension and flexion with mild swelling. Laboratory tests reveal a positive C-reactive protein and a WBC of 22,000 with a left shift. EKG shows a prolonged PR interval. Which of the following tests would be positive for the microorganism responsible for his patient's illness?

A. Catalase test.
B. Coagulase test.
C. Sensitivity to bacitracin.
D. Sensitivity to optochin.

Answer: C. Sensitivity to bacitracin.

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Sandpaper rash
4. Hyaluronic acid can also break connective tissue

Down

1. Is based on the M protein
2. Outer membrane proteins M, T and R

10 NEISSERIA

Introduction 00:00:02

- Gram negative cocci.
- All the cocci are gram positive except
  - Moraxella
  - Veillonella
  - Neisseria
- Two types of Neisseria
  - Neisseria meningitidis
  - Neisseria Gonorrhoeae

Refer Table 10.1

Oxidase Test 00:02:26

Oxidase Test

- Deep purple color is considered positive
- Paper strip method / Kovac's test
- Niesseria do not have flagella but it has a twitching motility because of the Pili

Modified Thayer Martin media 00:06:38

- Contains
  - Vancomycin
  - Colistin
  - Nystatin
  - Trimethoprim
- Trimethoprim: added in later part which makes it modified.

Neisseria meningitidis 00:07:24

- Most cases are asymptomatic and carriers.
- It results in Pyogenic meningitis
  - Fever
  - Neck rigidity
  - Purpuric rash (due to endotoxin)
- Can cause Waterhouse Friedrichsen Syndrome-bilateral adrenal gland hemorrhage
- Long term
  - Chronic meningo-coccemia
  - Post Meningococcal arthritis

Diagnosis 00:08.44

- CSF sample
  - Lumbar puncture is done
- Nasopharyngeal swab

Treatment 00:09:04

- For asymptomatic- IV ceftriaxone
- For carriers- IV or IM ceftriaxone
- If allergic - chloramphenicol

Vaccine 00:09:24

- Has A, C, Y, W135 valency
- Not B- the person is not protected against B type strain
- It is conjugated with DT and TT to increase the Immunogenicity
- It is given in:
  - Hypogammaglobulinemia
  - HIV
  - Age (elderly)
  - Habitant of Africa
  - Asplenia
    - → Splenectomy patient are given capsular organism in vaccine
    - → Spleen protects against capsular organism and if it is damaged then this vaccine needs to be given
  - Terminal complement deficiency
    - → Membrane attack complex

Neisseria Gonorrhoeae 00:12.04

- Clinical features
  - Ophthalmia neonatorum (mother to child)
    - → While the child is passing through vaginal canal it can get inoculated in the eye of the child
  - Female Vaginitis, Urethritis, Cervicitis-Water Can perineum
  - Male - Prostatitis, Seminal vesicles
  - Anorectal infection
- Most common in female - Cervicitis
- Most common in male - Urethritis
  - Flow of seed discharge (like semen)
- Multiple discharging sinuses can be present on the skin
  - Water can perineum

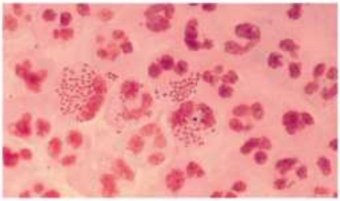
Complications 00:14:06

- Fitz - Hugh - Curtis syndrome
  - Involvement of Perihepatitis
    - → Also seen with chlamydia
- Disseminated Gonococcal infection

- Protein cause it - PORB1a
- Involvement of
  - → Skin - Dermatitis
  - → Joints - Arthritis, Tenosynovitis
  - → Brain - Meningitis
  - → Heart - Endocarditis

Diagnosis
00:15:57

- Sample (depends on site of infection)
  - Genital discharge
  - DGI - blood sample
  - Brain - CSF sample
- Microscopic examination



- Gram-negative Diplococci - present in pairs

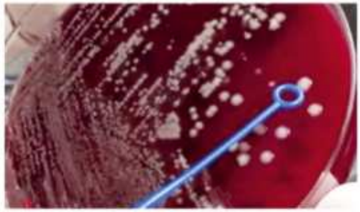
Treatment
00:16:50

- Ceftriaxone + Azithromycin

Moraxella
00:17:09

Moraxella Catarrhalis
00:17:14

- Seen in COPD exacerbation
  - Otitis media can occur
- A hockey puck sign is seen
  - The bacteria colony will move when pushed



- Treatment
  - Amoxiclav

Moraxella Lacunata
00:18:17

- Responsible for causing angular conjunctivitis

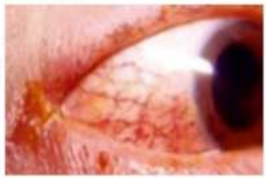
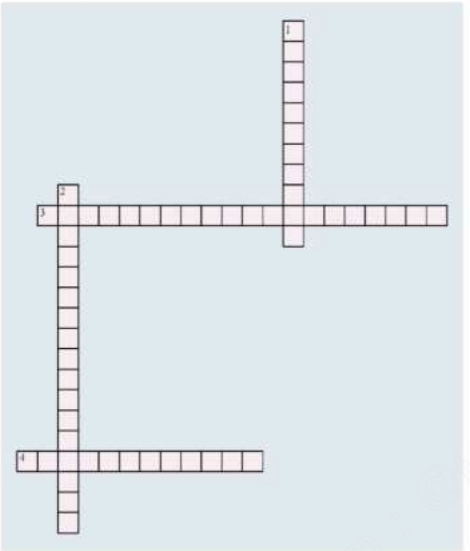


Table 10.1

Features	Neisseria meningitidis	Neisseria Gonorrhoeae
Habitat	Normally present in Nasopharynx	Genitals
Disease	Meningitis	Gonorrhoea
Fermentation	Maltose and glucose	Only glucose
Shape	Lens shape with Capsule	Bean or Kidney shaped organism
Catalase	Positive	Positive
Oxidase	Positive	Positive
	Strict Aerobe	Facultative anaerobe
Virulence factor	Capsular polysaccharide (as it has a capsule) • A, B, C, Y, W135	Not present
Outer membrane protein	Present	Present • Protein 1 o Protein 1a (PORB1a) → Cause disseminated infection o Protein 1b (PORB1b) → Localised infection • Protein 2
	IgA1 protease	IgA1 protease
	Endotoxin present	Endotoxin present
	Lipooligosaccride and Pili present	Lipooligosaccride and Pili present
Transport media (both are charcoal base)	• Amies media • Stuart media	• Amies media • Stuart media
Selective media	Modified Thayer Martin media	Modified Thayer Martin media

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Famous for COPD exacerbation
4. Can cause long term Chronic meningo-coccemia

Down

1. Clinical feature : Ophthalmia neonatorum
2. Responsible for causing angular conjunctivitis

11 GRAM POSITIVE BACILLI PART-1

- All Bacilli are Gram Negative Bacilli except for:
  - Mycobacterium
  - Anthracis bacillus (spore forming)
  - Clostridium Species (spore forming)
  - Corynebacterium diphtheria
  - Nocardia (Filamentous)
  - Actinomyces (Filamentous)
  - Listeria
  - Diphtheroids

Corynebacterium

00:01:36

- Known as Kleb Loeffler's Bacillus
- Gram positive
- Non-Motile
- Non sporing
- Non capsulated

Types

00:02:19

1. C-diphtheriae
2. Others (Non-Lipophilic):
   a. C. Ulcerans:
   - Lungs involvement

   b. C. Pseudotuberculosis
   - Other name is Preisz Nocard Bacillus
   - Causes Infection in sheep

   c. C. Minutissimum
   - Shows erythrasma (red rash in folds such as axilla, groins)
   - Gives Coral Red fluorescence under Wood lamp due to Coproporphyrin III

3. Others (Lipophilic)
   a) C. Jeikeium
   - Seen in Immunocompromised individuals
   - Causes- Prosthetic Valve Endocarditis

   b) C. urealyticum
   - Causes Struvite stone

C-Diphtheriae

00:06:51

Diphtheria Toxin

00:07:00

- Released by:
  - C- Diphtheria
  - C. Ulcerans
  - C. pseudotuberculosis
- Goes into all cells except RBCs
- Mediated by:
  1. Phage mediated
  2. TOX gene mediated
  - If bacteria doesn't have TOX gene, it will not have Toxin & it will be non-toxigenic.
  - If it has Tox gene, it will have toxin and it can be transmitted by lysogenic Conversion which involves toxins:
    → A- S. pyogenes
    → B- Botulinum toxin
    → C- Cholera toxin
    → D- Diphtheria toxin
    → E- EHEC

3. Iron levels

- Toxin has 2 parts (Bipartite):
  - Fragment A (action)- ADP Phosphorylation of EF2 → protein synthesis inhibition
  - Fragment B (binding)
- DT = acidic / formalin → toxoid → used in vaccination
- Strain- Park William 8 strain

Toxins- Decreasing Protein Synthesis

00:11:22

- Diphtheria Toxin and Exotoxin A of Pseudomonas

1. They cause ADP Ribosylation of EF2 & decreases protein synthesis

- Shiga Toxin and Verocytotoxin (Shiga like toxin)

2. Acts on 60s ribosome & decreases protein synthesis

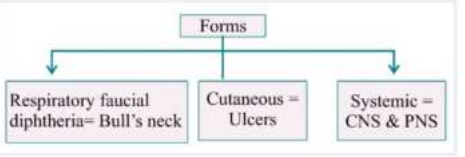
Diptheria

00:13:55

- IP- 3-4 Days
- Transmission – through secretion
- Droplet infection
- Source- Human

Forms

00:14:25



3. Respiratory / Faucial Diphtheria (Bull's Neck)

- There is a pseudo membrane present in the soft palate & mouth region
- If present in larynx- can cause respiratory problem
- Lots of Lymph nodes present causing Bull's Neck

4. Cutaneous: Ulcers
5. Systemic: CNS & PNS

Complications 00:15:35

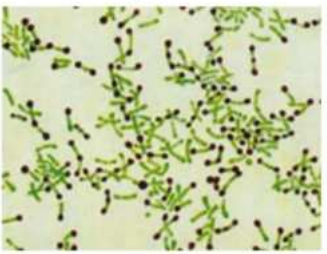
- Myocarditis (7-10th day)- M/c cause of death
- Neurological-Oculomotor Paralysis (spontaneous recovery)
- Minor complications:
  - Hemorrhage (Thrombocytopenia)
  - Obstructive (asphyxia)

Diagnosis 00:16:42

1. Sample- 2 swabs from beneath the membrane
2. Microscopic examination: Gram positive Bacilli (purple)

- V & L shaped giving a Chinese letter / Cuneiform appearance due to "snapping division."
- Ends are little prominent and has the Volutin granules.

Volutin Granules 00:18:28

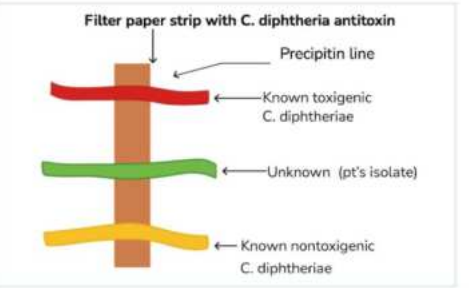


- Bipolar or Metachromatic or Babes Ernst Granules
- These granules are made up of Polymeta-phosphate (energy reserves)
- Stains:
  a) Ponder's
  b) Loeffler Methylene Blue- best
  c) Albert Stain: has 2 solutions-
  - Albert solution 1-
    → Green: Malachite green
    → Purple: Toluidine Blue- shows metachromasia.
  - Albert solution 2-
    → Has iodine
  - First add Toluidine blue Iodine Malachite green
  - On Albert's staining- organism is blue in color and volutin granules are purple in color

  d) Neisser's
  - Volutin Granules are also seen in:
    → Gardnerella
    → Yeast
    → Spirillum
    → MTB
    → Yersinia

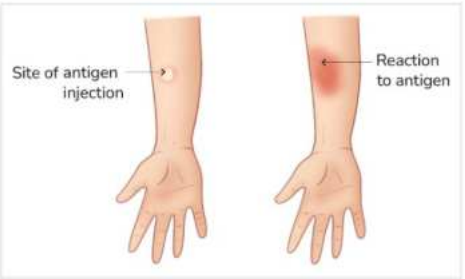
3. Medium:
   - Enriched Medium- Loeffler Serum Slope (6-8 hrs)
   - Selective Medium- Best
     → Macleod
     → Hoyles
     → Tinsdale medium
     → PTA (Potassium Tellurite Agar)
     → Black colonies
     → Take 48 hrs
4. Biochemical Test
- HISS Serum Sugar Test

5. Toxins-
   a) Elek's Gel Precipitation Test-for knowing toxigenicity of bacteria



- There is an agar
- There is a filter paper with an antitoxin ©. diphtheria)
- On top of it, there are 3 more filter papers of which 1 is toxigenic C. Diphtheriae
- Precipitin line is seen because of toxin
- Another filter paper with non-toxigenic C. diphtheria is kept
- No precipitin line is seen
- Now, the third one is kept which is patient's sample.
- Precipitin line were formed which means toxin is present

b) Inoculation in Guinea pig
c) SCHICK Test: To know the susceptibility of person.



- Toxin-antitoxin reaction.
- We put toxin injection; it reacts→ means susceptible (positive schick test)
- If we put toxin and there is no reaction→negative schick test

Treatment 00:32:02

Type of disease	Diphtheria Antitoxin	Antibiotics	Adjunctive
Pharyngeal / Laryngeal	20000-40000 IM or IV	Penicillin or erythromycin	Droplet precaution
Nasopharyngeal Disease	40000-60000 IM or IV	Penicillin or erythromycin	Droplet precaution
Extensive disease	80000-120000 IM or IV	Penicillin or erythromycin	Droplet precaution
With neck disease / Disease > 3 days	80000-120000 Half IM, Half IV	Penicillin or erythromycin	Droplet precaution

- During Convalescence:
  - DTap (higher dose of Diphtheria toxin- 10-25 flocculation units): <7 yrs children
  - Tdap (lower doses of DT): > 7 yrs children

Bio-Typing of Cyanobacteria (Types) 00:35:05

- Based on PTA

1. **C. gravis**
   - Daisy head
   - Starch fermentation
2. **C. intermedius**
   - Frog egg
   - Non-Hemolytic
3. **C. mitis**
   - Poached egg
   - Least virulent organism

MCQ's

Q. 1. The corynebacterium species causing erythrasma?
A. C. Jeikeium
B. C.urealyticum
C. C. minutissimum
D. C hoffmani

Correct Answer - C. Minutissimum

Q. 2. The corynebacterium species which are lipophilic?
A. C. Jeikeium and C. Urealyticum
B. C. ulcerans and C. diphtheria
C. C. Hofmanni and D. diphtheria
D. C.xerosis and C. Ulcerans

Correct Answer- C. Jeikeium and C. Urealyticum

Q. 3. The corynebacterium species causing prosthetic valve endocarditis is?
A. C. Jeikeium
B. C. Ulcerans
C. C.hoffmanni
D. C.xerosis

Correct Answer- C. Jeikeium

Q. 4. Preisz-nocard bacillus is?
A. Mycobacterium Bovis
B. Corynebacterium Ulcerans
C. Corynebacterium pseudotuberculosis
D. Bacillus cereus
Correct Answer- Corynebacterium pseudotuberculosis

Q. 5. The mechanism of action of the exotoxin produced by corynebacterium diphtheria can be characterized by which of the following?
A. Acting as a superantigen that binds to MHC class II protein and the T-cell receptor
B. Blocking the release of acetylcholine causing flaccid paralysis
C. Blocking the release of glycerine (inhibitory neurotransmitter)
D. Inhibits protein synthesis via EF-2 adenosine diphosphate (ADP) ribosylation

Correct Answer- Inhibits protein synthesis via EF-2 adenosine diphosphate (ADP) ribosylation

Q. 6. True about corynebacterium diphtheria except?
A. Iron has no role in toxin production
B. Toxin is responsible for local reaction
C. Non sporing/ non capsulated and non motile
D. Toxin production is by lysogenic conversion
Correct Answer- Iron has no role in toxin production

Q. 7. A12 year old child presents with fever and cervical lymphadenopathy Oral examination shows a grey membrane on the right tonsil. Which of the following will be ideal for the earliest diagnosis?

A. MacConkey medium
B. Tellurite blood agar
C. Loeffler's serum slope
D. Lowenstein jensen medium

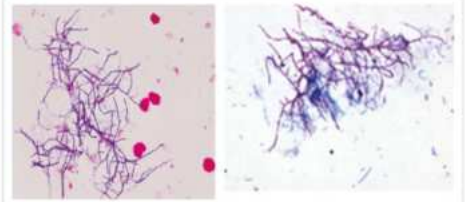
Correct Answer- Loeffler's serum slope

Gram Positive Filamentous Bacteria 00:40:35

Nocardia and Actinomycetes 00:40:46

Nocardia	Actinomycetes
Strict Aerobe (O2)	Strict Anaerobe
1% acid fast	Nonacid fast
Exogenous (Soil)	Endogenous (mouth flora)
• In immunocompetant patients- cutaneous lesions • In Immunocompromised persons – o M/c - Pulmonary o Extra-pulmonary: Brain	• M/c - Jaw • Oral cervical actinomycetes
Culture- Paraffin bait technique (carbon source)	• On solid media - Spider / molar tooth • In liquid media-Fluffy ball
Sulfonamides	Penicillin

- **Nocardia**



- Gram positive- Purple in color
- Filaments present
- Zn stain is used

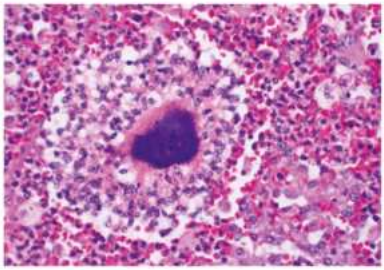
- **Actinomycetes-**

- Present in mouth
- Colonies present

Types of Actinomyces 00:45:45

1. Orocervical-
- Referred as Lumpy Jaw
- Shows swelling of jaw
- Multiple sinuses from which discharge with sulphur granules is present
2. Thoracic
3. Abdominal
- Following surgery
4. Pelvic
- Eg: IUCD

Histopathology of Actinomyces



- Can be in any location
- Gram positive purple color filamentous organism
- There is pink zone around it referred as Splendore hoeppli's phenomenon and it indicates Ag-Ab reaction

Treatment 00:48:32

- Nocardia → Sulphonamide
- Actinomycetes → Penicillin

Actinomycetoma 00:49:05

- Nocardia and Actinomycetes occuring together
- Can be caused by fungus too.

Actinomyces Israelii 00:49:44

- Seen in IUCD users.
- Results in Pelvic inflammatory Disease
- Yellow sulphur granules are seen.
- Sometimes it can be Acid fast.

Listeria Monocytogenes 00:51:11

- Toxin or virulence factor - Listeriolysin O
    - Shows β hemolysis
- Shows Tumbling Mobility at 25° & not 37°
    - motile at 25degree because of peritrichous flagella
- Treatment - Ampicillin + Gentamicin

Modes of spread 00:54:31

- Contact with animals
- Inhalation/ ingestion of contaminated food
- Milk and milk products
- Soft cheese
- Cold food

Clinical Features 00:54:55

- Gastroenteritis
- Pregnant females
    - Abortion/ stillbirth
    - PROM
- Early neonatal period - sepsis
- Late neonatal period –
    - Meningitis
    - Granulomatous infanti septicum

Lab diagnosis 00:55:21

- Specimen –
    - Cervical
    - Vaginal
    - Meconium
    - Cord blood
    - CSF
- Shows ß-Hemolysis
- Cold enrichment at 4°C
- CAMP test- in S. agalactiae & Listeria
- Catalase +ve
- Anton test +ve :
    - Animal test
    - Take rabbit's eye and put Listeria into it
    - Rabbit's eye will end up having KeratoConjunctivitis
    - Same as Sereny Test which is done for entero-invasive E.coli.
- Serology: ELISA Treatment(heading): Ampicillin & Gentamicin

12 GRAM POSITIVE BACILLI PART-2

Spore Forming Bacteria 00:00:16

1. Bacillus
   - Obligate Aerobe
   - Non bulging spores (not bulging out)
2. Clostridium
   - Obligate Anaerobe
   - Bulging spores (bulging out)

Bacillus 00:01:40

- M/c lab contaminant of culture media
- All are motile, unencapsulated and hemolytic except - B. Anthracis
- Types –
  1. B. anthracis
  2. B. cereus
  3. B. stearothermophilus- For autoclave, Plasma sterilization
  4. B. subtilis- For hot air oven
  5. B. pumilus- For radiation
  6. B. globigii- For Gas Sterilization

Bacillus Anthracis 00:04:00

- First pathogenic bacteria which was
  - Seen under microscope
  - To have live attenuated vaccine
  - Isolated in culture media.
- It is the largest pathogenic bacterium
- Category A Bioterrorism agent

Virulence factors 00:04:58

- It is a capsulated organism:
  - Pxo2 Plasmid
- It has Anthrax toxin:
  - Pxo1 plasmid
- It is plasmid coded.
- Loss of plasmid→ loss of Virulence→ Useful in making vaccine (sterne vaccine)
- Capsule:
  - Made of polyglutamate polypeptide.
- Anthrax Toxin- It is Tripartite:
  - Edema factor: increases cAMP (MOA)
  - Protective factor: attachment
  - Lethal factor: cause death of cells

C/F & Pathogenesis 00:08:04

- It is a zoonotic disease by:
  - Cutaneous (M/C)
  - Inhalation
  - Ingestion
- Infectious forms - Spores

1. Cutaneous Anthrax

  - Hide Porters Disease
  - Lesions seen on neck.
  - Referred to as Malignant Eschar (black color)

2. Pulmonary Anthrax

  - Wool Sorter's Disease
  - Results in Hemorrhagic mediastinitis
  - Complications-
    → Pericarditis
    → Septicemia
    → associated with Bioterrorism.

3. Intestinal Anthrax

  - Caused by eating undercooked meat.
  - Hemorrhagic enteritis

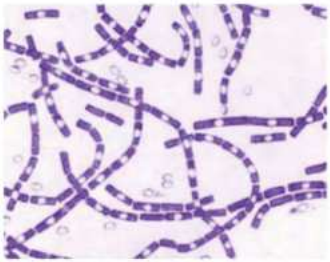
Complications 00:12:04

- CNS / Meningitis / Meningoencephalitis
- Hemorrhagic CSF
- Hemorrhagic mediastinitis
- Hemorrhagic enteritis

Diagnosis 00:12:30

- Specimen:
  - Skin
  - Blood
  - CSF
  - Sputum
  - Stool
- Animal: If an animal has Anthrax- Never perform Autopsy.
  - We might take: Blood sample/ cut one ear → should be kept it biosafety cabinets (BSL II/ III cabinets)

Microscopy

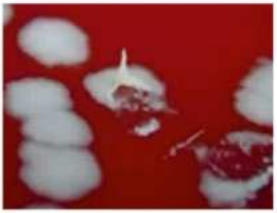


- Bamboo stick appearance or box car appearance
- There are holes which are spores (Gram poor)
  - For spores- Schaeffer & Futon stain is used
- McFaydean Reaction -
  - This uses Polychrome methylene blue stain
  - Results in capsular material (purple material around organism)

Blood culture morphologies-

1. Blood agar

Frosted Glass Comet Tail



Beaten Egg

a) Frosted glass appearance
b) Comet tail appearance
c) Beaten egg appearance
d) Medusa Head appearance- seen at the edge of the colony.

Medusa head appearance

2. Blood agar + Penicillin

- String of Pearls appearance
- Agar + 0.5 -0.50 u/ml of Penicillin
- Cells → Spherical

3. Gelatin Liquefaction

- At surface of tube there is more oxygen than bottom, so it will have more energy at surface
- Gelatin Liquefaction maximally at surface and keep on diminishing as moving down.
- It shows inverted fir tree appearance.

4. PLET

- Selective media
- Polymyxin Lysozyme EDTA Thallous acetate.

Serology 00:20:52

- Ascoli's ring thermo precipitin test
- ELISA

CDC guidelines 00:21:30

- Any gram +ve bacillus, non-Motile, non-Hemolytic, catalase +ve → Gives Presumptive diagnosis of Anthrax

Precautions 00:22:27

- Biosafety:
  - Sanitization of factory
  - No autopsy for anthrax died animals.
  - Buried deep in quicklime / cremated to prevent soil contamination.
- Duckering
  - Disinfection of wool is done by:
    → 2% formaldehyde at 30-40° C for 20 minutes

Treatment 00:23:29

- Immunization of:
  - Vets / occupational exposure:
    → AVA biothrax (human vaccine)
    → Anthrax Vaccine Adsorbed.
  - Animals:
    → Pasteur anthrax vaccine
    → Sterne vaccine (spores)
    → Mazzucchi vaccine (Carbazoo strain)
- Rx:
  - Uncomplicated cutaneous: Ciprofloxacin or doxycycline (7-10d)
  - Systemic: Add antitoxin
    → Raxibacumab (M.ab)
  - Anthrax meningitis: 60-day antibiotic
    → Ciprofloxacin + Meropenem + Clindamycin
  - For PEP (post exposure prophylaxis): AVA Biothrax + 60 day antibiotics

Bacillus Cereus 00:27:04

- Causes food poisoning.

Features	Emetic type	Diarrheal type
IP	<6 hours	8-16 hours
Food	Chinese fried rice	Meat and milk
Heat	Heat Stable	Heat labile
MOA	Increased CGMP	Increase CAMP
Toxins	Cerulide → Vagomimetic action	Nhe toxins → secretory Diarrhea

Lab Diagnosis 00:30:44

- Motile, Hemolytic
- Non encapsulated
- Selective media:
  - MYPA: Mannitol, Yolk Polymyxin, Agar
  - PEMBA: Polymyxin, Mannitol, Bromothymol Blue, Agar

MCQs

Q. Which of the following is not a virulence factor of bacillus anthracis?
A. Labile toxin
B. Edema toxin
C. Lethal toxin
D. Capsular polypeptide

Correct Answer- Labile toxin

Q. All of the following are characteristics of bacillus anthracis except?
A. Motility on wet mount examination
B. Medusa head colonies
C. Poly-d-glutamic acid capsule
D. Absence of hemolysis on 5% sheep blood agar

Correct Answer- Motility on wet mount examination

Q. All true about bacillus anthracis, except?
A. Non-motile
B. Arranged in chains
C. Central/subterminal bulging spores
D. Capsulated

Correct Answer- Central/subterminal bulging spores

Q. Which of the following is not a cultural characteristic of B. anthracis?
A. Medusa head appearance
B. String of pearl reaction
C. inverted fir tree appearance
D. Bamboo stick appearance

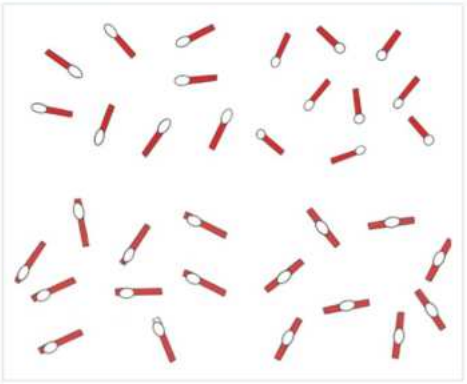
Correct Answer- Bamboo stick appearance

Q. Food poisoning has occurred due to ingestion of contaminated fried rice; most likely organism being?
A. Staphylococcus aureus
B. Bacillus cereus
C. Vibrio parahaemolyticus
D. Yesenia

Correct Answer- Bacillus cereus

Clostridium

00:35:24



- Obligate Anaerobe
- Gram +ve Bacilli
- Bulging spores
  - M/c location of spore is subterminal.
  - Terminal spores-
    → C. tetani (round: drumstick)
    → C. tertium (oval: tennis racquet)
  - Central spores-
    → C. bifermetans
- Mostly encapsulated except for:
  - C. perfringens
  - C. butyricum
- All are Motile except:
  - C. Perfringens
  - C. Tetani VI

C. Perfringens

00:39:51

- Non-motile
- Encapsulated
- Gram +ve Bacilli
- Subterminal spores

Virulence Factors

00:40:22

Invasive + Toxigenic		
4 major toxins: • β • ε • ι • α or Lecithinase or Phospholipase C	8 minor toxins: γ, δ, θ, κ, λ, η, μ, ν	Enzymes: Histaminase, neuraminidase

Clinical Features

00:41:26

1. Gas gangrene

Wound Infection		
Simple wound contamination	Anaerobic cellulitis	Anaerobic myositis (gas gangrene- type of myonecrosis)

Mechanism

00:42:10

- It requires:
  - Anaerobic environment
  - Wound contamination
  - Rarely spontaneous
- Road accident happens→ wound→ wound contamination → alpha toxin (lecithinase) of C. Perfringens will damage cell membrane→ Capillary permeability increased → muscle tension → Further anoxia
- Alpha toxin→Hemolytic→RBC breakdown→ Hemoglobinuria

Features of Gas Gangrene:

- Foul smelling, black, dirty looking tissues
- Pain
- Discharge
- Gas bubbles (Crepitus)
- Shock

Incubation Period of Organisms causing Gas Gangrene

00:44:55

- C. perfringens - 10-48 hrs
- C. septicum - 2-3 days
- C. novyi - 5-6 days

Treatment of Gas Gangrene

00:45:45

- Surgical Debridement - Removal of Dead muscles
- IV Penicillin + Clindamycin for 10-14 days
- Hyperbaric oxygen
- Passive Immunization can be given in some cases- Anti-gangrene serum

2. Food Poisoning

- Because of heat resistant Spores
- Can be caused due to cold/ warmed up meat.

3. **Gangrenous appendicitis**
- Caused by Type A
4. **Necrotizing enteritis**
- Caused by Type C

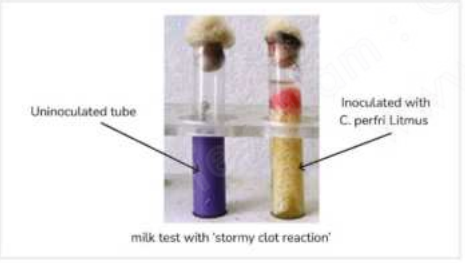
5. **PIGBEL:** 00:47:48

a. Common in European countries.
b. Abdominal pain & Diarrhea
c. Caused by consumption of pork and sweet potato together.
d. Sweet potato has trypsin inhibitors which prevents the breakdown of Beta toxin of pork in the intestine.
e. Rx- IV Penicillin + Metronidazole

Lab Diagnosis 00:49:55

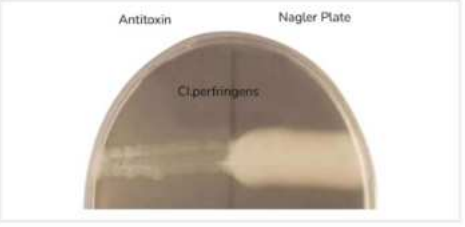
- Specimen:
  - Necrotic tissue
  - Muscle fragments

- Microscopically:
  - C. Perfringens shows- Subterminal spores.
  - C. Septicum shows- Citron body
- Media:
  - Robertson Cooked meat broth
  - Red/ Saccharolytic)
- Litmus Milk
  - Stormy clot formation on litmus milk



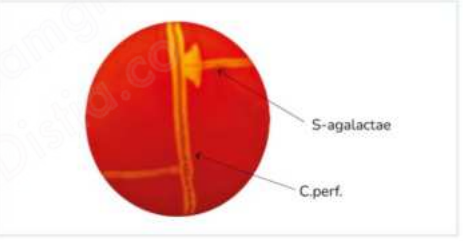
milk test with 'stormy clot reaction'

- Naegler's Reaction



  - Nagler plate has Egg yolk (lecithin) based agar
  - We divide the medium into 2 parts
  - C. perfringens has Lecithinase & egg yolk has lecithin which will break and opaque area is seen
  - On the other side, no opacification is seen as there is antitoxin which neutralizes.
- Target Hemolysis

  - Double zone of Hemolysis
  - Inner complete part is due to Theta toxin
  - Outer incomplete part is due to alpha toxin
- Reverse Camp Test +ve



  - In the reverse camp test, the horizontal line is of C. Perfringens
  - There is an arrowhead formation at junction.

Clostridium tetani 00:57:41

- Causes - Tetanus (muscle spasm)
- It has terminal spore (drumstick appearance)

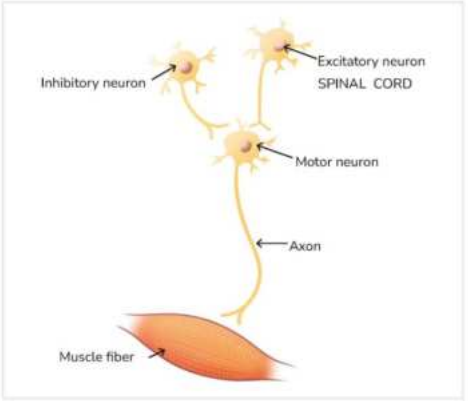
Virulence Factors 00:58:19

- 2 Exotoxins
  - Tetanolysin
  - Tetanospasmin

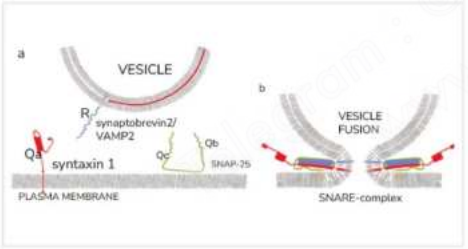
Tetanolysin
- Oxygen labile
- Heat labile

Tetanospasmin
- Causes spasm
- Oxygen stable

- Heat labile
- Muscle Contraction
- Neurotoxin→ acts presynaptically at inhibitory neuron terminal→ prevents release of GABA & glycine.



- Tetanus is gone in muscle (sores in muscle)
- Release of TT (Tetanospasmin)
- Retrograde movement
- It goes till Inhibitory neuron



- Tetanospasmin inhibits the fusion of the SNARE proteins such as synaptobrevin, SNAP25, and Syntaxin. As a result, the vesicles will not fuse and the mediators such as GABA and Glycine will not be released.
  In the absence of GABA, Glycine the muscle will not relax. However since Ach is released the muscle will continue to contract leading to muscle spasms
- No relaxation happen due to muscle spasm

Important Information

- Strychnine Poisoning
  - Acts post synaptically

Transmission 01:04:11

- By Injury: Unsterile RTA
- No person to person transmission
- Incubation Period- 6-10 days
  - Tetanus is also known as 8th day disease

Clinical Features 01:04:27

- 1st Symptom is Increased tone of masseter muscle → Lockjaw / Trismus
- With progression of disease:
  - Limb Spasm
  - Descending spastic paralysis
  - Deep tendon reflexes exaggerated.
  - Autonomic disturbance (2nd week; Increased BP, PR, Sweating)
  - M/c cause of death- Respiratory Failure

Complications 01:06:12

- Risus Sardonicus (looks like patient is smiling but it's muscle contraction)
- Opisthotonos

Diagnosis 01:07:01

- Specimen: Necrotic Tissue
- Microscope examination:
  - Gram +ve Bacilli
  - Spores with Drumstick appearance- Terminal Spores

- On blood agar- Concentric movement is seen- Swarming
- Gelatin Stab:
  - Fir tree appearance
  - Organism will work maximally at the bottom.
- Robertson's Cooked Meat Broth –
  - Black color
  - Proteolytic reaction

Fildes Technique 01:10:28

- To obtain pure colonies of C. tetani

Prevention after injury 01:11:32

		Simple wound (Duration- <8 hrs)	Dirty wound (Duration- >8 hrs)
Category A	TT / Booster in last 5 yrs	Nothing	Nothing
Category B	5-10 years	Toxoid 1 dose	Toxoid 1 dose
Category C	Beyond 10 yrs	Toxoid 1 dose	Toxoid 1 dose + HTIg
Category D	Unknown status	Complete Toxoid dose	Complete Toxoid dose + HTIg

C. Botulinum 01:14:40

- Most toxic substance.
- Bottled and canned foods

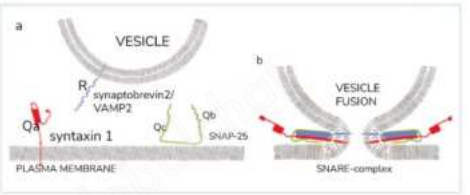
Virulence Factors 01:15:15

- Botulinum Toxin
- Serotype: 9
  - A, B, E – cause infections in humans
  - A- most severe
  - All are chromosomal mediated
  - C1, C2, D phage coded
  - All are neurotoxin except C2 which is enterotoxin
- Botulinum toxin: Produced intracellularly appears in medium only on cell death

Botulinum toxin
↓
Released as protoxin
↓
Activated by trypsin

Mechanism: Flaccid paralysis → blocks release of Ach

Snare Protein



- Excitatory Neuron→Botulinum toxin will come and cut off Snap 25, Syntaxin 1, Synaptobrevin→No fusion and acetylcholine is not released →No contraction →Floppy muscles

Clinical Manifestations

- No contraction of muscle (floppy muscles) → flaccidity is noted.

Types of Botulism 01:19:25

1. **Food borne botulism:**
- Bottled and canned food (heat Labile toxins)
- IP- 12-36 hrs
- Results in-
  - Diplopia
  - Dysphagia
  - Dysarthria
  - Descending Flaccid Paralysis
  - Dilated pupils.
  - GI symptoms are also present.

2. **Wound botulism**
- IP- 7-10 days
- No GI symptoms

3. **Infant botulism:**
- Bottle fed / honey / baby food
- IP: 1-2 days
- First symptom is Constipation.
- Floppy baby syndrome → flaccid muscles

4. **Iatrogenic botulism:** Surgeries

Lab diagnosis 01:23:37

- Gram +ve Bacillus
- Spores seen
  - Subterminal
  - Oval
  - Bulging
- Anaerobe

Treatment 01:23:55

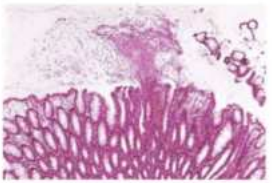
- Toxoid Antiserum

Clostridium Difficile 01:24:09

- Normally present in the gut
- Pseudomembranous enterocolitis is caused.
- Virulence factors:
  - Toxin A: enterotoxin (gut attached)
  - Toxin B: cytotoxin

Mechanism 01:25:17

- We have toxin A & B coming in system.
- They will soon attack the GTP binding proteins (Rho, Rac, CDC 42)
- There will be a damage to actin cytoskeletal → Cell death.
- Toxins will stimulate IL8 production → attract neutrophils recruitment.
- This will form dirty yellow necrotic membrane present on Mucosa.
- The dead cells will burst out like a volcano eruption (in microscopic vision)



Pseudomembranous Enterocolitis 01:28:01

- Risk factor - Long term usage of antibiotics (3rd generation cephalosporins)
- Clinical feature- Watery Diarrhea acute colitis with watery diarrhoea

Diagnosis 01:28:50

- Colonoscopy
  - 100% specificity
  - 50% sensitivity

- Toxin-
  - Tissue culture Assay
  - ELISA
  - PCR

- Media:
  - CCFA- Cefoxitin Cycloserine Fructose Agar
  - CCYA- Cefoxitin Cysteine Yeast Extract Agar
- Histopath- Volcano like eruption is seen.

Treatment 01:30:27

- Now Fidaxomycin is used.
- Recommended treatment in severe cases is oral vancomycin (500 mg four times daily) in conjunction with intravenous metronidazole and rectal vancomycin enemas in cases of severe ileus (Fulminant CDI)

MCQ

Q. Gas gangrene- is caused by all except?
A. C. Perfringens
B. C. Tetani
C. C. Novyi
D. C. Septicum
Correct Answer- C. Tetani

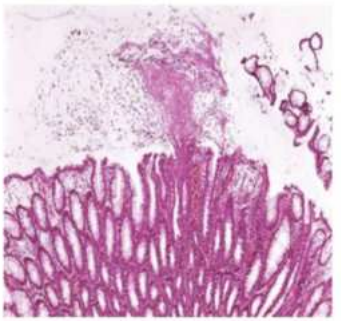
Q. Nagler reaction is predominantly associated with which toxin of Perfringens?
A. Theta toxin
B. Cytokine
C. Desmolase
D. Lecithinase
Correct Answer- Lecithinase

Q. Which of the following is incorrect about the toxin leading to the disease shown below?

A. It is heat labile
B. It is oxygen stable
C. It acts post synaptically
D. Inhibits the release of GABA

Correct Answer- It is oxygen stable

Q. Hospitalized patients developed severe watery diarrhea and dehydration following a long-term course of antibiotics. The histopathological image is shown below. Which of the following
toxins are responsible for the production of this condition.



A. Collagenase
B. Fibrinolysin
C. Lecithinase
D. Toxin A and B

Correct Answer- Toxin A and B

13 GRAM POSITIVE BACTERIA PART-3

Mycobacteria

- Mycobacterium tuberculosis.
- Mycobacterium leprae.
- Non-tuberculous mycobacteria.

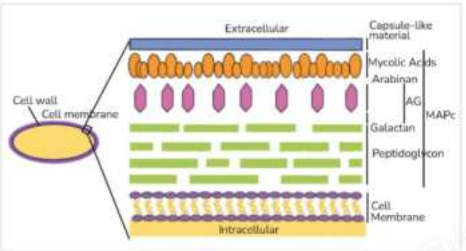
Mycobacterium tuberculosis

00:00:29

Mode of transmission

- Droplet nuclei (<5 micron).
- Ingestion.

Structure of M tuberculosis



- Cell membrane.
- Peptidoglycan → Gram positive bacteria.
- Arabinogalactan.(AG)
- Lipoarabinomannan (LAM).
- Mycolic acids.

Antigenic properties

- **Mycolic acid-**Acid fastness.
- **Cord factor-**Virulence.
- **LAM-**
  1. Attachment to host cells.
  2. Inhibition of phagolysosome formation.
  3. LAM-urine assay used to test TB in HIV+ patients.

Clinical Features

Pulmonary TB

Primary	Secondary	Disseminated
Subpleural location.- Lower part of UL & Upper part of LL.	presents at the Apex has a cavity in the lung	Affects different parts of the body such as the liver.
	Reactivation TB.	Miliary TB.
		Hematogenous spread.

Note:

Lung cavities occur in:

- Secondary TB.
- Histoplasmosis.
- Bronchiectasis.
- Squamous cell carcinoma.

Aspergilloma spp invades pre-existing cavity.

Extrapulmonary TB.

- Most common extrapulmonary TB is lymph node TB.
- Other affected sites:
  - Kidney → Putty kidney.
  - Urinary bladder → Thimble bladder.
  - Skeletal → Pott's spine.
  - CNS.
  - Skin → Lupus vulgaris, Scrofuloderma.
    → Scrofuloderma involves spread through the skin from an underlying focus.

Foci

- Ghon foci → Involvement of lung only.
- Ghon complex→ Involvement of lung and hilar lymph nodes.
- Ranke complex → Calcification present in Ghon complex.
- Apex → Simon focus.
- Supraclavicular → Puhl's focus.
- Infraclavicular → Assman focus.
- Liver → Simmond focus.
- Blood vessels → Weighert's focus.
- Brain → Rich's focus.

Hosts

- Humans.
- Cattle.
- Dogs.
- Cats.

Diagnosis of pulmonary TB

00:11:53

Specimen-Diagnosis of pulmonary TB

- Two sputum samples taken:
  - Spot sample.
  - Early morning sample.
- 2-5ml of sputum sample required.
- Sample should not have blood or saliva.
- If sputum is unavailable:
  - Laryngeal aspirate.
  - Bronchial secretions.
  - Bronchoalveolar lavage.
- In children, gastric lavage may be done.
  - Early morning gastric lavage taken.
  - Test should be done within 4 hours of specimen collection.

Diagnosis of extrapulmonary TB

- Lymph node fine needle aspiration cytology (FNAC) to examine pus.
- Pleural tap to examine pleural effusion.
- Cobweb reaction CSF test to examine for meningitis.
- Urine:
  - Intermittent 2-3 days sample.
  - Centrifugation with study of sediment.

Concentration

Petroff method

- Sputum + 4% NaOH.

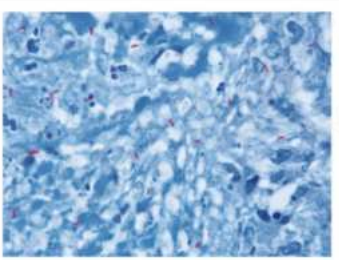
Further modification

- NALC + 2% NaOH.
- NALC-N-acetyl L-cysteine.

Microscopy

- Ziehl-Neelsen (ZN) stain.
  - Carbol fuschin.
  - Heat.
  - Acid H_2SO_4.
  - Methylene blue.
- Sensitivity → 10^4 acid-fast bacteria/ml.

Acid fast stain



Microscopic slide demonstrating ZN staining of M. tuberculosis>

- Organism → Red.
- Background → Blue.
- Reason → Mycolic acid presence.
- Appearance:
  - M. tuberculosis → Beaded appearance.
  - M. bovis → Uniform appearance.

Principle

- M. tuberculosis is acid fast and resists decolorization by 20% acid H2SO4.
- M. tuberculosis is alcohol fast and resists decolorization by 3% acid in 95% alcohol.
- TB is acid fast and alcohol fast.
- Atypical TB is only acid fast.

Grading

Number of bacilli	Result	Grading
>10/field	Positive	3+
1-10/field	Positive	2+
10-99/100 field	Positive	1+
1-9/100 field	Positive	Scanty
None	Negative	Negative

Microscopic fluorescent stain

- Stains include:
  - Auramine.
  - Rhodamine → Mycobacterium tuberculosis.

Culture

Egg-based culture	• Lowenstein-Jensen medium. • Dorset egg medium.
Blood-based culture	• Tharshi medium.
Serum-based culture	• Loeffler's medium.
Potato-based culture	• Pawlowsky medium.
Liquid media	• Middlebrook's medium. • Sula and Sauton's medium. • Proskauer and Beck's medium.

Lowenstein-Jensen medium

- Composition:
  - Coagulated hen's egg.
  - Mineral salt solution.
  - Asparagine.
  - Malachite green.
- Rough, buff (cream-colored) and tough colonies.
- 6-8 weeks to grow.
- Sensitivity → 10-100 bacilli/ml sputum.

Liquid culture media

- Long serpentine cords formed due to present cord factor.

Newer automated methods

- Take 8-14 days.

Bactec MGIT

Bactec machine.-used for diagnosis and measure the resistance

- MGIT → Mycobacterial growth indicator tube.
- Uses Middlebrook 7H9 broth.
- Fluorometric technique.
- Principle:
  - Fluorescence quenched in the presence of oxygen.
  - Mycobacterial growth uses the oxygen, leading to florescence.

BacT/alert

- Colorimetry-based technique.

Versatrek

- Detects pressure changes in media due to gas production.

Sensitivity

- Pyrazinamide added to test sensitivity.

Typing methods

- RFLP → Restriction fragment length polymorphism.

RFLP Is16110

- Target sequence/strain.
- Cannot differentiate active v/s latent TB.

Line probe assay

- Diagnosis and resistance.

Genexpert

- It is a type of CBNAAT → Cartridge-based nucleic acid amplification test (contains the sample).
- Useful for diagnosis and rifampicin resistance.
- Rifampicin drug resistance can be detected based on RpoB gene.
- Turnaround time → 2 hours.
- Sensitivity → 88%.
- Specificity → 99%.
- Detection limit → 131 organisms/ml.

Diagnosis of latent TB

00:36:54

Two tests can be done:

- Tuberculin test/Mantoux test.
- IGRA.

Tuberculin test/Mantoux test

- Type IV hypersensitivity reaction or cell-mediated hypersensitivity reaction.
- Antigen → Purified protein derivative (PPD).
- Procedure → Intradermal injection of 0.1ml PPD.
- Site → intradermal Flexor aspect of forearm.
- Reading taken after 48-72 hours.
- Measurement done perpendicular to the long axis of the forearm.
- What to measure → Induration i.e. thickened area.
- What not to measure → Erythema i.e. redness.

Width of induration	Result
≥ 10 mm	Positive.
6-9 mm	Equivocal.
< 5 mm	Negative.

- Interpretation of test:
  - Positive test → Present or past infection.
  - False positive → Recent BCG vaccine, Non-tuberculous mycobacteria.
  - False negative → Early TB, Low immunity as in HIV.

IGRA

- Interferon gamma release assay measured using ELISA.
- Sample → T-lymphocytes of suspected individuals (Th1 cells targeted).
- T-lymphocytes exposed to antigens:
  - ESAT6.
  - TB 7.7.
  - CFP 10.
- Exposure stimulates release of interferon gamma that is measured by ELISA.
- Advantage → Negative in BCG vaccination.
- Disadvantage→ Cannot differentiate between latent and active infection.

MPT 64 test

- Transdermal patch test.
- Only positive in active TB.
- 100% specificity.
- 98% specificity.

LAM assay-LIPOARABINO MANNAN

- Urine.
- Principle- Immunochromatography
- Good for HIV patients with TB.

Prophylaxis 00:44:26

- BCG → Bacillus-Calmette-Guerin.
- DANISH 1331 strain recommended by WHO.
- Central BCG lab located in Chennai.
- Dose → 0.1 ml.
- Site → Above the insertion of the left deltoid.
- Immunity → 15-20 years.
- Scar → After 6-12 weeks.

- **Onco-tice strain** of BCG:
  - Used in treatment of superficial bladder cancer and early-stage urinary bladder cancer.
  - Intravesical BCG.

Non-tuberculous mycobacteria 00:46:33

- Atypical mycobacteria or tuberculoid mycobacteria or Environmental mycobacteria
- Reservoir → Soil and water.
- No human-to-human transmission.
- Positively acid-fast.
- Negatively alcohol-fast.

Runyon's classification

- Photochromogens.
  - Pigment production only in light.
- Scotochromogens.
  - Pigment production in light and dark.
- Non-photochromogens.
  - No pigment production.
- Rapid growers.
  - Grow within 7 days in LJ medium.

Biochemical test

- All are catalase positive.

Culture

- Orange pigment in Lowenstein-Jensen medium.

Photochromogens

Organism	Effect
M. marinum	Swimming pool/fish tank granuloma (Warty skin lesion).
M. asiaticum	Respiratory infection.
M. simiae	Respiratory infection.
M. kansasii	Upper lobe scarring/cavity formation.

Scotochromogens

- M.szulgai.
  - Scotochromogen features at 37°C.
  - Photochromogen features at 28°C.
- M. Scrofulaceum → Cause Scrofula.
- M. Gordonae → Tap water scotochromogen.

Non-photochromogens

- M. Avium.
  - Most common cause granulomatous disease.
- M. Intercellulare.
  - Known as battey bacillus.
  - It is the only mycobacterium to produce an exotoxin i.e. mycolactone.
- M. ulcerans.
  - Causes Buruli ulcer which produces Exotoxin mycolactone.
- M. xenopi.
  - Grows at high temperature (45°C) in hospital water.
- Mycobacterium indicus pranii (MIP)

Lady Windermere syndrome

- Mycobacterium avium complex (MAC).
- Cough suppression led to non-clearance of secretion and bronchiectasis.
- Increased risk of MAC infection.

Rapid growers

- M. fortuitum.
- M. smegmatis.
- M. chelonae.
  - Associated with risk of postoperative abscesses.
- M. phlei.

Mycobacterium leprae/Hansen's bacillus 00:56:45

- Obligate intracellular organism.
- Obligate aerobe.
- Cannot be grown on culture media,can only be grown in animal.

Mode of transmission

- Nasal droplets – most common.
- Contact.
- Breast milk.

Ridley-Jopling classification

LL	Lepromatous leprosy.
BL	Borderline lepromatous leprosy.
BB	Borderline leprosy.
BT	Borderline tuberculoid leprosy.
TT	Tuberculoid leprosy.

	LL	TT
Bacillary load	Multi bacillary.	Pauci bacilliary.
Bacteriological index	Higher load. 4-6+	0-1+
Skin lesions	Leonine facies. Alopecia.	Few.
Nerve lesions	Late.	Early.
Cell-mediated immunity	Low.	Normal.
Lepromin test	Negative.	Positive.
CD4/CD8	1:2	2:1
Humoral immunity	Increased.	Normal.
Macrophages	Negative.	Present.
Giant cells	Negative.	Present.

Diagnosis

01:01:45

Specimen

- 6 smear samples:
  - 4 from the skin (forehead, cheek, chin and buttock).
  - 1 from the ear lobe.
  - 1 from nasal mucosa.
- Slit skin smear.
- Biopsy nodular lesion and thickened nerves.

Interpretation

- Lepromatous leprosy microscopy shows:
  - Virchow cells.
  - Cigar bundles (globi).
- Stain used is Fite foracco stain.
  - Ziehl-Neelsen (ZN) stain with 5% H_2SO_4.

Grading

	Grade
1-10 bacilli in 100 fields	1+
1-10 bacilli in 10 fields	2+
1-10 bacilli per field	3+
10-100 bacilli per field	4+
100-1000 bacilli per field	5+
>1000 bacilli/clumps/globi in every field	6+

- Bacteriological index = No of (+S) scored in all smears / number of smears.
- Morphological index = % of SFGB out of total bacilli.
  - SFGB → Solid fragmented granular bacilli.
- Morphological index preferred over bacteriological index for prognosis determination.

Animal cultivation

- Grown in a nine-banded armadillo or footpad of mice.

Serology

- Antibody against PGL-1 (Phenolic glycolipid).

Lepromin test

- Type IV hypersensitivity reaction or cell-mediated reaction.
- 0.1ml of lepromin antigen given intradermally in the forearm.

Interpretation

- Fernandez's reaction.(Early reaction).
  - >10 mm induration after 48 hours.
- Mitsuda's reaction.(Late reaction).
  - >5 mm nodule at 21 days or 3 weeks.

Treatment

- DCR regimen.
- Dapsone.
- Clofazimine.
- Rifampicin.

Lepra reaction

Type I	Type II
Type IV hypersensitivity reaction.	Type III hypersensitivity reaction (erythema nodosum leprosum).
Usually seen in BB. Downgrading occurs followed by reversal/upgrading.	Seen in BL and LL.

Can be seen before or after treatment.	Seen after treatment.
Most common feature is edema. Most common affected nerve is ulnar nerve.	Crops of tender inflamed nodules due to TNF-α.
Th1.	Th2.
Glucocorticoids.	Thalidomide.

- Tinel sign positive in type I.
- Pressure exerted on ulnar nerve in type I causes tingling sensation.

MCQs

Q: All of the following are correct matches except?
A. M. kansasii → Photochromogen.
B. Battey bacillus → Non-photochromogen.
C. M. szulgai → Scotochromogen.
D. M. xenopi → Photochromogen.
Answer: D. M. xenopi → Photochromogen.

Q: Mycobacterium TB is differentiated from other mycobacteria by which of the following cells?
A. Niacin.
B. PAS stain.
C. AFB stain
D. None of the above.
Answer: A. Niacin.

Differences between M. tuberculosis and M. bovis

	M. tuberculosis	M. bovis
Niacin	Positive.	Negative.
Nitrate reduction	Positive.	Negative.
Catalase	Positive.	Negative.
Colony	Rough, buff and tough.	Smooth, white and breaks easily.
Oxygen requirement	Strict aerobes.	Microaerophilic.

Q: A 66 year old man underwent hip replacement following degenerative joint disease. 5 days later, he presented with fever and pain. There is tenderness and swelling in the hip joint. The hip joint showed fluid accumulation which was aspirated and sent for microbiological assessment. Culture shows positive growth in 4 days and organism is acid-fast. Most likely diagnosis based on the above clinical and microbiology scenario is?
A. Mycobacterium Chelonae.
B. Mycobacterium tuberculosis.
C. Mycobacterium leprae.
D. Mycobacterium kansasii.
Answer: A. Mycobacterium chelonae.

14 GRAM NEGATIVE BACTERIA PART 1

Bacteriology - Enterobacteriaceae

Edward and Ewing classification

00:00:15

For enterobacterial family, there is an Edward and Ewing classification:-

- Escherichieae: Escherichia, shigella
- Edwardsielleae: Edwardsiella
- Salmonelleae: Salmonella
- Citrobactereae: Citrobacter
- Klebsielleae: Klebsiella, enterobacter, serratia, hafnia
- Proteeae: Proteus, morganella, providencia
- Yersiniae: Yersinia
- Erwinieae: Erwinia

Enterobacteriaceae

00:01:29

- They are gram negative
- Non sporing
- Aerobic
- Motile bacteria with Peritrichous flagella
  - Except shigella, Klebsiella, Salmonella Gallinarum, Salmonella Pullorum - all non motile
- Glucose fermentation (acid and gas/ acid)
- Nitrate → Nitrite
- Catalase +ve Oxidase -ve.
  - Except shigella dysenteriae (type 1)- catalase negative

Classification

00:03:08

- Lactose fermenter
  - E.coli
  - Klebsiella
  - Enterobacter Citrobacter
- Late lactose fermenter
  - Shigella sonnei
- Non lactose fermenter
  - Salmonella, shigella, proteus, yersinia

Testing for lactose fermentation

00:04:06

- MacConkey Agar
  - Pretty Pink Plant (mnemonic)
  - P- Peptone
  - L- Lactose fermentation -differential media
  - A- Agar
  - N- Neutral red (indicator dye) - indicator media
  - T- Taurocholate (Bile salts) - selective media.
- CLED -Cysteine Lactose Electrolyte Deficient
  - It is also lactose fermenter
  - Lactose fermentation gives yellow colour.
  - Indicator media - BTB (Bromothymol blue)
  - CLED is considered better than MacConkey because it is less inhibitory.
  - It allows both gram positive and gram negative to grow.
- EMB -Eosin Methylene Blue
  - Shows lactose fermentation
  - It gives blue black colonies with green sheen around it.

Note:

Which is the better media between MacConkey and CLED?

- CLED is the better media than MacConkey

MacConkey	CLED
Gives pink colour on lactose fermentation	Gives yellow colour on lactose fermentation
Neutral red is the indicator	BTB is the indicator

Escherichia Coli

00:09:29

- Lying only in humans/animal intestine
- Classification in terms of its antigens:-
  - Somatic O antigen (present in cell wall)
  - Flagellar H antigen
  - Capsular K antigen
    - → Type 1 - antiphagocytic
      - As a result, the patient will have Meningitis, sepsis, and other features.
    - → Type 2
- Toxins:-
  - Hemolysin
    - → Produced more commonly by virulent strains.
    - → Role in pathogenesis +/-
  - Enterotoxins
    - → LT (Labile toxin)
    - → ST (Stable toxin)
    - → VT/SLT (Verocytotoxin/shiga like toxin)

Toxins	Plasmid coded	Phage coded
LT	ST	VST
Heat labile ↓ Fragment A (1) CAMP ↑ Fragment B (Five) Gm1 ganglioside	Heat stable STA: cGMP	Verocyto/shiga like toxin) • Has mechanism similar to shigella • E.coli(enteroinvasive) can cause dysentery similar to shigella. MOA: 60S Ribosomes decreases → protein synthesis decreases

CLED is Better than macconkey as it is less inhibitor. It allows both gram +Ve and grm -Ve organisms to grow

Decreasing protein synthesis

- D- Diphtheria toxin
  - Mechanism:- Cause ADP Ribosylation of EF2
- P- Exotoxin A of Pseudomonas
  - Mechanism:- Cause ADP Ribosylation of EF2
- S- ST (shigella) SLT (Shiga like toxin) VST
  - Inhibits the 60S Ribosomes
- Ultimate goal is to decrease protein synthesis

Toxin testing:-

LT	ST	VST
Heat labile	Heat stable	Verocyto/shiga like toxin
← INVIVID → Ligated Rabbit Ileal loop test ⇒ 18 hrs (+) Invitro (+)	⇒ 6 hrs (+) Invitro (-)	↓ • ELISA • Hela, Vero ↓ (African Green Monkey)

- In vivo - grown in ligated rabbit ileal loop test
  - 18 hours ie in LT (Labile toxin) → Invitro +ve
  - 6 hours ie in ST(Stable toxin) → Invitro - ve
- VST - Verocyto/ Shiga like toxin
  - It is actually grown on vero cell lines, so called Verocyto.
  - ELISA
  - Hela,vero - (African green monkey)

In vitro tests of E.coli LT

- Show Steroid production in mouse adrenal cell
- Elongation in Chinese hamster ovary cells.

Clinical features of E.coli 00:16:46

- UTI
- Diarrhoea
- Meningitis
- Sepsis
- Pneumonia

Strains (capable of causing diarrhoea)

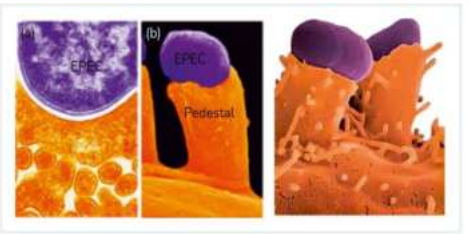
- EPEC
- ETEC
- EIEC
- EHEC
- EAEC
- DAEC

Refer Table 14.1

Note:

PYQ AIIMS - EPEC shows A/E lesions

- A/E - attachment/effacement lesions



Sereny Test 00:23:06

- Inoculation suspension of bacteria into guinea pig's eye.
- Severe mucopurulent conjunctivitis and severe keratitis indicates a positive test.

Sereny test is done for:

- Enteroinvasive E. coli
- Shigella
- Listeria monocytogenes (anton test)

SMAC test

- S- Sorbitol fermenters (pink)
- Mac - MacConkey Agar
- But for O157:H7, sorbitol will not be fermented, and no reaction will happen with the SMAC test.

Laboratory diagnosis 00:30:34

Associated with UTI

Sample: Urine

- Fresh clean catch midstream urine
- Suprapubic aspiration (Obstructive uropathy)-if person cannot pass urine
- For example:
  - Needle is put above the pelvis into the urinary bladder.
- Catheter specimen not recommended

Culture 00:31:55

- Blood Agar: Haemolytic
- MacConkey: pink
- CLED: yellow
- Selective media: DCA
- Broth: Turbidity and heavy

KASS Criteria 00:32:37

- Significant bacteriuria > It has more than 100000 CFU/ml.
- Exception:-
  - S- Suprapubic aspiration

- P- Gram POSITIVE Cocci (staph, candida)
- A- already on Antibiotic course T/T
- On diuretic T/T

Laboratory diagnosis 00:35:35

Quantitative method:

- Pour plate method (count/ml)
- Complicated; not done routinely.

Semi quantitative method:

- Standardized loop method.
- Performed more frequently

Screening tests:

- Microscopic examination (GNB)
- Dip slide culture method
  - Took slide with Agar present on it.
  - Then, dipped that slide in a urine sample.
- Catalase + (bubbles)
- Griess nitrite test +
- TTC test (triphenyl tetrazolium chloride): detects metabolically active organisms (using Myocardial infarction)

Biochemical test: 00:37:58

- IMViC testing:-
- I- Indole
  - Tryptophan (tryptophanase) → indole (red colour +ve)
- M- Methyl red
  - Glucose utilised by mixed fermentation pathway result in acid+ production Red colour signifies positivity.
- V- Voges proskauer test
  - Glucose utilised by non- mixed fermentation pathway.
- C- Citrate utilisation
  - Bacteria utilise citrate as a source of carbon and blue colour will indicate positivity.
- Ferments glucose/lactose/maltose/sucrose (acid and gas)
- Laboratory diagnosis for E.coli

  - ++-- = E.coli (lactose fermentation)
- Laboratory diagnosis for Klebsiella
  - --++ = Klebsiella (lactose fermentation)

Treatment

- Rx: Uncomplicated: Cotrimoxazole or Ciprofloxacin
- This depends on sensitivity. (AST)

Shigella 00:43:00

- Non motile
- Non sporing
- Non encapsulated
- Gram negative bacilli
- Infective dose: low 10-100 bacilli (small infectivity dose)
- No lactose fermenter ability

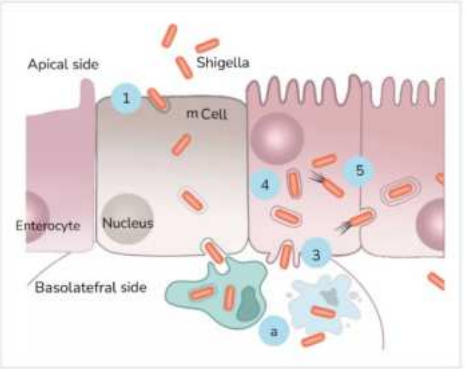
Different Shigella species

- S. Dysenteriae
- S.flexneri: most common in India
- S.boydii
- S.sonnei: most common in the world
- All of them are non Lactose fermenters.
- But S.sonnei is a late lactose fermenter.
- All of them are catalase positive and mannitol fermentation.
- Whereas Shigella Dysenteriae shows catalase negative but not mannitol fermentation.

Toxins for shigella 00:45:42

- Exotoxin
  - Enterotoxin ShET 1 & 2
    → Associated with shigella flexneri
  - Cytotoxin Shiga toxin
    → Associated with shigella dysenteriae
  - MOA-decreases protein synthesis by decreasing 60S Ribosomes.
- Endotoxin
  - Released by autolysis
  - Induced intestinal inflammation & ulceration

Mechanism of Action



- Shigella organism or shiga toxin is picked up by M cells.
- It is then given to the macrophages in the body. So, it will rupture the macrophage.

- Due to which shiga bacteria will be out and enter the next cell via Basolateral route.
- This is known as Basolateral endocytosis.
- It is invading because of VMA (Virulence marker antigen) and IPA (Invasive plasmid antigen)
- Then, it will go cell to cell by Actin polymerisation.

Shigellosis 00:50:47

- Infection Phase
  Incubation period-1-4days
- Initial: watery diarrhoea → dysentery
- Complication
  - Toxic megacolon
  - Perforation
  - Metabolic complication - hypoglycemia, hyponatremia
  - Ekiri syndrome- toxic encephalopathy, delirium, confusion
  - HUS-hemolytic uremic syndrome
    → EHEC
    → Shigella
- In post infectious phase:-
- Reiter's syndrome
  - Reactive arthritis
  - Urethritis
  - Conjunctivitis

Diagnosis of shigella 00:52:49

- Sample - stool
- IMViC testing -+--
  - Mnemonic - M Shy
  - M- Only methyl red positivity will be seen in
  - S- Shigella
  - Y- Yersinia
- Transport media
  - SBGS-Sach's buffered glycerol saline
- Enrichment media / broth
  - Selenite F broth
  - Tetrathionate Broth
- Selective media
  - XLD (Xylose lysine deoxycholate)
  - DCA (deoxycholate agar)
  - SS (salmonella shigella agar)
  - HE (Hektoen enteric agar)
- All enrichment and selective media are S/S (salmonella/shigella) sisters.

Treatment

- Supportive
- If it does not work, take antibiotics.

Table 14.1

Strains	Disease	Extra
EPEC Enteropathogenic E.coli	Pediatric/ infantile diarrhoea	Bundle forming PILI (BFP). Plasmid coded makes BFP.
ETEC Enterotoxigenic E.coli	• Traveller's Diarrhoea • Diarrhoea in children • It has a very large infectivity dose (contaminated food).	Colonization factor antigen (CFA)
EIEC Enteroinvasive E.coli Test:- Sereny test	• Dysentery • Blood along with stool • It is also caused by shigella • Shigella Dysentery might not show you manitol fermentation or lactose fermentation.	Virulence marker antigen (VMA)
EHEC Enterohemorrhagic E.coli • Small infectivity dose • Less than 100 bacilli is enough to cause this infection.	• Most common cause of (HUS) hemolytic uremic syndrome • Hemorrhagic cystitis	• Associated with VST/SLT • Associated with strain O157:H7 • Test for O157:H7 does not show SMAC reaction.
EAEC EnteroAggregative E.coli	• Causes persistent diarrhoea for almost three weeks (>14 days)	• Due to EAST 1 toxin • On the hep2 cell line, it produces a stacked brick appearance.
DAEC Diffuse adherent E.coli		

15 GRAM NEGATIVE BACTERIA PART-2

Tribe Klebsiella

- Klebsiella
- Serratia
- Enterobacter cloaca
- Hafnia alvei
- Pantoea agglomerans

Types:

K. pneumoniae or Friedlander's bacillus.

- Pneumonia
- Red currant jelly

Sputum

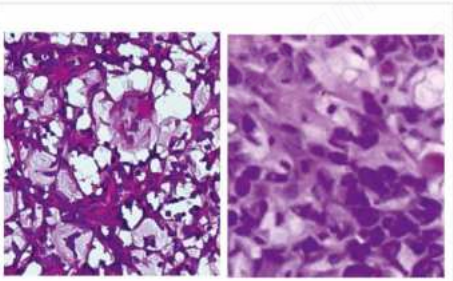
K. ozaenae.

- Atrophic rhinitis
- Foul-smelling discharge
- Anosmia

K. rhinoscleromatis

- Rhinoscleroma

Rhinoscleroma



- Gross findings
  - Woody nose or hebra nose
- Microscopic findings
  - **Mikulickz cell** → Foamy cell.
  - **Russell body** → Immunoglobulin production.

K. granulomatis.

- Painless genital ulcers.
- Also known as Donovanosis/Granuloma Inguinale (Donovan bodies).

Klebsiella pneumoniae

- Community-acquired, hospital-acquired or ventilator-acquired pneumonia.
- Chronic obstructive pulmonary disease (COPD) → Superadded infection.
- Catheter-associated urinary tract infection (CAUTI).
- Septicemia.

Wright-Geimsa stain

K. granulomatosis viewed microscopically

- Donovan bodies
- Pund cells (mononuclear cells)-Safety pin appearance of Donovanosis.
- Bipolar staining

Important Information

- Recent strain → Hyper-viscous, hyper-virulent strain of K. pneumoniae.
- Causes community-acquired pneumonia, meningitis and sepsis.
- Resistant to treatment.

Klebsiella pneumoniae 00:06:32

Morphology

- Non-motile rod
- Mucopolysaccharide capsule seen as a halo around the organism.

Diagnosis

MacConkey Agar (MCA)

- Positive lactose fermenter.

- Pink color after fermentation.
  - Pink color also seen in E. coli.
  - Capsule presence in K. pneumoniae can be used to differentiate it from E. coli.
- Mucoid colony
- String test positivity

Biochemical tests
- Urease positivity
  - Pink color
- IMVIC testing → - - + +
- Ferments all sugars with production of acid and gas.

Mnemonic Punch Kiss
- Proteus
- Ureaplasma
- Nocardia
- Cryptococcus
- Helicobacter
- Klebsiella
- Staphylococcus Saprophyticus
- Staphylococcus Epidermidis

Treatment
- Piperacillin + Tazobactam.
- Extensively drug-resistant (XDR) strains → Colistin or Polymyxin.

Serratia Marcescens 00:11:27
- Contaminated IV lines, surgical instruments and antiseptic solutions.
- Causes:
  - Pneumonia (pseudohemoptysis)
  - Contact lens keratitis(pink hypopyon)
  - Meningitis, cardiovascular problems, septicemia.

Culture
- Non-diffusible red pigment at room temperature.
- Pigment → Prodigiosin responsible for pseudohemoptysis.

Tribe proteeae 00:14:21
- Proteus.
  - Urease positive
  - Phenyl pyruvic acid (PPA) positive
- Providencia.
  - Urease negative
  - PPA positive
- Morganella.
  - Urease positive
  - PPA positive

PPA reaction

Phenylalanine acted on by phenylalanine deaminase to produce PPA.

Proteus
- Pleomorphic bacillus → Any shape
- Gram negative bacilli
- Non-capsulated
- Fishy odour

Swarming growth or motility

- Seen in: Mnemonic = PVCS
  - Proteus
  - Vibrio parahaemolyticus

- Vibrio Alginolyticus
- Clostridium tetani
- Bacillus cereus
- Serratia

- Inhibited by:
  - Firm agar (5-6% agar)
  - Chemicals e.g. boric acid and chloral hydrate.
  - MCA due to taurocholate bile acid present.

Epidermiological typing

- To determine strains in different patients.
- Same strains → Merging of swarming.
- Different strains → Line of demarcation.
- Known as Dienes phenomenon.
- Should not be confused with Diene stain done for Mycoplasma.

Uses of Proteus

- Non-motile strains especially Proteus mirabilis:
- OX 2, OX 19, OX K strains used in the Weil-Felix test for rickettsia.

Treatment

- Highly resistant bacteria.
- Antibiotic sensitivity testing required.
- P. mirabilis → Ampicillin and Cephalosporin sensitive.

Morganella

- Food poisoning due to uncooked fish.
- Scombroid food poisoning.

Tribe Erwinieae 00:24:09

Erwinia Herbicola

- Causes UTI
- Associated with Yellow pigment.

Yersinia 00:24:39

Refer Table 15.1

Note:

Differential motility seen in:

- Yersinia enterolitica
- Yersinia pseudotuberculosis
- Listeria

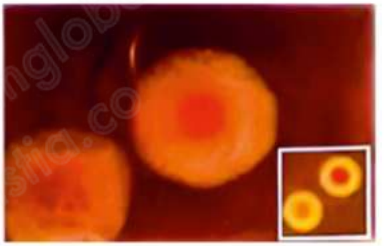
Yersiniosis 00:27:26

- 3 human diseases:
  - Self-limiting gastroenteritis.
  - Mesenteric adenitis, terminal ileitis (child).
    → Pseudo appendicitis → Mimic appendicitis.
  - Systemic diseases (adults).

Laboratory diagnosis of yersiniosis

Culture

- MAC agar
- Y. enterolitica is a lactose fermenter
- Selective CIN agar → Bullseye colonies.



Differential motility

- Motile at 22°C
- Non-motile at 37°C
- Phenomenon also seen in listeriosis

Cold enrichment

- Optimum temperature for growth is 25°C.
- Incubation at 4°C for 1 week makes isolation easier.
- Same phenomenon seen in listeriosis.

Note:

Both **listeriosis & Yerniosis** shows

- Cold enrichment
- Diff. motility

Yersinia pestis 00:29:44

Endemic foci for plague

- Kolar (Tamil Nadu)
- Beed Latur (Maharashtra)
- Rhoru (Himachal)
- Uttaranchal

Plague

- Zoonotic disease i.e. animal-to-human transmission.
- Reservoir → Wild rodents.
- Vector → Rat flea (Xenopsylla cheopis, Xenopsylla astia).
- Spread → Bite of rat flea.
- Flea index = Number of fleas/Number of rats.

Life cycle

- Flea gets infected from rat → Enters stomach → Multiplies → Comes back and block proventriculus of flea → Regurgitation in blood meal.
- Propagative vector.
- Extrinsic incubation period.
  - Time between ingestion and blockage.
  - 2 weeks for X. cheopis.

Virulence

- Protein envelope/Protein capsule antigen F1.
  - Heat labile, plasmid coded.
- V, W antigen inhibit phagocytosis.
- Coagulase positive.
- Fibrinolysin positive.
- Toxins: Endotoxin murine toxin.

Cycles of plague

Urban/Domestic cycle

- Between rodents and humans.

Wild/Sylvatic cycle

- Between wild rodents.

Types of plague

Bubonic plague	2-7 days incubation period. Lymph node painful enlargement and fever.
Pneumonic plague	1-3 days incubation period. Respiratory symptoms. Associated with bio-terrorism.
Septicemic plague	2-7 days incubation period. Aka Black death. DIC, shock and multi-organ failure.

Diagnosis:

Sample

- Stool
- Sputum
- Bubo

Microscopic findings

- Safety pin appearance
- Bipolar staining
- Wayson stain (new methylene blue NMB stain).
- Wright stain
- Giemsa stain

Organisms that show safety pin appearance

- Yersinia pestis
- Vibrio parahaemolyticus
- Burkholderia mallei
- Burkholderia pseudomallei
- Haemophilus ducreyi – Chancroid
- Klebsiella granulomatis – Granuloma inguinale

Culture

- Optimum temperature → 27°C.
- Development of envelope → 37°C.
- Blood agar/Chocolate agar → Dark-brown color due to absorption of hemin.
- Nutrient broth with oil/ghee (Ghee broth) → Stalactite growth.

Biochemical reactions

- Fermentation [+ acid production - gas production].
  - Glucose
  - Maltose
  - Mannitol
- Catalase positive
- Oxidase negative
- IMViC - + - -

Serology

- Passive hemagglutination by antibody against F1 antigen.
- Titre > 128 Å in a single sample (1:128).

Isolation from rats

- Carcass dipped in 3% Lysol to destroy infected fleas.

Vaccine:

Recombinant Rf1v vaccine

- Pre-exposure prophylaxis.

Sokhey's modification of haffkine vaccine

- Formalin-killed

- Subcutaneous route
- Protects only against bubonic plague.
- Protection for 6 months.

Treatment
- Streptomycin
- Fluoroquinolones →for CNS and cardiovascular involvement.

Salmonella
00:44:21

- Two types:
  - Typhoidal
  - Non-typhoidal

Kaufmann and White scheme
- Used to classify salmonella.

A	S. paratyphyi A.
B	S. paratyphyi B. S. typhimurium.
C1	S. paratyphyi C.
C2	S. muenchen.
D	S. typhyi. S. enteritidis.
E2	S. anatum.

Antigens
O (somatic)
- Complex made up of polysaccharide, protein and lipid.
- Boivin antigen.

O (somatic)	H (flagella)	Vi (vaccination)
Heat stable.	Heat labile.	Heat labile.
Less immunogenic.	More immunogenic.	Envelopes O antigen.
Reaction with antisera is: Slow. Chalky, granular clumps.	Reaction without antisera is: Fast. Loose, fluffy clumps.	S. typhi will not agglutinate with O antigen.
Same for S. typhi, S. paratyphi A and S. paratyphi B.	Different.	Used for vaccination

Antigenic variation

Loss of H antigen
- Loss of flagella

Loss of Vi antigen
- V antigen (agglutinable with Vi antiserum) changes to W antigen.
- Intermediate forms VW.

Loss of O antigen
- Smooth to rough variation of cell wall.
- Loss of virulence.

Enteric fever
00:51:09

- Typhoid fever

Pathogenesis
- Mode of transmission is feco-oral.
- Infective dose is 10^7-10^8 bacilli.

Life cycle
Binds to: M cells
↓
Penetrates submucosa
↓
Resists intracellular killing
[Invasion, survival, spread is regulated by Salmonella pathogenicity Island]
↓
Mesenteric lymph node
↓
Via thoracic duct: Blood [bacteria]

Clinical features
- Step-ladder pattern fever.
- Longitudinal ulcers in the GIT.
- Pea soup diarrhea.
- Faget's sign → Fever with bradycardia.
- Soft, palpable spleen and liver.
- Rose spots seen in 2nd or 3rd weeks that fade with pressure.

Serology
- **Relative lymphocytosis/neutropenia.**

Carriers
- Shed bacilli in feces and urine.

Convalescent carrier
- 3 weeks to 3 months of recovery.

Temporary carrier
- 3 months to 1 year of recovery.

Chronic carrier

- > 1 year of recovery.

Diagnosis

Week 1(B)	Blood culture.
Week 2(A)	Antibody test (WIDAL).
Week 3(S)	Stool culture.
Week 4(U)	Urine culture.

Blood culture

- Blood: Culture fluid used in ratio 1:10.
- Bile broth or glucose broth used.
- Add sodium polyanethol sulfonate (SPS) to remove antibiotic effect.
- Castaneda medium can be used.

WIDAL test

- Highly sensitive but poorly specific.
- Biological false positive occurs in:
  - Infectious mononucleosis.
  - Malaria.
  - Systemic lupus erythematosus (SLE).
- Antibody titre against O antigen → 1:60.
- Antibody titre against H antigen → 1:180.
- Flagellar antigen(H antigen) is more immunogenic.
- Paired testing done in a gap of 2 weeks with a 4-fold increase in titre suggesting positivity.

Types

- Slide WIDAL test.
- Tube WIDAL test (Kahn tubes).

Principle

- T_O → O antigen
- T_H → S. typhi H antigen
- A_H → S. paratyphi A H antigen.
- B_H → S. paratyphi B H antigen.
- 1st to appear in blood → O antibodies.
- Next to appear in blood → H antibodies.
- 1st to disappear in blood → O antibodies.
- Last to disappear in blood → H antibodies.

Refer Table 15.2

New tests

- Card-based tests.
- **Typhidot**
  IgM/IgG against outer membrane proteins.
- **Dot blot**
  IgG against flagellar membrane proteins antigen.

Culture Media:

Enrichment media

- Selenite F broth
- Tetrathionate B broth

Selective media

- Xylose lysine deoxycholate (XLD).
- Deoxycholate agar (DCA).
- Salmonella-shigella agar (SS).
- Hektoen enteri agar (HE).

All these media are used for shigella except Wilson and Blair media.

- Best media for S. typhi.
- Jet black colonies.

Biochemical tests

- IMViC - + - +

Bacteriophage typing

- S. typhi phage A, E1.
- S. paratyphi type 1 and 2.

Vaccine:

Typhoral

- Live oral vaccine.
- Ty21a-Lacks enzyme UDP-galactose epimerase.
- On ingestion, self-destruction occurs after 4-5 divisions and there is no illness.
- Immunity is established.

Typhim Vi

- Vi vaccine
- Ty2

Salmonella gastroenteritis

- Non-typhoidal salmonella.
- Most common cause:
  - S. typhimurium > S. enteritidis.
- Source → Food e.g. meat and milk.
- Incubation period → 24 hours.

Clinical features

- Fever
- Vomiting
- Invasive diarrhoea

Treatment

- Usually supportive.

Salmonella septicemia

- Caused by S. Choleraesuis.
- Clinical features
  - Endocarditis
  - Pneumonia
  - Osteomyelitis
- Fatality rate of 25%

Summary

Refer Table 15.3

***Note:**

Salmonella has non motile members

- S.Gallinarum
- S.Pullorum

MCQs

01:16:02

Q. Indicator used in CLED?

A. Neutral red

B. Bromothymol blue

C. Phenol red

D. Azure

Q. Indicator used in MacConkey agar?

A. Neutral red

B. Bromothymol blue

C. Phenol red

D. Azure

Q. CLED medium is considered better than MacConkey agar for the following reasons?

A. It differentiates lactose fermenters from non-fermenters.

B. It prevents swarming of Proteus.

C. It inhibits growth of commensals.

D. It allows Staphylococcus and Candida to grow.

Q. Which organisms cause hemolytic uremic syndrome?

A. Neisseria

B. Salmonella

C. Pseudomonas

D. E. coli

Q. Diagnostic test for EIEC is?

A. Sereny test

B. Sorbitol MacConkey agar

C. WIDAL test

D. Blood examination

Q. 65 year old diabetic man presents to the emergency room with a severe productive cough producing thick bloody sputum resembling currant jelly-like appearance. Culture using MacConkey agar shows pink colonies which are mucoid in appearance. Which of the following organisms are responsible for this patient's condition?

A. Proteus

B. E. coli

C. Klebsiella

D. Pseudomonas

Q. Which of the following shows stacked brick appearance on cell lines?

A. ETEC

B. EAEC

C. DAEC

D. EHEC

Q. The blood of a patient suspected to have pyogenic live abscess was sent for culture. The colony morphology shows large mucoid colonies and a positive string test. The most likely etiologic organism is?

A. Escherichia coli

B. Klebsiella pneumoniae

C. Vibrio alginolyticus

D. Streptococcus milleri

Table 15.1

Features	Y. pestis	Y. enterocolitica	Y. pseudotuberculosis
Disease	Plague.	Yersiniosis.	Yersiniosis.
Family	Miscellaneous bacteria.	Enterobacteraciae family.	Enterobacteraciae family.
Motility at 22°C	Non-motile.	Differential motility. Motile at 22°C. Non-motile at 37°C.	Differential motility. Motile at 22°C. Non-motile at 37°C.
Urease	Negative.	Positive.	Positive.

Table 15.2

	T_O	T_H	A_H	B_H
S. typhi	+	+	-	-
S. paratyphi A	+	-	+	-
S. paratyphi B	+	-	-	+
Early infection	+	-	-	-
Late infection	-	+	-	-
Vaccine	-	+	+	+

Table 15.3

Member	Motility	Lactose fermenting/ Non-lactose fermenting	IMViC	Urease
E. coli	Motile.	Lactose fermenting.	+ + - -	Negative.
Klebsiella	Non-motile.	Lactose fermenting.	- - + +	Positive.
Enterobacter	Motile.	Lactose fermenting.	- - + +	Negative.
Citrobacter	Motile.	Lactose fermenting.	V	Negative.
Shigella	Non-motile.	Non-lactose fermenting.	- + - -	Negative.
Salmonella*	Motile.	Non-lactose fermenting.	- + - +	Negative.
Proteus	Motile.	Non-lactose fermenting.	V	Positive.
Yersinia	Motile/Non-motile.	Non-lactose fermenting.	- + - -	Negative.

16 VIBRIO AND NON FERMENTERS

Vibrio

00:00:19

Types

- Halophilic
- Non Halophilic

Halophilic vs Non-Halophilic

00:00:34

Halophilic	Non-Halophilic

• Need Salt 7-10%
• Includes
  ○ V. Parahaemolyticus
  ○ V. Alginolyticus
  ○ V. Vulnificus
	• Cannot grow at high salt
	• Includes-
	  ○ V. Cholerae
	  ○ V. Mimicus

Gardner and Venkatraman Classification

00:01:23

Vibrio can be divided into

1. V. Cholerae: On the basis of O antigen, it's divided into-
   - O^1
   - O^{139}
   - O^2-O^{138}
2. Others

Biotypes of O^1

1. Classical
   - Ogawa
   - Inaba
   - Hikojima
2. Eltor
   - Ogawa
   - Inaba
   - Hikojima
3. Eltor variants

Differentiation between Classical and Eltor

00:02:49

	Classical	Eltor (Mostly +ve, resistant and carrier state)
Polymyxin B Sensitivity	Sensitive	Resistant
Phage IV susceptibility	Susceptive	Resistant
B-Hemolysis on sheep blood agar	Negative	Positive
Chick erythrocyte agglutination	Negative	Positive
VP Test	Negative	Positive
CAMP test	Negative	Positive
Mortality	More	Less
2nd attack rate	More	Less
Carrier subclinical cases	Lesser	More

Epidemiology

00:04:33

- 1992- In Chennai, there was a Non-O^1 outbreak
- 1993- In Bengal O^{139} (Capsulated) occurred
- In India, the eltor variant has come up
  - Rishikesh- Classical

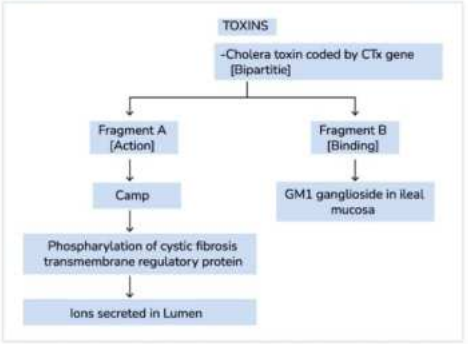
Pathogenesis

00:05:14

- In the vibrio Cholerae, there is Rice water stools.
- Transmission- Contaminated Food and water
- Infectivity dose: 10^8 CFU/ml (quite high ID, it is because most of the vibrio are killed by acid in stomach)
- Mucus crossed by mucinase
- For attachment – Pili are present.
- Intestinal epithelium adhesion- For attachment there is Toxin Coregulated Plus (TCP)
- Zona occludens toxin which disrupts the tight junctions.

Toxin

00:07:31



- Cholera toxin is coded by CTx gene and it is bipartite
  - Fragment A (action)
    → Activate Adenylate Cyclase
    → Phosphorylation of cystic fibrosis Transmembrane Protein
    → Ions secreted in lumen, responsible for Rice Water Stool
  - Fragment B (binding)
    → Binding occurs at GM^1 Ganglioside.

Refer Image 16.1

- A has two parts- A^1 & A^2
  - A^1 & A^2 helps in Activating Adenylate Cyclase & cAMP.
- B has 5 subparts
  - For binding, it has to bind to GM^1 Ganglioside.
- Because CFTR is affected by cAMP, all the water and chlorine is going into lumen causing rice water diarrhoea.

Toxins increasing cAMP

00:10:16

- Cholerae
- Anthrax
- Enterotoxigenic E.coli (Labile)
- Pertussis

Clinical Features

00:11:07

- IP: 1-5 days
- Reservoir is human.
- Carrier: Eltor > Classical

- Rice water stools:
  - Mucus flaky turbid water having fishy odor & sudden in onset

Diagnosis

00:12:15

- Sample: Stool or rectal swabs (when stool not available)
- Transport media:
  - VR medium
  - Cary Blair medium

Microscopy

- Comma or curved shaped organism is seen
- Reddish because they are gram -ve
- Flagella is coming out
- Darting motility / shooting star motility
  - Also shown by Campylobacter

- These are Monotrichous
- There is Fish in stream appearance

Culture

00:15:19

- MacConkey Agar: NLF
- Blood Agar: Greenish Hemolysis
- Nutrient Agar: Translucent colonies (bluish tinge in light)
- Gelatin Swabs: Shows Napiform / Turnip Liquefaction

Important Information

- Gelatin swab culture of:
  - Bacillus anthrax- Inverted fir tree appearance
  - C. tetani- Fir tree appearance

Enrichment

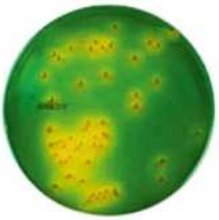
00:16:52

- Alkaline peptone water
- Monsour's taurocholate Tellurite peptone water

Selective Media

00:17:52

- TCBS- Thiosulfate citrate bile salt sucrose
- ABSA- Alkaline bile salt agar
- GTA- Gelatine Taurocholate Trypticase Tellurite Agar (black center and halo)
  - Also seen in Corynebacterium diptheria
- Thiosulfate Citrate Bile salt Sucrose



Vibrio cholerae on TCBS agar

Vibrio pharahaemolyticus on TCBS Agar

- Helps in differentiating sucrose vs non sucrose fermentation.
- Because V. Cholera is sucrose fermenter while others are non-sucrose fermenters.
- For sucrose, the color is yellow
- and the indicator used is Bromothymol Blue

Biochemical Tests 00:21:11

- V. cholera shows

Mnemonic CCOINSS

- Cholera Red reaction
  - Red color / Nitroso Indole compound formed by: Vibrio + APW + H^2So^4
- Catalase +ve
- Oxidase +ve
- Indole test +ve
- Nitrate reduction +ve
- Sucrose Lysis +ve
- String Test

Positive String Test

- Rice water stools + 0.5% deoxycholate Stringiness appears.
- Vibrio → Damage → DNA comes out (gives Stringiness)
- String test is done in:
  → V. Cholera
  → Klebsiella (because of presence of mucoid colony)
  → Giardia lamblia.

Test for toxins 00:25:54

- Animal based tests
  - Adrenal Tumor (Y1) cell assay
  - ELISA
  - Chinese Hamster ovary Cell Assay

Treatment 00:26:17

- Supportive treatment
  - Give ORS with or without IV fluids.
- Antibacterial if needed.
  - Doxycycline in adults
  - Azithromycin in children or pregnant females

Vaccine 00:26:41

- Live oral Vaccine

Vibrio Mimicus 00:26:56

- Seen in USA
- Due to Seafood consumption
- Grows best at low salt concentration.

Halophilic Organisms 00:27:42

Vibrio Parahaemolyticus	Vibrio Vulnificus	Vibrio Alginolyticus
Gastro-enteritis (seafood)	• Gastro-enteritis in normal people • Sepsis in HIV/ liver cirrhosis patient	• Conjunctivitis • Otitis media
All three are capable of Wound infections		
• Motile / mobile • Shows Kanagawa phenomena. • Shows Swarming	Only Lactose fermenter	• Most tolerant to salt (Nacl): >10% • Shows Swarming

Kanagawa Phenomena 00:31:19

- Wagatsuma Agar (blood agar having 2-4% salt) is used.
  - Pathogenic strain- Shows Hemolysis
  - Non Pathogenic strain- No Hemolysis

Non-Fermenters 00:32:48

- Use sugar oxidatively / does not ferment sugars.
- Organisms
  - Pseudomonas
  - Burkholderia mallei

- Burkholderia pseudomallei
- Acinetobacter Baumannii

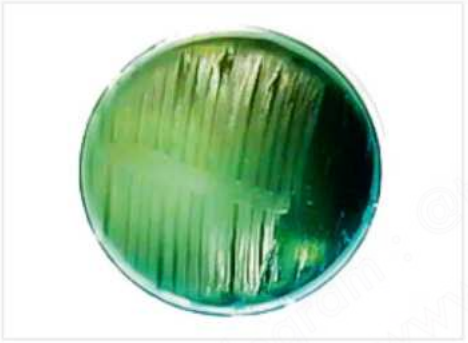
Pseudomonas 00:33:27

- Gram -ve Bacillus
- Catalase +ve
- Oxidase +ve

Virulence Factors 00:33:47

- Has Capsule
- Pigments

1. Pyocyanin (m/c)
   - Seen in P. Aeruginosus
   - Blue-green pigment
   - Can be enhanced on King's medium.
   - Diffusible pigment



2. Pyoverdine
   - Greenish Yellow
   - It is a siderophore.
3. Pyorubin
   - Red pigment
4. Pyomelanin
   - Brownish - black pigment

Important Information

PIGMENTS

- S. Aureus- Golden
- Serratia- Red
- Pseudomonas - Blue- green & Diffusible

- Alginic Acid
  - Found in mucoid strains of pseudomonas
  - Helps in biofilm production.
- Toxins
  - Exotoxin A decreases the protein synthesis by acting on elongation factor 2:
    → Fragment A
    → Fragment B
- Enzyme: Proteases, Elastases
- Other toxins such as Hemolysin, Enterotoxins
- Slime layer
- Pilli

Clinical Features 00:38:13

- Pneumonia (HAP, VAP)
- Green Nail syndrome
- Burns
- UTI, especially CAUTI
- Meningitis
- Contact Lens a/w corneal ulcers
- Hot tub folliculitis
- Swimmer's ear (Simple otitis externa)
- Shanghai Fever: Sepsis along with enteric disease

WHO priority organisms causing Nosocomial Infection 00:40:04

Mnemonic ESKAPE

- Enterococcus Faecium
- S. aureus
- Klebsiella pneumoniae
- Actinobacter Baumanni
- Pseudomonas Aeruginosa
- Enterobacter species

Diagnosis 00:41:11

- Microscopy
  - Gram -ve Bacillus
- Pigments enhanced by King medium

- Sweet odor / Fruity odor
- Colonies
  - Shows Iridescence
  - Metallic shine
- Use sugar oxidatively.
- MacConkey Agar: Non lactose fermenter
- Selective media: Cetrimide agar
- Blood agar: Beta Hemolysis
- Motile with Polar flagella

Susceptibility 00:44:37

- Susceptible to silver salts
  - Silver sulfonamides in topical burn creams
- E Test
  - Done for antibiotic Sensitivity testing

Burkholderia Pseudomallei 00:45:09

- Causes Melioidosis
- Also known as Vietnam Time Bomb
- Whitmore Bacillus
- Bioterrorism agent
- Culture media is Ashdown Agar / ASA (Crystal violet + gentamicin)
- Shows safety pin appearance
- Treatment is Carbapenem and Imipenem
- Clinical features
  - Skin ulcers
  - Pneumonia
  - Lymphadenopathy

Burkholderia mallei 00:47:41

- Causing disease in Animals known as Glander's disease
- Diagnosis is done by Strauss reaction:
  - If you give an inoculation in Guinea pig, it will result in testicular swelling.
- C/f includes skin ulcers with Pneumonia.

Burkholderia Cepacia 00:49:31

- Most common
- Most potent
- Associated with
  - Chronic Granulomatous Disease
  - Cystic Fibrosis
- Antibiotic resistance

MCQ

Q. All of the following are Halophilic vibrio except?

A. Vibrio Vulnificus

B. Vibrio Parahaemolyticus

C. Vibrio cholera

D. Vibrio Alginolyticus

Q. Statement about cholera which is true?

A. El tor biotype is VP positive

B. Chick erythrocyte agglutination cannot differentiate classical and el tar

C. Kanagawa phenomenon is used to differentiate classical and el tor

D. All are true

Q. Which of the following statements is true about vibrio cholera enterotoxin?

A. Appears to produce its effect by stimulating adenylyl cyclase

B. Causes destruction and invasion of intestinal cells

C. Causes influx of ions and water from the tissue into the large intestine lumen

D. Has a MW of 2,88,000

Q. Channel activated by cholera toxin?

A. Adenylate cyclase

B. Guanylyl cyclase

C. ABC transport channel

D. Iron transport channel

Q. Transport media used for vibrio is?

A. Selenite F broth

B. Tetrathionate broth

C. Nutrient broth

D. Venkatraman ramakrishnan medium

Q. Which of the following is used to differentiate colonies of vibrio cholerae and vibrio parahaemolyticus?

A. Macconkey agar

B. Bile salt agar

C. TCBS

D. DCA

Q. You receive a urine culture a report from the laboratory on a 45 year old patient who is quadriplegic with an indwelling urinary tract catheter. The urine culture is growing 1,00,000 oxidase positive gram negative bacilli. The most likely an organism is?

A. E.coli

B. Enterococcus

C. Klebsiella

D. Pseudomonas aeruginosa

Q. Mechanism of action of exotoxin A of pseudomonas aeruginosa is?

A. To activate acetylcholine esterase

B. To block Ef2

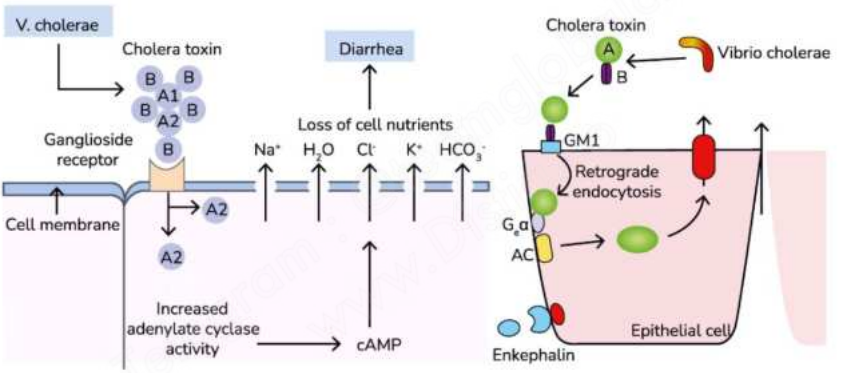
C. To from pores in the WBC and increase cation permeability
D. To increase intracellular cyclic adenosine monophosphate

Q. Fluorescent pigment produced by pseudomonas aeruginosa?
A. Pyomelanin
B. Pyorubin
C. Pyoverdine
D. Pyocyanin

Q. Burkholderia cepacia is typically associated with?
1. Cystic fibrosis
2. CGD
3. Multiple myeloma
4. MPO deficiency

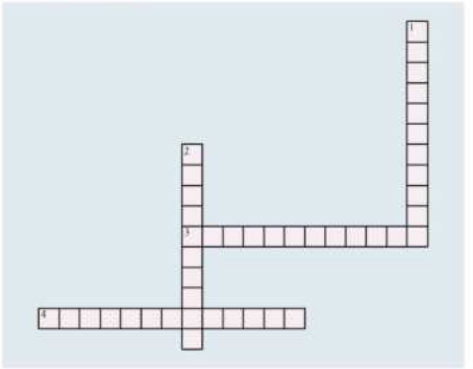
A. 1,2
B. 2,3
C. 1,3
D. 3,4

Image 16.1



CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Zona occludens toxin which disrupts the tight junctions
4. Related to Seafood consumption

Down

1. Gram -ve Bacillus
2. Need Salt 7-10%

- All are Oxidase +ve

Mnemonic: Oxidase +ve organisms
- **P - P**seudomonas
- **V - V**ibrio
- **N - N**eisseria
- **C - C**ampylobacter
- **H - H**elicobacter, HBB

Haemophilus 00:01:50
- Gram -ve
- Non motile
- Non sporing
- Oxidase +ve

H. influenzae 00:01:53
- **Other name:** PFEIFFER's bacillus
- **Virulence factors**
  - Capsular polysaccharide
  - Has 6 sub types (A-F)
  - H1B is most virulent and requires vaccination (H1B Polyribosyl Ribitol Phosphate)
  - Induces IgG,IgM,IgA which is used foe immunization
- **Consists**
  - Outer membrane proteins
  - Lipooligosaccharides
- **2 types**
  - Invasive
  - Non-invasive

Invasive vs Non-invasive

Invasive	Feature	Non – invasive
Encapsulated	**Capsule**	Non-encapsulated
Children	**Age**	Adults
Blood	**Spread**	locally
Positive	**Blood culture**	Negative
Hib	**Type**	Non – typeable
Meningitis, pneumonia, sepsis	**Infections**	Otitis media, sinusitis, exacerbation of COPD

Clinical Features
- Meningitis
- Pneumonia
- Laryngotracheobronchitis (Croup)
- Suppurative lesions

Diagnosis and Culture
- **Specimen:** CSF, blood, sputum
- **Precaution:** No refrigeration of the sample

To Remember:
In Yersiniosis and Listeriosis, refrigeration of samples is preferred.

  - **Microscopy:** Gram -ve coccobacilli
  - **Culture Characteristics**
    → The culture requires
- X factor (hemin)
- V factor (NAD or NADP)

Satellitism
- When a streak of Staph aureus is applied to the culture medium, narrow zone of hemolysis takes place
- Due to hemolysis factor X and factor V are released from RBC which facilitates the growth of Haemophilus.

Treatment
- **Antibiotics**
  - Ceftriaxone
  - Cefotaxime
- **Precaution**
  - Hib PRP vaccination

H. Aegyptiacus 00:09:00
- It is also called as Koch week's bacillus

To Remember: Koch's bacillus is T.B
- **It causes**
  - Pink eyes
  - Brazilian purpuric fever (septicemia due to conjunctivitis)

H. Ducreyi 00:09:40
- It causes Chancroid (soft sore)
- It is a painful soft genital ulcer

To Remember:
Mnemonic =Syphi Less
In syphilis, chancre is seen but it is hard and painless
- **Culture media**
  - Chocolate agar supplemented with isovitalex (growth factor), fetal calf serum
- **Tissue biopsy and staining**
  - Bipolar staining : safety pin appearance
  - Tram track/school of fish

To Remember:
Fish in stream appearance is seen in Vibrio cholera

Treatment
- Azithromycin

Factors	X	V	CO_2	Hemolysis
H.influenzae	+	+	-	-
H. Aegypticus	+	+	-	-
H.Ducreyi	+	-	-	-
H. Parainflenzae	-	+	-	-
H. Hemolyticus	+	+	-	+
H. Parahaemolyticus	-	+	-	+
H.Aphrophilus	+	-	+	-
H. Paraphrophilus	-	+	+	-

Haemophilus characteristics.
To Remember:
- **Hemolysis-**H. hemolyticus,H. Parhaemolyticus
- **Co2**-H.aphrophilus,H.paraphrophilus
- Factor X-H.Ducreyi(X-10;Dus;Duc)
- Factor V-H.Parainflenzae ,H. Parhaemolyticus, H.Paraphrophilus

To Remember:
Normal mouth commensals also cause infective endocarditis.
- HACEK (Mnemonic)
  - **H** - Haemophilus
  - **A** - Aggregatibacter
  - **C** - Cardiobacterium
  - **E** - Eikenella
  - **K** - Kingella

Bordetella 00:16:29
- Non-motile
- Non-sporing
- It has capsule
- It **causes**
  - Pertussis (Whooping cough/100 day cough)

Virulence Factors
- Agglutinogens
  - Helps in attachment
- Filamentous hemagglutinin
  - Also helps in attachment
- Pertacin
  - It is an outer membrane protein
- **Toxins**
  - Pertussis toxins
- Expresses on surface
- Exhibit
  - LPF (lymphocytosis producing factor)-Causes lymphocytosis
  - HCF (histamine sensitizing factor)
  - IAP (Islet activating factor) - Leads to hypoglycemia
- Pertussis toxin has fragment A and B
- Adenylate cyclase: It increases cAMP
  - Heat labile toxin
  - Tracheal cytotoxin
  - Lipopolysaccharide

To Remember:
cAMP is elevated in Cholera, Anthrax, E.coli (labile toxin), Pertussis

Clinical features
- **Incubation period:** 1-2 weeks
- **Phases of symptoms**
  - **Catarrhal**
  - Patient experience low grade fever and dry cough Subconjunctival hemorrhage
  - Patient is highly infective (maximum infectivity)
  - **Paroxysmal**
  - Bouts of cough
  - Inrush of air into lungs (whoop)
  - Post Tussive vomiting (characteristic)
  - **Convalescent**
- Lesser severity
- **Complications**
  - Pressure effect due to cough Subconjunctival hemorrhage
  - Respiratory problems Lung collapse
  - Brain complications Convulsions and coma

- **Diagnosis**
  - **Specimen**
  - Nasopharyngeal swab
  - Per nasal
  - Per oral
  - Cough plate method
  - Placing a plate in front of the patient and asking the patient to cough.
  - 6 swabs should be taken and are transported in casamino acid solution /Stuart media
  - Cotton swabs should not be used, instead Dacron or calcium alginate swabs are used.
  - **Culture**
  - Bordet-gengou (mostly preferred)
  - Regan-lowe media
  - Charcoal blood agar
  - In culture
  - Mercury drop/bissected pearls are observed
- Thumb print appearance is also seen

- If culture is allowed a little more time, then there will be **aluminum paint appearance (Confluent stage)**
  - Culture is preferred in the initial 2-3 weeks of infection
  - PCR can be used for infections until 4-5 weeks
  - Serology also done in later stages

- **Treatment and prevention**
  - Drug of choice Erythromycin and Azithromycin
  - Close contacts, irrespective of vaccination, are also given with the same treatment
  - **Prevention**
  - **Whole cell killed vaccine**
- DPT (Diphtheria-Pertussis vaccine)
  - Complication is Post vaccination encephalopathy,hence it is avoided
  - **Acellular vaccine**
- DtaP
- Made from Agglutinogens, Filamentous hemagglutinin, pertacin and Pertussis toxin
- They have no complications

Brucella 00:27:23

- It is Zoonotic infection (animal to human spread)

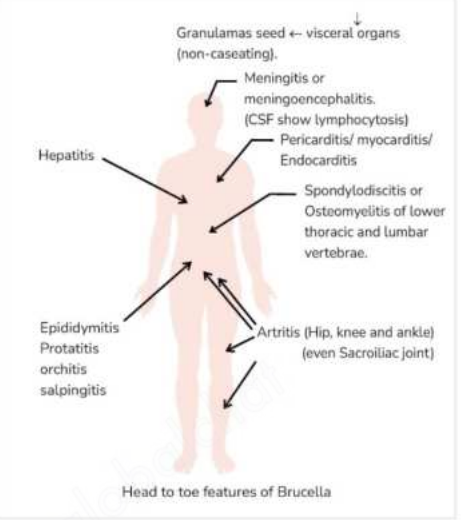
NOMEN system (DNA hybridization)

Mel itensis	Abortus	Suis	Canis	Ovis	Neutamae
↓	↓	↓	↓	↓	↓
Sheep goat	Cattle	Pigs	Dogs	Sheep	Rodent
↓					
Most Pathogenic					

- **Antigens**
  - **M.antigen:** Brucella melitensis
  - **A.antigen:** Brucella abortus

- **Virulence factors**
  - Lipopolysaccharides
  - BvrR/BurS for binding

- **Clinical features**
  - Zoonotic disease
  - May be due to direct contact or raw milk consumption
  - Hepatosplenomegaly-Due to invasion of reticuloendothelial cells(macrophages)
  - Night sweats
  - Arthritis
  - Low platelet count
  - **Incubation period:** 1 week to 1 month



Head to toe features of Brucella

- **Stages of infection**
  - Latent
    → No clinical evidence but serology is positive
  - Acute/Subacute
    → Fever
    → Myalgia
    → Arthralgia
    → Night sweats
    → Hepatosplenomegaly
    → Low platelet count contribute to bleeding
  - Chronic
    → Low grade

To Remember:
Brucella have a predilection in placenta(grows in placenta due to presence of erythritol in placenta)which has a stimulant effect

- **Diagnosis** (in humans)
  - Culture medium used is
    → Castaneda medium (biphasic)
- Liquid part is Trypticase soy broth
- Solid agar plate is placed on the side where organism grows
  - Automated (**VITEK**) cultures
  - COU(Catalase, Oxidase and Urease) positive
  - **Serology diagnosis**
    → Standard agglutination test
- To serum dilutions (patient antibodies), standardized killed antigen is added
- It is incubated for at 37° Celsius for 24 hours

- **Titre > 1:160:** Significant (IgM+IgG)
- To measure only IgG, treat with 2-mercaptoethanol, IgM is destroyed.

To Remember (cat sat on a mat-my bi lii)
- CAT - Cold agglutination test: Mycoplasma
- SAT - Standard agglutination test: Brucella
- MAT - Microscopic agglutination test: Leptospira

- **Diagnosis** (in animals)
  - **Milk ring test**
    → To detect Brucella antibody in the animal milk
    → Milk is added with standard antigen and if agglutination occurs as a purple color ring, then it is specific.
  - Bengal card test
  - Whey agglutination test

- **Treatment**
  - Adults
    → Doxycycline for 45 days + Streptomycin (I.M daily) for 14 days
- Doxycycline+Rifampin for 6 weeks (WHO regimen)
- If neurobrucellosis is present: Doxycycline + Rifampin + Ceftriaxone

MCQs

Q 1. Which of the following are observed on milk ring test
A. Brucellosis
B. Salmonella
C. T.B
D. All the above
Answer: Brucellosis

Q 2. For brucellosis, 2 ME agglutination is used for
A. IgG
B. IgM
C. IgA
D. IgE
Answer: IgG

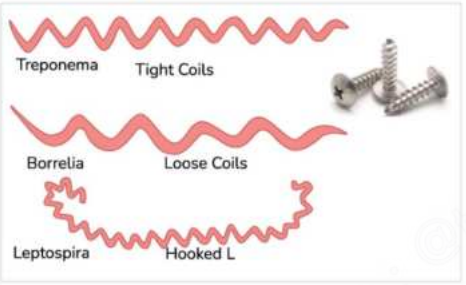
Q3. All of the following are true about bordetella pertussis except
A. Most common cause is B. Pertussis
B. It may occur in children and adults
C. Bacterial culture from nasopharyngeal swab is definitive diagnosis
D. Presence of antibodies in the serum is diagnostic of disease
Answer: Presence of antibodies in the serum is diagnosis of disease.

18 SPIROCHETES

- MRCS-bacteria that do not stain well with Gram (called Gram poor)
- M - Mycoplasma
- R - Rickettsia
- C - Chlamydia
- S - Spirochetes
- Spirochetes
  - Spiro - spiral, chetes – hair
  - Spiral and thin like hair

Classification of Spirochetes

00:01:03

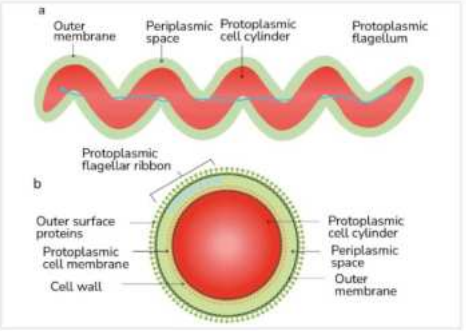


- Treponema
- Borrelia
- Leptospira

Treponema	Borrelia	Leptospira
• one with tight coils • has a corkscrew motility	• lashing motility • It has loose coils	• It is motile • It has hooked ends

Endoflagella

00:02:48



- The flagella that are hiding inside
- Also called axial filaments
- Hiding in the periplasmic space

Treponema

00:03:38

- **Classification**
  - Treponema pallidum
    → Causes venereal syphilis
- **Sexually transmitted disease (STD)**
- **Non - STD**
  - Treponema endemicum
    → Causes endemic syphilis
  - Treponema pertenue
    → Yaws
  - Treponema carateum
    → Pinta

Feature	Yaws	Endemic syphilis	Pinta
Transmission	Skin to skin	Kissing	Skin to skin
Age	Early childhood	Early childhood	Late childhood
Features	Ulcerative papilloma	Rare	Non - Ulcerative papule
Sites	Extremities	Oral	Extremities
Relapses	Common	Uncommon	No relapses

Note:

- Trick - Yawning kid called PINTU
  - Yawning - yaws
    → We yawn with stretched hands - Extremities
    → Kid - occurring in children
    → Yawning causes others to yawn - relapsing
  - Pintu - Pinta
  - Yawning from mouth so oral

Treponema pallidum

00:07:28

- Venereal syphilis - Sexually transmitted disease
- Incubation period
  - 9 -90 days
- Stages
  - Primary
  - Secondary
  - Tertiary

Primary Syphilis

- Genital ulcer -painless
- Hard chancre / Hunterian chancre
  - Indurated
  - Genital ulcer
    → Painless
- An inguinal lymph node is also painless
- Covered with thick glary exudate
- In most cases it is avascular

Note :

- Soft chancroid
  - Haemophilus ducreyi - very painful
- Heal in 10 - 40 days
  - Leaves thin scar
- Patients can also enter into secondary syphilis

Secondary syphilis

- Occurs after 1-3 months of primary syphilis
- Patients suffer from condylomata lata at the Mucocutaneous junction
- Papular skin rashes occur on palms and soles
  - Involvement of 2 hands and 2 feets

Latent Syphilis

- Sometimes after secondary stage, instead of tertiary, the clinical features disappear, but serology shows the presence of syphilis
- The sleeping phase is called latent syphilis, which can cause problems later
- Clinical feature-not present.

Tertiary syphilis

- Benign tertiary syphilis
  - Results in the formation of Gummas in the bone and skin
    → Microscopically shows Granuloma
- Plasma cells are in abundance
- Cardiovascular syphilis
  - Obliterative endarteritis of vasa vasorum
    → The artery supplying the artery - vasa vasorum
  - Ascending aorta and arch of the aorta are involved - aneurysms
  - Aortic valve -shows aneurysms
- Neurosyphilis
  - Aseptic meningitis seen in TB
  - Tabes Dorsalis (posterior column)
  - General Paresis

Note:

- Where does one see the tree bark appearance in Aorta?
  - In tertiary syphilis
    → Tree - three

Non-venereal syphilis and Congenital syphilis 00:15:01

Non-venereal syphilis

- Seen in
- Health care workers: Primary Chancre at Extra genital site
  - Transmission by touch
  - The non-sexual roots can cause chancre in hands
- Blood Transfusion patients: No primary chancre
  - No body-to-body touch

Congenital syphilis

- Mother-to-child transmission
  - Transplacental
  - Manifest late as body lesions (osteoperiostitis)
    → Saddle nose
    → Saber shin (tibia)

Lab diagnosis 00:16:46

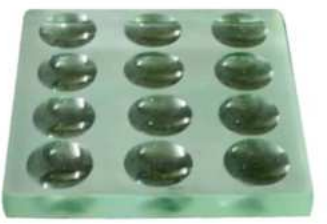
- Specimen
  - Depends on the stage
  - Genital ulcer in primary stage, Blood/CSF in secondary stage
- Microscopy
  - Dark field
    → Due to the presence of the thin organism
- Forward and backward movement is seen
- Can show bending at 90 degrees (right angles)
- Silver impregnation is done
  → Fontana's method
- FILMS- Levaditi's method
- Tissue samples
  - It gives a black colour
    → Done to see under light microscope

Serological Diagnosis

Non specific

- Wassermann test
  - Compliment fixation technique
- KAHN test
  - Tube precipitation test
- VDRL
- RPR

VDRL



- Venereal Disease Research Lab
  - Done on slide
  - Very difficult in real life
    → Need to heat activate the serum from a patient
- Special antigen called cardiolipin antigen is added
- Cardiolipin antigen
  - Li - related to Lipid
    → Lipid extract of beef heart
    → Lecithin + Cholesterol
- Then the blood is mixed and then rotated, and clumps are seen on microscopy

VDRL false positive

- VDRL False Positive - Mnemonic
  - Viral infections (IM)
  - Drugs (procainamide)
  - Rheumatic fever
  - Lupus
  - Leprosy
  - First trimester of pregnancy can also shows false positive VDRL
    → Early pregnancy

Differences in VDRL and RPR

VDRL	RPR-rapid plasma reagin
Antigen to be used within 24 hours	No fluid prepared
Preheating of serum is needed	Not needed
Blood, serum, CSF	All except CSF
Miscrscopically read Slide flocculation test	Not needed Card test
Cheaper	Costly

RPR card

Specific Tests 00:26:42

- FTA - ABS
  - Fluorescent treponemal antibody absorption
- TPI
  - Treponema pallidum immobilisation
- TPHA
  - Treponema pallidum hemagglutination assay
- TPPA
  - Treponema pallidum Particulate assay
- Most sensitive
  - FTA - ABS
- Most specific
  - TPI
    → TPI >> TPPA
    → TPI is not used as it uses live treponemes and thus cannot be used all the time.
- Most important for congenital syphilis
  - FTA - ABS
  - Among all VDRL is preferred
    → VDRL >> FTA - ABS
- Strain that is used for treponema straining
  - Nicole strain

Treatment

- Benzathine penicillin
  - Drug of choice for Primary, Secondary Syphilis and Latent stages of syphilis
- Neurosyphilis - Penicillin G
- Can induce Jarisch Herxheimer Reaction

Borrelia 00:31:32

- Borrelia have loose spirals
  - Lashing motility
- Borrelia Burgdorferi
  - Causes Lyme's disease
- Borrelia Recurrentis
  - Causes relapsing fever
- Borrelia vincenti
  - Causes Vincent's angina

Borrelia Burgdorferi

Lyme Disease

- Most common vector-borne disease in the USA
- Vector - Hard tick or Ixodid ticks
- Incubation period - 3 - 30 days
- Presentation
  - Rash where the tick has bitten

Clinical Feature

- Localised infection
  - Site of tick bite - Annular/ Bull's eye lesion (target rash) - Erythema Chronicum Migrans (ECM)
    → Reddish color will be seen
    → Migrans-redness will gradually increase towards outside
- The centre will become more clear

- Disseminated infection
  - Small lesions are seen on the periphery called satellite lesions other than ECM.
  - Disseminated - going to other organs
    → CVS
- Can cause AV block and myocarditis.
  - CNS
- Facial nerve palsy
  - Joints
- Involved in the third stage when patients have lyme's arthritis
- Persistent infection
  - Lyme's arthritis is seen.

Complication of Borrelia burgdorferi

- It can show Meningeal involvement.
  - Bannwarth's syndrome -Brain involvement
  - Result in painful radiculoneuritis
    → Pain and burning sensation along the dermatome.

Lab Diagnosis 00:37:26

- Sample - depend on the organ involved
  - CSF, Blood
- Biopsy
  - Arthritis - synovial tissue
  - ECM - Skin
- Stain
  - WSSS - Warthin Starry Silver stain
- Real time or conventional PCR can be used
- Serology
  - ELISA is done

Treatment

- Ceftriaxone

Borrelia recurrentis

- Causes Relapsing fever
- Epidemic Louse borne
- Human to human

Borrelia duttoni

- Tick-borne -endemic due to Tick bite
- Tick Relapsing fever
- Between Tick and animal

Pathogenicity 00:41:23

- Relapsing fever
- Incubation Period → 2-10 days
- Fever lasts for 3-5 Days
- Abundant organisms in Blood
- Afebrile for 4-10 days [Blood → no organism]
- Relapsing fever [organism reappears → 3-10 relapses]

Laboratory diagnosis

- Sample: blood (during pyrexia)
- Microscopy: Dark field microscopy
- Stain → Silver stain or Giemsa/leishman (Borrelia)
- Culture not done
- Serology: ELISA
- Treatment
  - Tetracycline, chloramphenicol
  - No vaccine

Borrelia Vincentii 00:43:40

- Causes Vincent's Angina
  [Ulcerative gingivostomatitis or Oro-pharyngitis
- Normally also present in mouth
- Diagnosis
  - PCR
  - Dark field Microscopy
- Treatment:
  - Penicillin & Metronidazole

Note: DOES B. Vincentii have a symbiotic relation with fusiform ?

- + FUSOSPIROCHETOSIS
- Fusobacterium fusiform + B. Vincentii

Leptospira 00:44:53

- Causes WEIL's disease (ictero-hemorrhagic fever)
- Also known as hepato-renal syndrome.
- Classification
  - L. Interrogans (pathogenic)
- Pathogenesis (zoonotic)
- Rat urine-contaminated rainy water, associated with Rice/paddy field workers, they will get infected.

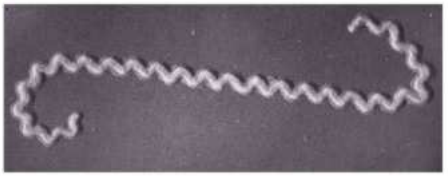
Mnemonic- RRRR

Severe illness/WEIL's disease:-

	First stage/septicemic phase	Second stage/immune phase
Duration	3-10 days	10-30 days
Features	High grade fever Jaundice Raised liver enzymes Haemorrhages- pulmonary, skin, conjunctival Renal- raised creatinine	Similar to first stage
Sample	Blood, CSF	Urine
Serology	IgM absent	Present
Antibiotics	Doxycycline	Refractory penicillin

Microscopy and culture media

- Microscopy: dark field Microscopy



- Culture media :
  - EMJH - Dingers ring
  - FLETCHER
  - KORTHOFF
- Serology (best)
- Microscopy Agglutination Test (MAT)-for leptospira (preferred)
  - Standard agglutination test - for brucella
  - Cold Agglutination test - for mycoplasma

Note:- National Referral centre for leptospira is PORT BLAIR

Laboratory Diagnosis

- Faine's criteria
  - Clinical data
  - Epidemiology
  - Lab finding (IgM)

MCQ'S

Q. Which of the following statements about diagnostic modalities of treponema pallidum is incorrect?

1. VDRL is positive in secondary syphilis
2. RPR is used as a diagnostic modality in CSF samples (Neurosyphilis - VDRL)
3. Antigen for VDRL is prepared from killed T. Pallidum (Cardiolipid)

A. 1,2,3
B. 1,2
C. 2,3
D. 1,3

Ans. c. 2,3

Q. Identify the stain used for the diagnosis of the lymph node of a patient with suspected syphilis?

A. GMS
B. Levaditi stain
C. Giemsa stain
D. Warthin starry stain

Ans. b. Levaditi stain

19 RICKETTSIAE

Introduction

00:00:29

- Obligate intracellular organisms
- Cannot grow on artificial media
- Grow on cell lines only
- Arthropod Borne transmission
- Rash is present
- **Exception:** Coxiella
  - No Arthropod
  - No rash
  - Cause due to inhalation

Pathogenesis

Reservoir Host → Vector → Humans (accidental)
↓
Multiply locally and enter blood stream
↓
Localise at the endothelium
↓
Degenerate and thrombosis
↓
Occlusion

Organisms and Vectors

Disease	Organism	Vector
Epidemic typhus	R. Prowazekii	Louse
Endemic typhus	R. Typhus	Flea
RMSF (Rocky Mountain Spotted Fever)	R. Rickettsii	Tick
Indian tick typhus	R. Conori	Tick
African tick typhus	R. Africae	Tick
Rickettsial pox	R. Akari	Mite
Scrub typhus	Orientia tsutsugamushi	Mite

Note:

Vectors - Mnemonic: **LET FEN TRIA PSM**

- L - Louse; ET - Epidemic typhus
- F - Flea; EN - Endemic typhus
- T - Tick; RIA - RMSF, Indian tick typhus, African tick typhus
- PS - Pox, Scrub; M – Mite

Typhus Group

00:07:19

01. Epidemic Typhus

- Other name: Jail Fever
- Caused by R. Prowazekii
- **Vector:** Human body Louse
- **Incubation period:** 5-15 days
- Clinical features: Fever and chills
- 40% cases are fatal

Note:-

- LOUSE is LEAVING
  - Leave body when Fever comes
  - Louse Sensitive to heat
- This is not a zoonotic disease
- Infection is transmitted by entry of LOUSE FECES into body via abrasions (Lazy Louse)

To Remember

- Rash is seen in all, firstly on trunk
  - Later seen on limbs
  - Not on face, palms, and soles
  - Except in **RMSF**-Rash starts in extremities to the trunk

- **Reactivated epidemic typhus**
  - Brill Zinsser disease/Recrudescent typhus
  - Less sensitive
  - No vector

02. Endemic Typhus

- **Mode:** Bite/ Saliva/ Feces/ Aerosols of dried feces/ Food ingestion (contaminated)

Neil Mooser Reaction/Tunica reaction-**To Differentiate Epidemic and Endemic**

- **R. Prowazekii-negative reaction**
- **R. Typhi-positive reaction**

Pt blood sample
↓
Injected in guinea pig
↓
Scrotum inflammation
↓
+ve in Endemic typhus

To Remember:

- Least severe form is Rickettsia pox

03. Scrub Typhus

- Other name: Chigger borne disease (larvae of mite is Chigger)
- Vector-Mite
- **Zoonotic Tetrad**
- Chigger Borne Disease

Zoonotic tetrad

Transported by trombiculid MITES
↑
Carry infection in Rats/ Squirrel ↔ Chiggers / Larvae Infects Humans
↕
Present in vegetation shrubs

Pathogenesis:

Mite larvae
↓
Seek the moist area of body(axilla,groin)
↓
After 1-3 weeks
↓
Eschars (black lesion), Fever, Nausea, Vomiting
↓
Compilation: Pneumonia, ARDS, Shock

Diagnosis of Rickettsial Infections

- **Specimen:** Blood, Tissue, Skin biopsy
- **Microscopy:** Gram poor
- **Special stains:** Giemsa, Castaneda, Gimenez, Macchiavello
- **Culture:**
  - Yolk sac of Embryonated Hen's egg (or)
  - Cell lines -Vero, Hela, Hep2, Detroit 6 (as these are obligate intracellular)

Serology

- **Serology:** IgM [ELISA]
- Weil Felix reaction/ Tube agglutination test
  - Non Motile Proteus Strains
    → OX 19
    → OX 2
    → OX K

	OX 19	OX 2	OX K
Epidemic	+++	+	-
Brill Zinsser	-	-	-
Endemic	+++	+/-	-
Spotted	++	++	-
Scrub	-	-	+++

Table showing Weil Felix reaction

- OX K is +ve only in Scrub (Skrub)
- Reactivated epidemic typhus/ Brill Zinsser disease - Not +ve to any
- False +ve in case of Proteus infection
- Gold standard: IFAT (Indirect Fluorescent Antibody Test)

Treatment

- Doxycycline or Tetracycline are given

Genus Coxiella 00:19:55

- **Organism:** Coxiella Burnetii
- Intracellular Parasite
- **Has**
  - NO vector
  - NO Weil Felix Reaction
  - NO Rash

Pathogenicity

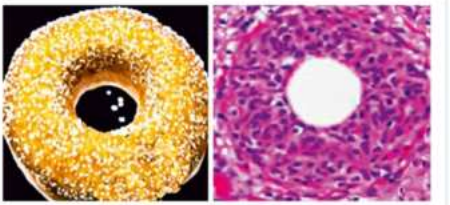
- **Humans**
  - Inoculation-(Animals)
  - Inhalation (Dried Feces)
  - Ingestion (Meat, Milk)

Important: For INICET

- Shows Antigenic Phase Variation
  - Fresh isolates (Phase I)
  - Repeated passage through yolk sac (Phase II)
  - Passage through Guinea Pig (back to Phase I)
  - Phase I is more powerful immunogen

Disease

- Q. Fever
- **Histopathology:** Donut granuloma/Fibrin ring granuloma



Interstitial pneumonia

- **Chronic:** Hepatitis, endocarditis

Lab Diagnosis and Treatment

- **LAB DIAGNOSIS**
  - **Microscopy:** Giemsa, Gimenez, Castaneda, Machhiavello
  - **Serology:** IgM (IIFT), IgG
- **TREATMENT:** Tetracycline (DOC)
- **VACCINE:** Q-vax (Australia) for >15 years

Arthropod Borne Infections

00:24:24

- Bartonella bacilliiformis
- Bartonella Quintana
- Bartonella henselae

Note:

- All rickettsia are obligate intracellular except BARTONELLA
- They will grow in blood agar

Bartonella Bacilliformis

- a/k/a **OROYA** fever/Carrion's disease
- Transmitted by sandfly
- C/F:
  - Fever
  - Hemolytic anaemia

Bartonella Quintana

- Five day fever
- Trench fever
- Only in humas ,no animal reservoir
- Transmission by-Body louse

Bartonella Henselae

- Cat scratch disease(shows Stellate Granuloma)
- Bacillary angiomatosis
- Bacillary peliosis-sinusoidal dilation in liver, spleen
- Stain: Warthin starry silver stain(WSSS)

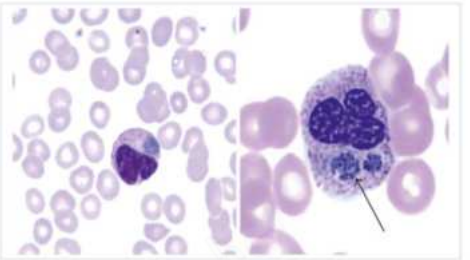
Note: WSSS is used for staining H. Pylori and B.Henselae

Genus Ehrlichia

00:29:03

- Human lymphocytic Ehrlichiosis
  - Neorickettsia sennetsu by ingestion of fish
  - Fever and lymph node enlargement
- Human monocytic Ehrlichiosis
  - E.Chaffeensis by Amblyomma tick
- Human Granulocytic anaplasmosis
  - Anaplasma phagocytophilum by Ixodid ticks
  - Affect neutrophils
- C/F:
  - Fever
  - Headache
  - Myalgia
  - Rash
  - Renal failure
  - DIC

Note: Morula (Mulberry Inclusion)
Monocyte shows -Echaffeensis
Neutrophils shows Anaplasmosis



MCQ

Q. Neil Mooser reaction/tunica reaction is useful to differentiate between?
A. R. Typhi and R. prowazekii
B. R. Typhi and R.Rickettsia
C. R. prowazekii and R.Rickettsia
D. R. Rickettsia and R.Burnetii
Ans. a

Q. A man presented with fever, headache, myalgia and rash on the trunk. He gave a history of visit to the Uttarakhand forest about 10 days back. He was given ceftriaxone but worsened despite the treatment. Later, a lesion which was missed earlier was seen in the axillary region. What is the likely diagnosis
A. Cutaneous anthrax
B. Scrub typhus
C. Kyasanur forest disease
D. Fungal infection

Ans. b

Q. Which is the correct match ?
A. R. prowazekii; endemic typhus
B. R. akari; Brill-Zinsser disease
C. R. Conori; Scrub typhus
D. B. Quintana; trench fever
Ans. d

20 CHLAMYDIA

Introduction 00:00:20

- Mnemonic: **ABC** organisms
- **A**typical bacteria
- Do not grow on **A**rtificial media - obligate intracellular
- **A**TP from host (**A**TP parasite)
- **B**asophilic bacterial inclusions
- **C**annot be **C**ultured
- No peptidoglycan **C**ell wall (gram poor)

Types of Chlamydia 00:02:20

Classification	
Chlamydia	**Chlamydophila**
Chlamydia trachomatis	• Chlamydophila pneumoniae • Chlamydophila psittaci

- Every chlamydia has 2 forms

Elementary body	Reticulate Body
Extracellular (Entry, Exit)	Intracellular
RNA = DNA (Equal)	RNA > DNA
Metabolically Inactive	Metabolically active
Small	Large
Infections form	Replicating form
Mnemonic: EEEII	Mnemonic: All opposite

Flow chart of 2 forms

- Elementary body (ER) enters host cell
- ER converted to Reticulate Body (RB)for replication
- RB converted to EB for exit

Refer Image 20.1

Serotypes 00:05:31

- **C.trachomatis**
  - A, B, Ba, C
    → Causes Trachoma (M/C infectious cause of blindness in India)
    → M/C is **B** form
  - D, E, F, G, H, I, K
    → Causes Nongonococcal urethritis
    → May come in contact with hand which causes inclusion conjunctivitis (does not cause blindness)
    → In children -ophthalmia neonatorum, infant pneumonia
- **C.trachomatis**
  - L1, L2, L3
    → Causes lymphogranuloma venereum,
    → Most common is L2

Mnemonic: The 2nd ones are common

- B is 2nd
- L2 is 2nd

- **C.pneumoniae**
  - One strain (TWAR strain)
  - Seen in Taiwan
- **C.psittaci**
  - Many strains

Lymphogranuloma Venereum (LGV) 00:08:38

- **A** - Asymptomatic (many cases)
- **B** - Bubos (painful lymph nodes)
  - Genital ulcer
  - Groove sign is seen

Inflamed bubos separated by inguinal ligament

- **C** - C.trachomatis (L1, L2, L3)
  - Reiter syndrome: **C**onjunctivitis, **U**rethritis, **P**olyarthritis (CUP syndrome)
- **D** - Doxycycline (DOC)
- **E** - Esthiomene (rectal and vulvar strictures)
- **F**
  - Fitz Hugh Curtis syndrome (perihepatitis)
  - Fries test (skin test)

- **G** - Groove sign
- **G** - Groove sign

Recall: Fitz Hugh Curtis syndrome is also seen in N.gonorrhoeae

Lab Diagnosis

00:11:45

- **Sample**
  - Conjunctival material
  - Urethral scrapping
  - Urine sample
  - Blood sample
  - Sputum
  - Buboes (LGV)
- **Microscopy**
  - **Other stains:** Giemsa, Gimenez, Castaneda, Machhiavello
  - Lugol's iodine can be +ve (glycogen matrix of C.trachomatis)
- **Culture**
  - No culture media
  - **Cell lines:** Hela, Hep2, McCoy
- **Antigen testing:** ELISA
- **Antibody:** Complement fixation test

Important Information

- PCR/NAAT are most sensitive and specific

Chlamydia Pneumoniae

00:13:27

- Causes pneumonia
- Has only one strain (Twar strain)
- Taiwan acute respiratory strain

Recall: Causes of Pneumonia

- 1st MC - S.pneumoniae
- 2nd MC - H.influenzae
- 3rd MC - C.pneumoniae

- **Associated**
  - Atherosclerosis
  - Asthma
  - Sarcoidosis

Recall: Infectious Atherosclerosis

- C.pneumoniae
- CMV
- Herpes

Chlamydia Psittaci

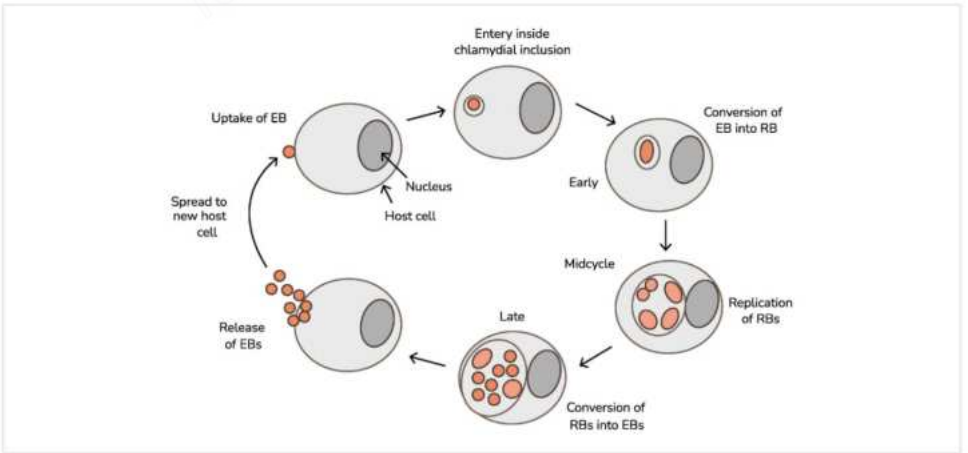
00:14:48

- Many serotypes
- Birds to human (mainly Parrots)
- No human to human
- Causes pneumonia

Important Information

- **P** for Psittaci **P** for Parrot

Image 20.1



21 MISCELLANEOUS BACTERIA

Mycoplasma/ Eaton's Agent

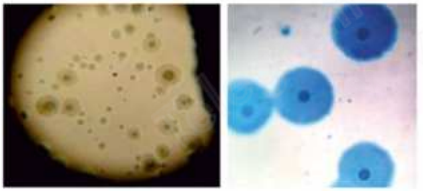
- My
  - M/C/C of walking pneumonia.
  - M/C/C atypical community-acquired pneumonia.
- Co
  - Cold agglutination test +ve
  - Cell wall –ve
  - Cell membrane has sterols.
- Pla
  - PPLO-Pleuropneumonia Pneumonia Like Organism.
  - PPLO agar (selecting agent-penicillium and thallium acetate) shows fried egg colonies.
- Sma
  - Stain - Diene's Stain (added on the surface of the culture)
  - Smallest bacteria can pass through bacterial filters.

Important Information

- NAAT (Nucleic acid amplification testing) - Most sensitive test

Agglutination test

CAT(heterophile)-cold agglutination test



Pulmonary Symptoms

- M/C/C of walking pneumonia.
- M/C/C community acquired atypical pneumonia
- Associated with cold agglutinin disease-AIHA (IgM-Mycoplasma).

Extrapulmonary Symptoms

- Meningitis.
- Encephalitis
- GBS
- Steven Johnson's Syndrome
- Carditis
- Treatment(heading)
- DOC:Macrolides

The Bacters

00:07:22

- Campylobacter
- Helicobacter
- COPS(HIGHLIGHTED IN PINK)
  - C-catalase +
  - O-Oxidase +
  - P-positive
  - S-all are Spiral rods

1. Campylobacter Jejuni

- **Thermophilic:** Grows at 42 degree C
- **Causes:** diarrheal disease
- **Route:** Ingestion (contaminated poultry)
- **Incubation Period:** 1-7 days
- **Clinical Features:** Fever, Abdominal pain, watery Diarrhea
- **Complication:** GBS (serotype O19)

Intestinal infection

- Implemented diarrhea.
- Abdominal pain
- Fever
- Pseudo appendicitis (due to more pain)
- Loose stools to bloody diarrhea (self-limiting).

Extraintestinal

- Due to C.fetus
- **Features:** Bacteremia, sepsis, meningitis, vascular infections

Lab Diagnosis

- **Microcopy**
  - **Gram negative**
  - **Shape:comma/'S'/ or GULL wing**
  - Darting motility or shooting star motility.

Recall: Darting motility is also seen in Vibrio cholera.

- **Stool Sample:** Fecal leukocytes (pus cells).
- **Selective media**
  - Skirrow/ campy BAP/ butzler
  - Contains charcoal.
  - Effuse droplet-like colonies are seen.

Treatment
- Symptomatic treatment
- **Azithromycin only in severe or prolonged illness**

2. Helicobacter Pylori
- Urease +ve and also COPS.
- **Virulence factors** (PYQ)
  - **Vac A**- Vacuolating cytotoxin A
  - **Cag A**-Cytotoxin-associated gene A (encodes for type IV secretion system).

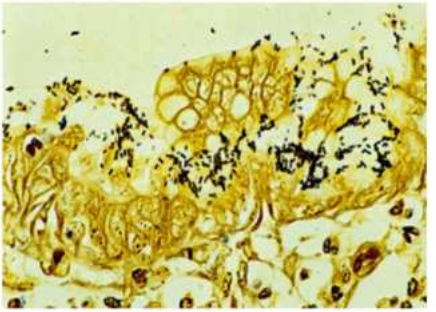
Recall: Shigella had a Type III secretion system

Pathogenicity
- **Route:** Feco-oral
- **Risk factors:** Poor hygiene, overcrowding
- **Site:** Antrum

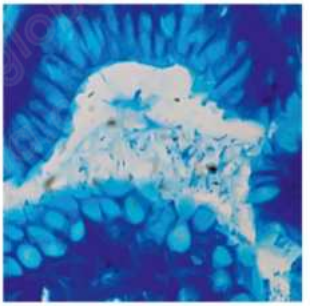
Diseases
- **Inflammation related.**
  - Chronic Gastritis B.
  - Peptic ulcer disease.
- **Cancerous**
  - Adenocarcinoma.
  - MALToma (marginal zone lymphoma).

Protective Role of H.Pylori
- **A** - Adenocarcinoma of the esophagus
- **B** - Barrett's esophagus
- **G** - GERD
- **Analysis** - Asthma

Lab Diagnosis
- **Invasive tests**
  - Biopsy (Antrum).
  - Microscopy (COPS).
    → Gram –ve spiral shaped



  - **Stains**
    → Warthin Starry Silver stain (black color H.pylori) - Best
    → Steiner Silver stain
    → Modified Giemsa stain - Less time-consuming.



Stomach Biopsy with Modified Giemsa Stain

Recall: Warthin Starry Silver stain is also used in Bartonella Henselae

- **Non-invasive tests**
  - IgM/ IgG ELISA
  - Capro antigen.
  - Urea breath test - Sensitive (urease +ve).
  - **Biochemical reactions:** Urease + Catalase + (in front of oxidase)
  - CLO test (to differentiate from Campylobacter, also called Campylobacter-like organisms).
  - **Culture** - Specific
    → Skirrow/ campy BAP/ butzler.

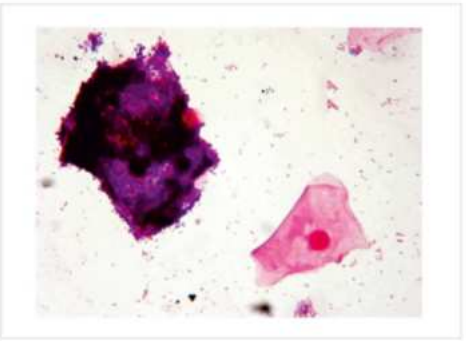
Treatment
- **OCA**-Omeprazole+Clarithromycin + Amoxicillin x 14 days
- **OBM** - Omeprazole + Bismuth Salicylate + Metronidazole x 14 days

- **OBTM** - Omeprazole + Bismuth Salicylate + Tetracycline + Metronidazole x 14 days

Gardnerella Vaginalis

00:25:18

- **CF:** Bacterial vaginosis
- **Diagnosis**
  - **Sample:** Discharge
  - M/E-On PAP smear CLUE cells are seen(,coccobacilli stuck on cells)



Amsel's Criteria

- **pH:** >4.5
- **Whiff test:** Discharge + 10% KOH → Fishy odour
- Clue cells

Nugent's Criteria

- Replacement of lactobacillus (doderlein bacillus) by pathogenic organisms like Gardnerella
- **Treatment:** Metronidazole

Rat Bite Fever

Streptobacillus Moniliformis	Feature	Spirillum Minus
Haverhill fever/ Erythemica/ Arthriticum/ Epidemicum	**AKA**	Sudoku fever
7-10 days	**IP**	1-3 week
Relapsing fever, rash, arthralgia	**C/F**	Local ulcer, lymphadenopathy, Relapsing fever, rash, arthralgia.
Penicillin	**DOC**	Penicillin

Pasteurella Multocida

- Gram –ve
- **CF**
  - A/w cat/dog bite
  - Local site inflammation - Swelling, tenderness, pus
  - Tenosynovitis
  - Osteomyelitis
  - Septicemia
- **Treatment:** Beta lactams

Legionella Pneumophila

00:33:03

Causes

- Legionellosis
  - Legionnaires disease (pulmonary form)-Black day for lung.
  - Pontiac non-pneumonic form.
    → Mild fever.
    → Myalgia.
    → Nonproductive cough.
    → Respiratory failure.

Risk factors

- Smoking
- Alcohol
- Hospitalizations
- Immunodeficiency

Infection route: Aerosols (AC)

Pathogenesis

Bacteria
↓
Alveoli
↓
Multiply inside macrophages.
↓
Dissemination via blood or lymphatics
↓
Contagious spread

- **IP:** 2-10 days

Lab Diagnosis

- **Specimen:** Sputum/ Bronchial washing
- **M/E:** Gram-ve coccobacilli.
- **Stains:** Silver impregnation stain - Black color
- **Culture:** BCYE agar (Buffered Charcoal Yeast Extract) - Black color.
- **Serology:** ELISA

Treatment: Macrolides

Culture Media

- **BCYE** - Legionella
- **CCFE/ CCYE** - C.difficile
- **MYPA/ PEMBA** - B.Cereus

Klebsiella Granulomatis

- **Other name:** Calymmatobacterium granulomatis
- **Pathogenicity:** Donovanosis/ granuloma inguinale
- **Incubation period:** 1-2 weeks
- **CF:** Painless papule (genitalia) → Ulcer → Chronic

Recall

- **Painful genital ulcers**
  - HSV2
  - H.Ducreyi
- **Painless genital ulcers**
  - Syphilis
  - Klebsiella
  - Lymphogranuloma Venereum
- **Microscopy**
  - Donovan bodies on wright giemsa stain

  - Safety pin appearance/ bipolar stain.
- **Treatment:** Tetracycline.

Erysipelothrix

00:40:43

- Non-Motile
- Non Sporing
- Non-Capsulated
- Formation of erysipeloid: At the site of inoculation/ cut/ abrasion after handling animal product

- **Lab diagnosis:** Tellurite medium/ tinsdale medium.
- **Treatment:** Penicillin (resistant to vancomycin).
- Mnemonic: Erysipelothrix
  - **Ery -** Erythema (Erysipeloid)
  - **Thrix -** Tellurite medium/ tinsdale medium

HACEK

- Gram-ve non-motile family.
- **Bacterias**
  - **H -** Haemophilus
  - **A -** Aggregatibacter
  - **C -** Cardiobacterium
  - **E -** Eikenella
    → Has twitching motility due to Type IV pili
    → Shows needle licker osteomyelitis.
  - **K -** Kingella (most aggressive)

Recall: Type IV pili are present in Neisseria (cocci) as well

- HACEK is known to cause Prosthetic valve endocarditis.

Acinetobacter Baumanii

- Non fermenter
- Present in hospital environments.
- **Leads to**
  - Opportunistic nosocomial infections
  - VAP - Ventilator acquired pneumonia.
  - Bloodstream infections
  - Post catheter UTIs
- An ESKAPE organism as per WHO

Francisella Tularensis

00:46:43

- **Disease:** Tularemia
- Zoonotic infection
- Similar to rodents plague
- **Route**
  - Contact with animal.
  - Aerosols inhalation
- **CF**
  - Ulceroglandular tularemia (most common).
  - Pulmonary tularemia.
  - Oropharyngeal tularemia
- Used as a Category-A bioterrorism agent.
- **Culture**
  - Difficult to culture
  - PCR is best.
  - Gram -ve coccobacilli
    → Catalase +ve
    → Bipolar stained appearance can be seen
- **Treatment:** Gentamicin

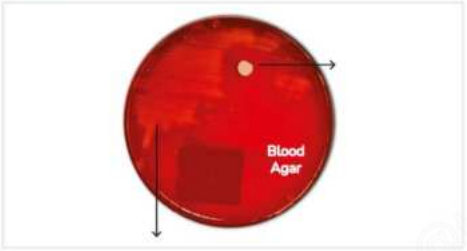
Important Information

- Can show safety pin appearance.
- Catalase +ve
- Ulceroglandular tularemia is most common

Q. A 12 year old boy presents with vomiting within 3 hours of consumption of food at a party. What is the likely causative agent responsible for the symptoms?
(NEET Jan 2020)

A. Staphylococcus aureus
B. Salmonella
C. Clostridium botulinum
D. Clostridium perfringens

Q. Identify the organism on Bacitracin sensitivity disc test given below? (AIIMS May 2018)



A. Streptococcus pyogenes
B. Staph aureus
C. Clostridium
D. Corynebacterium

Q. Which is correct about Diphtheria membrane? (FMGE June 2019)
A. Grey membrane that bleeds on removal
B. Grey membrane that does not bleeds on removal
C. Grey pseudo membrane that bleeds on removal
D. Grey pseudo membrane that does not bleeds on removal

Q. Diphtheria vaccine is type of?
(AIIMS June 2020)
A. Live attenuated vaccine
B. Killed vaccine
C. Toxoid
D. Polysaccharide vaccine

Q. Bacillus Anthrax is?
(FMGE June 2019)
A. Gram positive cocci in cluster
B. Gram positive rods with square ends
C. Gram positive bacilli with spherical ends
D. Gram negative cocci in cluster

Q. A 30 year old man reports four hours after acquiring a clean wound without laceration. He has received TT vaccination 10 years back. What do you advice regarding tetanus prophylaxis?
(NEET Jan 2020)

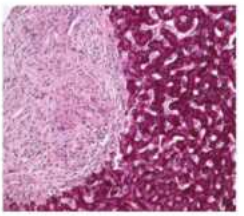
A. Single dose of TT
B. Full case of TT
C. 1 dose TT and TIG
D. No vaccination needed

Q. Diagnosis of Cl.difficile infection is made by which of the following methods?
(AIIMS Nov 2017)
A. Toxin gene detection by Polymerase Chain Reaction (PCR)
B. Culture
C. Enzyme – linked immunosorbent assay (ELISA)
D. Nagler's reaction

Q. Lipoarabinomannan (LAM) assay in urine is used for screening of?
(AIIMS Nov 2017)
A. Mycobacterium tuberculosis
B. Pneumocystis Jiroveci
C. Histoplasma Capsulatum
D. Cryptococcus neoformans

Q. Gene expert detects resistance due to mutation in following gene? (AIIMS June 2020)
A. rpo b
B. pnc a
C. Kat G
D. inh A

Q. Identify organism? (AIIMS Nov 2017)



A. Mycobacterium avium
B. Nocardiosis

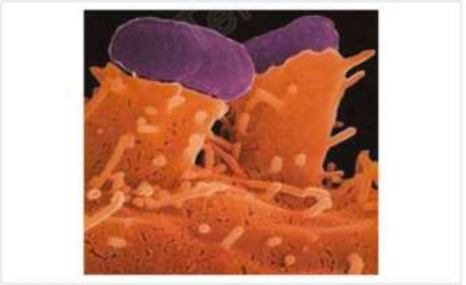
C. Actinomycetes
D. Pneumococci

Q. Which of the following is the most likely cause in case granuloma with positive AFB? (FMGE June 2019)
A. Cat Scratch disease
B. Trench fever
C. Leprosy
D. Syphilis

Q. Swollen foot with sinuses in a farmer? (INICET Nov 2020)

A. Mycetoma
B. Sporotrichosis
C. Melanoma
D. SCC

Q. Identify the organism which shows effacement & attachment of intestinal cells as shown below? (AIIMS May 2018)



A. EPEC
B. EIEC
C. Enterohemorrhagic E. coli
D. Diffusely enteroadherent E. coli

Q. Hemolytic Uremia Syndrome associate with? (FMGE June 2019)
A. E. Coli 0157
B. Malaria
C. Parvovirus B19
D. Bartonella henselae

Q. Which of the following is the cause of Donovanosis? (NEET Jan 2020)
A. H. ducreyi
B. K. granulomatis
C. Leishmania donovani
D. Treponema pallidum

Q. When should you perform Widal test in case of typhoid? (FMGE June 2019)
A. 1st week
B. 2nd week
C. 3rd week
D. 4th week

Q. 25-year-old man presented with diarrhoea& blood, on investigation of stool sample, the organism was gram negative, non-lactose fermenter, non-motile, oxidase negative, H2S negative diagnosis? (FMGE Dec 2020)
A. E. coli
B. Shigella
C. Salmonella
D. Y. pestis

Q. Organism most likely associated with VAP? (AIIMS May 2018)
A. Acinetobacter
B. Klebsiella
C. Clostridium
D. Mycobacterium TB

Q. Diarrhoea in vibrio cholerae is due to disruption of? (INICET Nov 2020)
A. Zonula occludens
B. Hemidesmosomes
C. Macula Densa
D. Intercalated disc

Q. What is the mechanism of action of cholera toxin? (FMGE Aug 2020)
A. ADP ribosylation of GTP
B. Decreases adenyl cyclase activity
C. Decreases CAMP
D. All of the above

Q. Urethritis in males is not caused by? (NEET Jan 2020)

A. **H. ducreyi**
B. Trichomonas
C. Chlamydia
D. Gonococcus

Q. All are true about H. Ducreyi induced chancroid except? (INICET Nov 2020)

A. Painful
B. Bleed on touch
C. Groove sign
D. School of fish

Q. Legionnaire disease cause? (FMGE June 2019)

A. Respiratory Disease
B. UTI
C. Retroperitoneal fibrosis
D. Acute Gastroenteritis

Q. Giemsa-stained smear cannot defect? (AIIMS Nov 2019)

A. Coxiella burnetti
B. Bartonella
C. Toxoplasmosis
D. E. chaffenensis

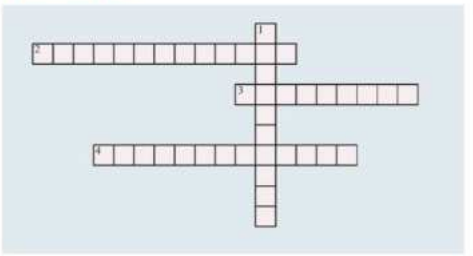
Q. Incubation period of LGV's is? (AIIMS May 2018)

A. 3-7 days
B. 7-10 days
C. 10-30 days
D. 30-90 days

Q. Which of the following statement is not correct regarding mycoplasma pneumoniae? (AIIMS June 2020)

A. Cannot be cultured easily
B. Serology is useful for diagnosis
C. Respond well to amoxycillin + clavulanic acid
D. Cause bilateral chest infiltrates on CXR

Crossword Puzzle



Across

2. Campylobacter Jejuni
3. Lab Diagnosis
4. Feco-oral

Down

1. Extrapulmonary Symptoms

22 VIROLOGY GENERAL PROPERTIES

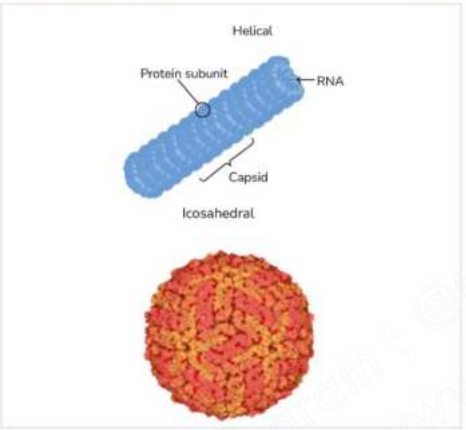
General Propertie

00:00:14

- Smallest infectious particles (only seen in E/M)
- No ribosomes present
  - Exception is Arenavirus which has non-functional ribosome (stolen from the body when enters)
- Obligate intracellular (grows only inside the cell)
  - Bacteria- Chlamydia & Rickettsia

Symmetry (Genome)

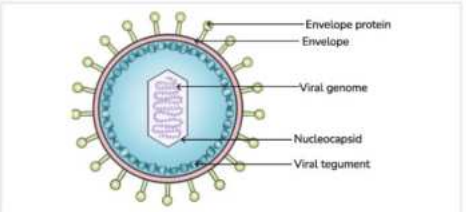
00:01:43



- All DNA viruses have Icosahedral symmetry
- The RNA viruses have helical symmetry
- Pox virus has a complex symmetry
- RNA- Helical symmetry:
  - M: Myxovirus
  - R: Rhabdovirus
  - F: Filovirus
  - A: Arenavirus
  - B: Bunyavirus

Structure

00:04:00



- On outside, there is Envelope & envelope proteins (known as Peplomers in many viruses) are coming out of it
- In the center, there is a capsid and it has a genome (nucleic acid). Together they are known as Nucleo Capsid
- Between envelope & nucleocapsid there is Tegument

Envelope

- Made of Lipoprotein
  - Lipid part is coming from host
  - Protein is from the virus
- Enveloped viruses are:
  - Ether & alcohol sensitive, heat Labile
  - Can be eliminated by soap, alcohol, sanitizer
  - If we eat them, our bile salt can break it
- Non enveloped viruses are:
  - DNA- Parvovirus, Adenovirus, Papovavirus (PAP)
  - RNA-Picornavirus, Astrovirus, Reovirus, Calicivirus, Hep. A, Hep. E (all a/w Gastro-enteritis (PARCH)

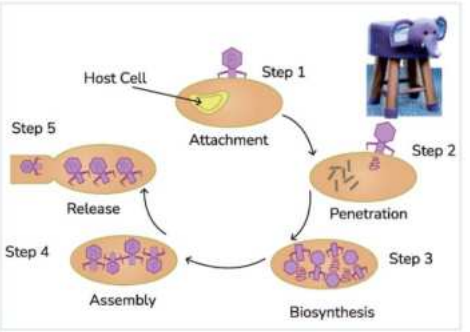
Viral Replication

00:09:29

- Virion: Nucleic acid with Capsid
- Viroid: Makes ss RNA without any Capsid
- Prion: Misfolded Proteins

There is no binary fission

- **Site:**
  - DNA- Nucleus except Poxvirus
  - RNA- Cytoplasm except retrovirus & orthomyxovirus



Steps:

- **Attachment / Adsorption**
  - Most specific step
  - Between Virus & host
  - Ex: HIV (has got gp120,41); Influenza (H & N); SARSCOV2 attached to ACE 2 Rc in human

- Penetration / Viropexia:
  - Phagocytosis by Macrophage (eaten up)
  - Membrane Fusion (HIV)
  - Injection of DNA / Genome by Bacteriophage
- Uncoating:
  - All Capsule lysed (lysozyme host)→ Nucleic acid released
  - This step is absent in bacteriophages
- Biosynthesis: New virions
- Assembly and Maturation
- Release:
  - Lysis of host cell
  - Budding out through host membrane
- Eclipse Phase: time b/w entry and first virus formation
- Bacteriophage- 15-30 mins
- Animal Virus- 15-30 hrs

Classification 00:21:51

- **DNA Virus: (Mnemonic: HHAPPPPy)**
  - Hepadnaviridae: HBV
  - Herpesviridae: HSV, HZV, EBV, CMV, HHV
  - Adenoviridae: Adenoviridae
  - Poxviridae: Smallpox, Cowpox, Molluscum
  - Papovaviridae: HPV, BK, JC, Polyoma, SV40
  - Parvoviridae: Parvovirus
  - Bacteriophage
- **RNA Virus:**
  - Picornaviridae
  - Paramyxoviridae
  - Caliciviridae
  - Togaviridae
  - Orthomyxoviridae
  - Bunyaviridae
  - Flaviviridae
  - Arenaviridae
  - Coronaviridae
  - Reoviridae
  - Rhabdoviridae
  - Retroviridae
  - Filoviridae

Size of Viruses 00:25:47

- **Determined by:**
  - Electron microscopy
  - Ultra-filtration
  - Ultracentrifugation
- Pox virus is the Largest virus (300 nm)
- Parvovirus is the smallest virus (20 nm)

Shape & structure of viruses

- Rabies: Bullet shaped
- Pox virus: Box/ brick shaped with Dumbbell DNA core
- Ebola virus: Filamentous/ bowl of spaghetti
- Tobacco mosaic virus: Rod shaped
- Adenovirus: Space Vehicle adenovirus
- Corona-virus: Crown with Peplomers
- Rotavirus: Rotating Wheel
- Astrovirus: Star

DNA Virus-Rules and Exception 00:30:46

- All DNA virus is dsDNA except Parvovirus (ssDNA)

RNA Virus-Rules and Exception 00:32:03

- All RNA virus has one copy of ssRNA which is unsegmented except
  - Retrovirus-has 2 copies
  - Reo/rota virus-has dsRNA
  - Bunyavirus(2seg), Influenza (8seg), reo/rotavirus (11seg), Arenavirus (2seg) - all are segmented
- Usually positive sense RNA except (MRFAB)
  - M: Myxovirus
  - R: Rhabdovirus
  - F: Filovirus
  - A: Arenavirus
  - B: Bunyavirus
- These negative sense RNA'S convert to Positive RNA by RNA dependent RNA Polymerase

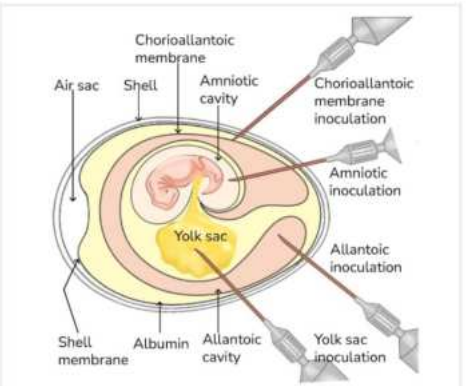
Genome size

- Largest genome-retrovirus
- Smallest genome- Hep D > Hep B

Virus Cultivation 00:39:19

- **Animal inoculation**

Eg: Coxsackie A, B; Arbovirus in brain of suckling mice
  - Coxsackie A- Flaccid paralysis
  - Coxsackie B- Spastic paralysis
- **Embryonated egg inoculation**



- Allontoic cavity-yellow fever, Vaccine Preparation of Influenza, Rabies
- Amniotic membrane-influenza isolation
- Yolk sac-Chlamydia, arbovirus, rickettsia
- Chorioallontonic membrane-vaccinia, variola, HSV 1 & 2

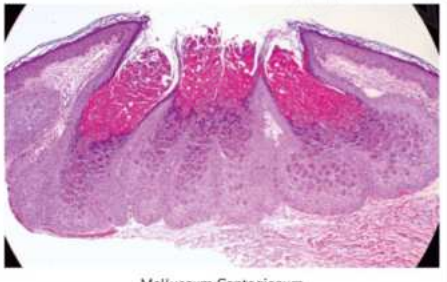
- **Tissue culture**
  - Cell lines

Primary cell lines	Secondary cell lines	Continuous cell lines
5-10 divisions	10-50 divisions	Continuous divisions
Rhesus kidney cell line Human Amniotic cell line Chick Embryo fibroblast	Human fibroblast cell line: CMV MRC5 WI38	Hela, HEP-2, KB, McCoy, Vero, Detroit 6, BHK, Chang C/I/L/K

Inclusion Bodies (Cyto Pathic Effect) 00:48:05

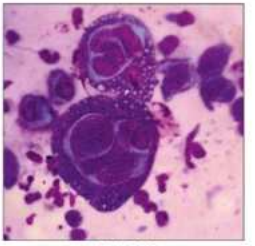
It is the effect of the virus on the cell

- **Intra cytoplasmic:**
  - Negri-Rabies (cerebellum, Hippocampus)
  - Paschen-variola
  - Guarnieri-vaccinia
  - Bollinger-fowl pox
  - Molluscum



Molluscum Contagiosum

- HP bodies (Henderson Patterson Bodies)
- Halbesteder Prowazeki (HP) body is seen in Trachoma
- Results in cup-shaped lesion
- **Intra-nuclear**:
  - Cowdry A in Yellow fever (Torres body) & Herpes (Lipschultz Body)
  - Cowdry B in Adenovirus & Poliovirus



HSV: CPE

→ Multinucleated
→ Molding
→ Margination of chromatin
→ Cowdry A

- Both intracytoplasmic and Intranuclear inclusion bodies seen in:
  - CMV (Cytomegalovirus)
  - Measles shows Warthin Finkeldey Giant Cell

Assays: To know Infectivity

Physical Methods	Chemical Methods
• PCR • Electron microscopy	• Qualitative: End point Assays • Quantitative: Plaque & Pock Assay

Interferons 00:58:59

- They are Cytokines produced by host
- Host specific

	IFN alpha	IFN Beta	IFN gamma
Source	Leukocytes	Fibroblast	Th1 cells
Function	Antiviral properties	Antiviral properties & used for multiple sclerosis	For Granuloma formation & used for treatment of Chronic Granulomatous disease
Chromosome	9	9	12
Chr. INF receptor	21	21	6

23 DNA VIROLOGY PART-1

Classification of Herpesviridae

00.00.22

- HHV1 - Human Herpes Simplex Virus 1
- HHV2 - Human Herpes Simplex Virus 2
- HHV3 - Human Herpes Zoster Virus
- HHV4 - Human Herpesvirus Epstein–Barr virus
- HHV5 - Human Herpesvirus Cytomegalovirus (largest member)
- HHV6 - Human Herpesvirus 6
- HHV7 - Human Herpesvirus 7
- HHV8 - Human Herpesvirus 8

Important Information

- Largest Virus: Pox (300 nm)
- Smallest Virus: Parvo (20 nm)

General Properties

00.01.43

- Size: 150-200 nm (second largest virus)
- Icosahedral Symmetry
- Double-stranded DNA

Classification

00.02.48

Subfamily	Species	Site of Latency
α	HHV1, HHV2, HHV3	Neurons
β	HHV5, HHV6, HHV7	Glands, T cells
γ	HHV4, HHV8	B cells

Herpes Simplex Virus 1 & 2

00.04.31

	HSV1 (Above waist)	HSV2 (Below waist)
Transmission	Mucosa/ Abraded Skin	Sexual/ Vertical
Latency	Trigeminal ganglia	Sacral ganglia
Age	Children	Adults
Clinical Features	• Orofacial Lesions • Skin Lesions above waist • Encephalitis • Meningitis	• Genital Lesions below waist • Neonatal Herpes (anything gone from mother to child)
Chick Embryo Fibroblast	Does Not Grow Well	Grows Well
Neurovirulence	Less	More
Drug Resistance	Less	More

Clinical features

00.07.17

1. Orofacial Mucosal Lesions:

- Most Common Site: Buccal Mucosa
- Most Common Primary lesion: Gingivostomatitis
- Most Common Recurrent lesion: Herpes Labialis

2. CNS Infections:

- Encephalitis
  - HSV 1 >>>HSV 2
  - Most common cause of acute sporadic viral encephalitis
  - Temporal lobe is affected.
- Meningitis
  - MOLLARET MENINGITIS (Recurrent Lymphocytic Meningitis)
  - Most commonly seen with HSV2

3. Ocular Lesions: HSV 1 > 2

- DENDRITIC CORNEAL ULCERS

4. Genital Lesions:

- Most commonly found in HSV2
- Bilateral
- Painful vesicles
- Painful Inguinal Lymphadenopathy

5. Other:

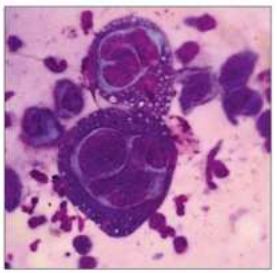
- Herpes Gladiatorum (also known as Wrestler's herpes)
- Eczema Herpeticum
- Erythema Multiforme
- Herpetic Whitlow (seen in health care workers in hands)

Lab diagnosis

00.12.17

1. TZANCK smear:

- Done for HSV1, HSV2 and Herpes zoster virus.
- Disadvantages:
  - Cannot differentiate between different Herpes.
  - Sensitivity is low.
- Procedure
  - Unroof the vesicles. (Try to take fresh vesicles)
  - Scrap the base of the lesion.
  - Staining. Stains used:
    - → Giemsa Stain (Romanowsky stain)
    - → Wright Stain (Romanowsky stain)
    - → Toluidine blue
- Microscopy:



  - Multinucleation
  - Moulding
  - Margination
  - Cowdry Type A (LIPSCHUTZ BODIES)

2. Virus Isolation

- Most Definitive
- Use of Cell Line

3. DNA Testing

- PCR (Can not differentiate between HSV1 and HSV2)
- Most Sensitive
- Can differentiate between HSV1 and HSV2

4. Serology

- ELISA (enzyme-linked immunoassay)
- If there is a fourfold increase in antibodies it is suggestive of herpes.

Treatment

00.16.08

- Drugs of choice: Acyclovir (inhibits DNA polymerase)
- If resistance to Acyclovir, then Foscarnet is given.

Human Herpesvirus 3 (HHV3)

00.16.52

- Known as Herpes Zoster/Varicella Zoster
- In children it causes chickenpox.
- In adults it causes Herpes Zoster/shingles.

Chicken Pox

00.17.17

- Incubation Period: 2 to 3 weeks
- **Rash Distribution:**
  - Bilateral
  - Diffuse
  - Centripetal (spread starts from trunk) and then centrifugal (spreads on extremities)
- Rashes come as Crops.
- There is fever with each crop.

- Macules
- Papules
- Vesicles
- Pustules
- Pleomorphic

- Dew Drop on a Rose Petal appearance (classical description of chicken pox rash)
  - Vesicle surrounded by an Erythematous Halo.
- If chicken pox occurs in adults it is more severe.
  - Hemorrhagic and Bullous Lesions are seen.
- **Period of Infectivity**: 2 days before and 5 days after rash.
- Immunity: Lifelong
- Reservoir: Humans
- Source: Patients
- Carrier: None
- **Complications**:

1. Most Common: Secondary Bacterial skin infection
2. Most Common Extra Cutaneous Complication: CNS Involvement (Benign Cerebellar Ataxia)
3. Most Serious: Varicella Pneumonia (especially in pregnancy)

Fetal / Congenital Varicella Syndrome 00.22.05

- Infection within 20 weeks of IUL
- **Clinical features:**
  - Cicatricial skin lesions
  - Limb hypoplasia
  - Microcephaly
  - Cortical Atrophy

Neonatal Varicella Syndrome 00.23.35

- Mother has varicella late in pregnancy.
- Risk of transmission is 5 days before to 2 days after delivery. (Maximum chances)
- Disseminated Disease
- Diagnosis: PCR from skin lesion.
- Treatment:
  - If lesions are absent- Acyclovir (Prophylactically)
  - If lesions are present
    → Vaccine: OKA strain (2 doses)
    → Varicella Zoster immuno-globulin is given maximum before 94 hours of exposure.

Herpes Zoster/Shingles 00.26.23

- Reactivation (in elderly people or when immunity goes down)
- **Rash Distribution**
  - Unilateral Rash
  - Segmental because of Dermatomal distribution (most common D3 to L2)
  - Painful

NOTE: If Dermatomal pain is present without skin lesions - Zoster Sine Herpete

- **Complications:**

1. Post Herpetic Neuralgia –
   - Most common
   - Pain at the local site.
2. Zoster Ophthalmicus –
   - Trigeminal Ganglion
   - Dendritic Ulcer

3. Ramsay Hunt Syndrome –
   - Involvement of the Geniculate Ganglion of 7th Nerve.
   - Facial Nerve Palsy
   - Vesicles at External auditory meatus.
   - Otalgia.
   - Loss of taste sensation in anterior 2/3rd of tongue.

- **Vaccines:** Zoster vaccine to enhance immunity.

1. Live attenuated Vaccine - Zostavax
2. Recombinant Vaccine - Shingrix

EBV: Epstein Barr Virus (HHV4) 00.32.48

- **Spread By:**
  - Close contact
  - Kissing
  - Sexual Intercourse
  - Blood transfusion
  - Bone marrow transplant
- It causes Polyclonal B cell activation.
- It enters the B cell through CD21/ Cr2.
- Molecules present:
  - LMP 1 (acts on CD40 pathway)
  - EBNA2 (activates the SRC proto-oncogenes)
  - vIL10 (stops inflammation)

Clinical Features 00.37.21

1. Infectious Mononucleosis

- Kissing disease/ glandular fever
- Incubation period is 4-8 weeks.
- Organs Infected
  - **S**plenomegaly
  - **H**epatitis
  - **E**ncephalitis
  - **F**ever
  - **S**ore Throat
  - **L**ymph Node
  - **A**rthralgia
  - **L**ethargy

2. Malignancy

- Carcinoma - Most common is Gastric Carcinoma.
- Lymphoma - It is of two types.

a) Hodgkin (mixed cellularity)

b) Non - Hodgkin Lymphoma (Burkitt's, DLBCL, Post Transplant Lymphoma, T/NK cell Lymphoma).

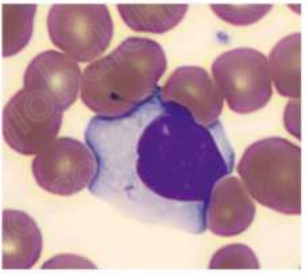
- Sarcoma - Leiomyosarcoma

3. Other Conditions:

- Duncan Syndrome (Lymphoproliferative disorder) - associated with people with SAP gene defect.
- Hairy cell leucoplakia/ Oral cell leucoplakia- associated with BRAF mutation
- Hemophagocytic Lymph Histiocytosis

Lab Diagnosis 00.43.30

1. Microscopic



- Blood sample: Atypical Lymphocytes (Downey cells)
- Ballerina skirt appearance
- Origin: CD8T Lymphocytes

2. Heterophile Agglutination Antibody Detection

- Paul Bunnell Test (different species or antibodies react with sheep RBC).
  - Titre of 1:256 is suggestive in Infectious Mononucleosis.
- Monospot Test (antibodies react with Horse RBC)

3. Specific Antibody Test

- Antibody to viral capsid antigen
- Antibody to early antigen
- Antibody to EBNA2

4. Most sensitive and specific: Nucleic Acid Hybridisation Test

Treatment 00.48.03

- Acyclovir for Oral hairy cell leucoplakia
- Rituximab

Cytomegalovirus (HHV5) 00.48.18

- Largest Virus of Herpesviridae
- Man is the only reservoir.
- Transmission:

1. Most Common: Oral Respiratory, Body Secretions
2. Less Common: Transplacental, Sexual

- Multiplies in:

1. Salivary Gland
2. Respiratory Tract
3. Kidney

- Latent in: Monocyte

Clinical features

00.51.03

1. Congenital:

- Most common intrauterine infection associated with congenital defects.
- Features:
  - Most Common:
    - → Hepatosplenomegaly
    - → Jaundice
    - → Petechiae/ Purpuric Rashes - Blueberry Muffin Baby
  - Less Common:
    - → Microcephaly
    - → Chorioretinitis
    - → Cerebral Calcification (Periventricular Calcification)
    - → Deafness

2. Perinatal

- Asymptomatic

3. Immunocompromised

- CMV chorioretinitis (most common opportunistic infection virus in HIV and transplantation)

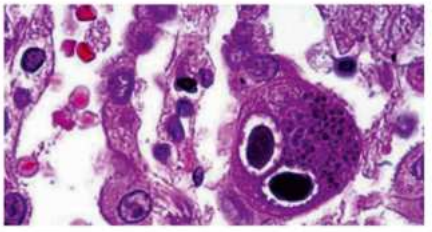
4. Immunocompetent

- Glandular Fever (IMN) like syndrome
  - Fever
  - Sore Throat
  - No Lymph node and spleen involvement is seen.
  - No heterophile antibodies are seen.
  - Paul Bunnell test / Monospot test negative

Lab Diagnosis

00.57.16

- Inclusion Bodies: Owl eye appearance



- Virus Isolation: Human Fibroblast cell line (2° Cell Line)
  - Specimen: Urine Washing
- Antibody Detection: ELISA
  - Antigen: Pp65
- PCR

Treatment

00.58.55

- DOC - Ganciclovir
- If resistance - Foscarnet

Human Herpesvirus 6 & 7

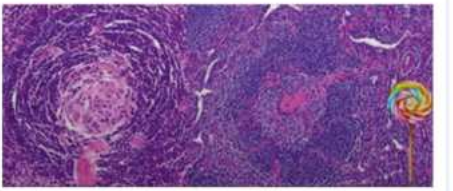
00.59.14

- Binds to T cells via CD46 receptor.
- Variants: 6A, 6B
- Mode of Infection: Oral Secretions
- In children: Sixth disease/ roseola infantum / exanthem subitum
  - Fever
  - NAGAYAMA SPOTS
    - → Erythematous, Rash after fever
    - → Seen on soft palate.
    - → Rose pink non pruritic papules

- In adults: Mononucleosis like syndrome

Human Herpesvirus 8 (HHV8)

01.02.53



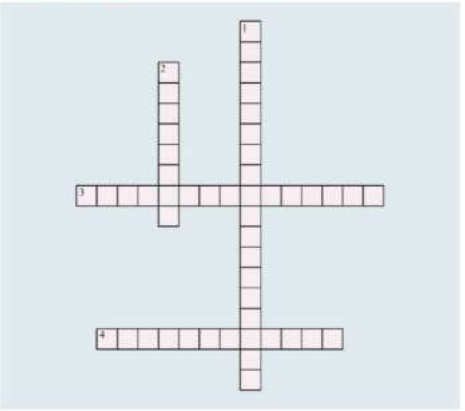
1. Kaposi Sarcoma (Vascular Tumour)
- Most commonly associated with HIV (Immuno compromised)
- Most common site - Lower Limb (skin)
- Second most common site - Lymph Node

2. Primary Effusion Lymphoma
- Clinically the patients have a history of lot of effusions (pleural effusion , pericardial effusion etc.)

3. CASTLEMAN Disease
- Occurs in Lymph Nodes
- Lollipop Follicles

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Largest Virus of Herpesviridae
4. Elderly people or when immunity goes down

Down

1. Congenital: Most common intrauterine infection associated with congenital defects.
2. Most common is Gastric Carcinoma

24 DNA VIROLOGY PART-2

Parvoviridae

- Smallest virus (20 nm)
- Only DNA virus which is single stranded
- Icosahedral symmetry as other DNAs

Spread

- Respiratory route
- Blood transfusion
- Transplacental transmission

Tropism

- P antigen on RBC precursors

3 Genotypes

- Parvoviridae 1 (m/c in world)
- Parvoviridae 2
- Parvoviridae 3
- 1 serotype

Conditions a/w Parvovirus B19

- Erythema Infectiosum (5th disease; slapped cheek appearance)

- Macular erythematous rash present on cheek
- Lacy rash on rest of body
- Self-limiting
- Prodrome: fever , sore throat
- Gloves & socks syndrome aka papular purpuric gloves & socks syndrome

- Arthritis, Arthralgia
- Aplastic Crisis (in Haemolytic Anaemia → Parvovirus B19)
  - Hemolytic crisis is a/w EBV
- Pure red cell aplasia
  - a/w Thymoma
- Hydrops Fetalis
- ELISA for antibody testing
- PCR for DNA virus

Diseases

- 1st disease: Measles
- 2nd disease: Scarlet Fever
- 3rd disease: German Measles
- 4th disease: DUKE (obsolete)
- 5th disease: Slapped cheek /erythema infectiosum
- 6th disease: Roseola Infantum, HHV 6

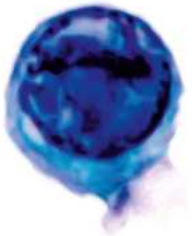
- RBC precursors showing dog ear projection

Papovaviridae

00:10:59

Papilloma Virus: HPV

- Polyoma virus
  - **MCV- Merkel Cell Carcinoma**
    → Neuro-endocrine Tumor around skin
    → CK 7- 20+
  - **SV 40- Non pathogenic to human**
    → a/w Mesothelioma
  - **JC- Attacks Oligodendrocyte**
    → Demyelinating Disorder
    → Progressive multifocal leukoencephalopathy
    → Myelin stain is Luxol Fast Blue
  - **BK- causes**
    → Interstitial Nephritis
    → Hemorrhagic Cystitis
    → Usually seen in post-transplant patients



Decoy cells

→ Urine m/e shows huge cells with high nucleo cytoplasmic ratio which mimics cancer cells

HPV

00:16:37

- Human Papillomavirus
- Non-Cultivable
- Non enveloped

Cutaneous involvement

- **HPV 1,2,3 (Low risk)**

Shows Plantar wart / Verruca

Filiform Wart (papillary projection in eyes)

- **HPV 5,8 (High risk)**

- Shows many warts
- Tree man Syndrome / Epidermodysplasia Verruciformis (a/w chr. 17 defect)
- While tree bark aorta is seen in tertiary syphilis.

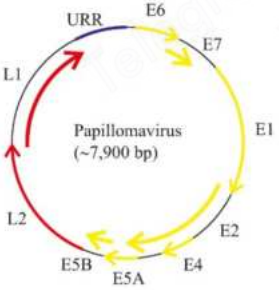
Mucosal involvement

- HPV 6,11 (Low risk)

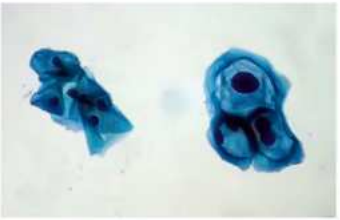
- Warts in vocal cord
  → Results in Laryngeal Papillomatosis, showing voice change

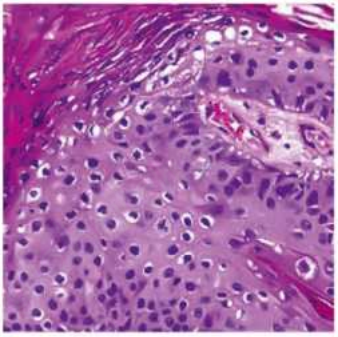
- Genital warts are Condyloma Acuminatum while Condyloma lata is seen in secondary syphilis.

- HPV 16,18,31,33,35 (High risk)
  - Results in Squamous Cell Carcinoma



- Structure of human Papillomavirus
  - L1 capsid protein is used for making a vaccine
  - E2 is controller
  - E4 & E5 are responsible for making a change in the cell called Koilocytosis.





  - There is a cell with Raisinoid nucleus
  - And the space left is called perinuclear halo
- E6 & E7 are responsible for Carcinogenesis as E6 inactivates the p53 and E7 inactivates the RB gene causing
  - Cervical cancer
  - Penile cancer
  - Anal canal cancer
  - Oral cancer
  - Laryngeal cancer
  - Esophageal cancer
- **Screening**
  - PAP
- **Confirmatory test**
  - Biopsy
  - Molecular tests like PCR and Hybrid Capture assay

HPV Vaccine

- Bivalent vaccine- Cervarix (Protection HPV 16,18)
  - Cervarix has Adjuvant. It goes and act on TLRs
  - ASO4 is added for immune enhance
- Quadrivalent vaccine- Gardasil (for HPV 6,11,16,18)
- Nonavalent vaccine- Gardasil 9

Poxviridae

00:31:10

- Size- 300 nm (largest)
- Shape
  - Box / brick shaped

- Has a Dumbell DNA core
- Symmetry: Complex
- Replication: Cytoplasm
- Ex- Vaccinia, Variola, molluscum contagiosum, monkeypox

Variola (Smallpox)

- IP- 10-12 days
- Eradicated
  - Typical c/f
  - No animal reservoir
  - No carrier
  - Vaccine

Features	Smallpox	Chickenpox
Crops	Not present	Rash in crops
Fever	Fever subsides with rash	Fever with each crop
Distribution	Starts centrifugal-centripetal (spread)	Centripetal (start) - centrifugal (spread)

- Eradication programme by WHO in 1980
- Vaccines
  - 1st vaccine- using Cowpox /vaccinia by Edward Jenner
  - Live vaccinia Vaccine- freeze dried preparation

Cowpox	Smallpox
Vaccinia	Variola
Guarnieri Body	Paschen Body
On CAM, vaccinia shows large, hemorrhagic, necrotic	Shows small, non haemorrhagic lesions (pocks)

Molluscum contagiosum

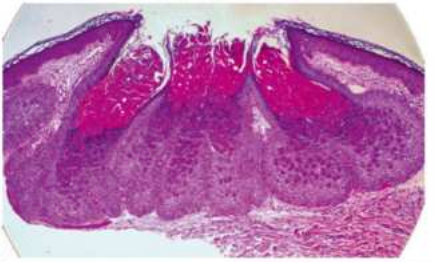
- Caused by Molluscum contagiosum virus

Transmission

- In child & adults- By contact
- All over body except Palms & soles
- Human Reservoir

  - Tan color umbilicated region with depression in the center
  - Curdy discharge on clicks

Skin scraping



- Cup shaped region
- Bodies inside it known as Henderson Patterson bodies (HP bodies)
- Stain for HP bodies is Phloxine Tartrate
  - Halberstaedter Prowazek body is seen in Trachoma
- PCR
- E/M
- Non-Cultivable

Monkeypox

00:43:31

- Comes from monkeys
- Rodents are reservoir
- By bites, scratches, consumption of meat
- Limited human to human transmission
- IP- 2 weeks

C/f

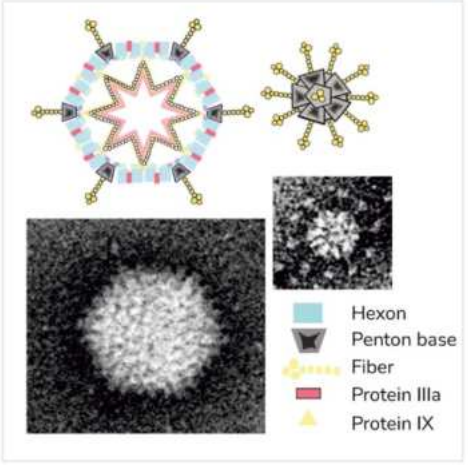
- Fever
- Rash
- Lymphadenopathy

Diagnosis

- Skin scraping
- PCR

Adenovirus

00:45:06



- Shape: Space Vehicle
- Some projections present like fibers comes from surface helps in attachment
- Non enveloped
- Hemorrhagic Cystitis is 11,21
- Infant Diarrhea is with 40,41

Eyes

- Epidemic Keratoconjunctivitis/ Shipyard eye: 8,9,37
  - Epidemic
  - Follicular conjunctivitis can be U/L or B/L
  - Corneal opacities can be seen
  - Preauricular lymphadenopathy

Pharyngo-conjunctivitis Fever/Swimming Pool conjunctivitis/ follicular conjunctivitis: 3,7,14

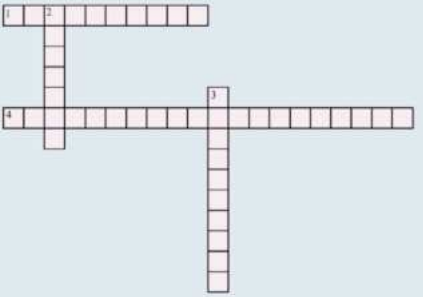
- History of swimming
- Patients have pharyngitis
- Preauricular lymphadenopathy

Respiratory Diseases

- URTI: 1,2,3,5
- Pneumonia: 3,7,21
- Transplant Recipients: 34,35

CROSS WORD PUZZLES

Crossword Puzzle



Across

1. ASO4 is added for immune enhance
4. Human Reservoir

Down

2. IP- 10-12 days
3. Replication- Cytoplasm

25 DNA VIROLOGY PART-3

Hepatitis

- **Types of hepatitis**
  - Viral
  - Alcoholic
  - NASH (Nonalcoholic steatohepatitis).
  - Autoimmune hepatitis.
  - Neonatal Hepatitis.

Refer Table 25.1

Important Information

Hepatitis G

- Does not cause known human Infection.
- Affects mononuclear cells.
- **Route of transmission:** Blood transfusion
- Protects against HIV

Viral Hepatitis

- Hepatitis A [27 nm]
- Hepatitis B [42 nm]/ Dane particle.
- Hepatitis C [60 nm].
- Hepatitis D
- Hepatitis E [30 nm]
- Hepatitis G

Hepatitis A

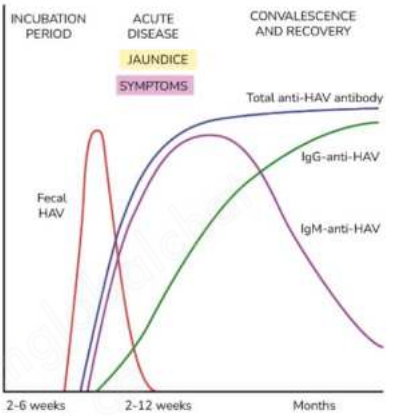
00:09:24

- Incubation period: 15-50 days.

Serotype: 1
Genotype: 6
Humans: 1 2 3
World: 1
India: 3

- Susceptible to boiling, chlorination, autoclave, and formalin.
- Fever
- Anorexia
- Nausea/Vomiting
- Jaundice
  - Dark urine
  - Pale feces

Laboratory Diagnosis



- HAV shedding in stool 2 weeks prior to 2 weeks after symptom onset.
- IgM appears with the onset of jaundice.
- 2-4 weeks later IgG seen.
- No liver biopsy
- It is an infection.

Important Information

- Hepatitis A shows an increase in plasma cells.
- **Acute hepatitis:** Faeco-oral route.
- Hepatitis C Shows cholestasis
  - Bile plugs/static

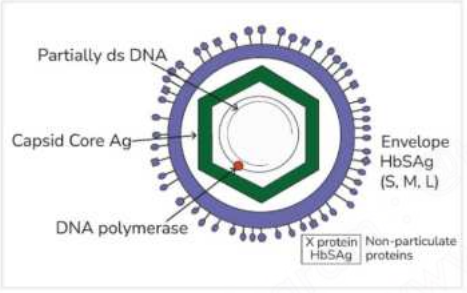
- **Inactivated Formaldehyde (IM)**
  - Given in 6-12 months.
  - Given to people above 12 months.
- **Live attenuated Vaccine**
  - Oh2
  - OLA1 strain
- **Immunoglobulins:** for all unvaccinated contacts.
  - <2 weeks exposure.
  - Protection for 1-2 months.

Hepatitis E

- Hepeviridae (RNA).
- Feco-oral route.
- Acute Hepatitis.
- **In pregnancy:** Fulminant hepatitis is 25%.
- Supportive treatment
- Vaccine available in China.
  - **Recombinant:** Capsid Protein

Hepatitis B (Dane Particle) 00:17:15

- 8 Genotypes(A-H).
- **D-Genotype is prevalent in India.**
- Serotypes
  - North and South India: ayw
  - East India: adr
- Hepadnaviridae.
- Only DNA.
- Partial Double Stranded.



- Two linear strands of DNA
  - Complete strand/ minus strand
  - Incomplete strand/ plus strand

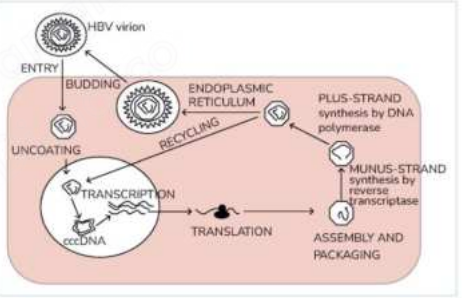
Important Information

- All DNA viruses are Double stranded.
- **Except:**
  - 1 single-stranded DNA virus is Parvo
  - Partial Double stranded virus is Hepatitis B

- Genome has
  - **P** - DNA Polymerase
    - → Largest
    - → Reverse transcriptase
    - → RNAse
  - **C** - Core gene
    - → Has a core area and a pre-core area. Core area code C antigen. Precor code E antigen.
  - **S** - Surface Antigen (Australia Antigen).
    - → These have different sizes(Small medium and large).
  - **X** - HBX responsible for carcinogenesis
    - → Liver
- Spherical particles - 42nm
  - Full virions/ DANE particles
  - Genome + infection
- Spheres/ tubular - 22nm
  - Genome no infection
- Filaments - 22nm
  - Genome: Infection - none

Mode of Infection

- Blood products.
- Sexual intercourse.
- Percutaneous.
- Mother to Child.
- Needle stick (Blood Stick Injury. BCI).
  - HIV 0.3%
  - HBV = 30%
  - HCV = 3%

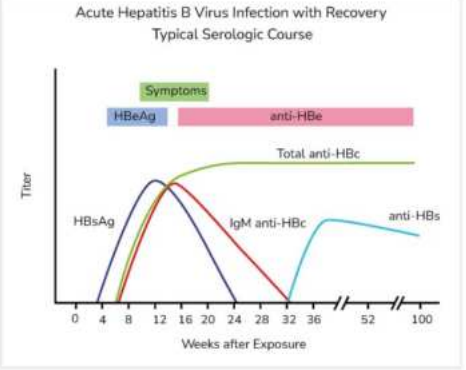


- HBV
- Comes inside from a process known as endocytosis.
- Uncoating happens.
- cccDNA is made (Covalently closed circular DNA).
- Translation occurs and RNA forms.
- Initially Capsid is made around Pg RNA.
- Reverse transcript and form DNA.
- One whole virus is made in the Endoplasmic reticulum.

Lab Diagnosis

Q. 1st Virological Marker In Blood?
Ans. HBV DNA

- 1st antigen is the Hepatitis B surface antigen.
- 2-6 weeks before onset of symptoms.
- Disappears in 1-2 months after onset of jaundice.



- **Epidemiological marker:** Hbs
- HBeAg Antigen appears.
  - Tells the infectivity rate.
  - Indicates active infection and high infectivity.
  - Anti HBe- indicates decrease in infectivity
- HBc core antigen is stuck inside.
  - Not detected in blood
  - Antibodies are made for it.'
  - IgM and IgG
  - Covers the window period.

Refer Table 25.2

- Acute Hepatitis: SEMA
  - E detects infectivity.
- Window period is the time when no one helps.

Hepatitis in Pregnant Women

- If the mother HBe positive- 90 percent risk of transmission.
- If mother is HBe antibody positive-10-15 percent risk of transmission
- When a neonate is born, on one arm we put the vaccine and on the other arm we put immunoglobulin.
- Schedule- 0, 1, 6 months.

Vaccine

- Hepatitis B vaccine
- Prepared in yeast (saccharomyces cerevisiae).
- Recombinant killed vaccine Schedule 0, 1, 6 months.
- Anti HbS >10 IU/ml vaccinated
- If >10 responder and <10 non responder.

Needle Stick Injury

HCW Status	Source: HBS antigen +	Source: HBS antigen -	Source N/K
Unvaccinated	Immunoglobulin + 0,1,6 months	0,1,6 months	0,1,6 months
Vaccinated and responder	x	x	x
Vaccinated and non-responder	Immunoglobulin + 0,1,6 months	x	x

Carriers

- HBs Antigen positive beyond 6 months.

Simple carriers	Super carrier
HBsAg positive	HBsAg positive
HBeAg negative	HBeAg positive
Low DNA P	High DNA P
-	Increased liver enzymes

Mutations 00:48:58

- **Escape mutants**
  - No HBs antigens.
  - All have HBs antibodies in body(Cannot neutralize escape mutant).
  - Mostly shown in healthcare workers
- **Precore Mutant**
  - HBe antigen absent
  - DNA copies> 1000 indicates infection.

Hepatitis D

- Co-infection
  - HBV + HDV infection together
  - Usually clears except I.V. drug abuser
- Superinfection
  - Patient is already infected with HBV get superadded infections with HDV
  - Fulminant infection (M/C/C)

Hepatitis C

- Highly mutable virus
- Most mutable protein E2
- "QUASISPECIES"
- No vaccine
- Spread
  - I.V. } Parenteral route
  - BT }

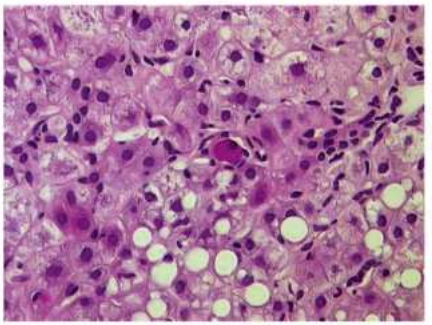
Laboratory diagnosis

- IgM HCV
- Hep C Tridot testing
- DOC: HCV RNA PCR

Morphological changes

- Acute hepatitis
  - Hep A = ↑ plasma cells
  - Hep E = Bile plugging's
  - Bodies (Councilman bodies)
  - Spotty Necrosis → Dropout Necrosis
  - Kupffer cell Hyperplasia (M)
- Chronic Hepatitis
  - Ito cell/ Stellate cells (space of disse) → TGF β → Fibrosis → Chronic Hepatitis

Councilman Bodies - Apoptotic body

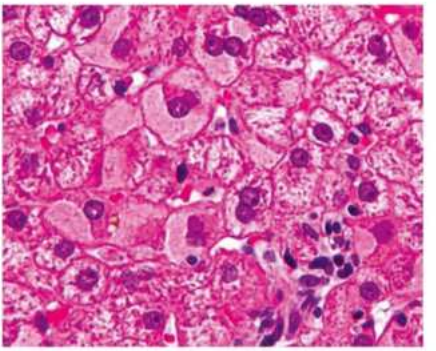


1. Cell Shrinkage
2. Pink ++ cyto
3. Blue++ nucleus

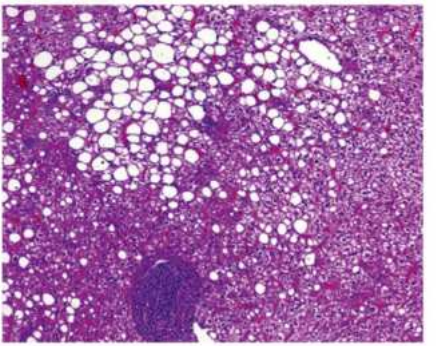
Chronic Hepatitis

- Hepatitis B
  - HbsAg/ Australia Antigen → Ground glass Hepatocytes
  - Shikata Orcein Antigen Stain
- Hepatitis C
  - Macrovesicular steatosis (Fatty changes)
  - Lymphoid follicles (prominent)
  - Hepatitis D
  - Microvesicular steatosis."

Ground Glass Hepatocytes (Diffusely pink cells)



HbsAg ++
↓
Orcein Shikata stain ++



Chronic Hep
Staging ← Biopsy Liver (Ix) → Grading
↓ ↓
Fibrosis Inflammation Necrosis

- M/c/c sporadic acute Hep in India? Hep E
- M/c/c fulminant viral hepatitis? HDV
- M/c/c fulminant viral hepatitis in preg? HEV
- M/c/c transfusion related hepatitis? HBV
- M/c/c of chronic Hepatitis? HBV
- Max risk & potential of chronicity? HCV
- M/c/c of carrier state? HBV
- M/c/c of viral HCC? HBV

- Most strong association with HCC? HCV

Extrahepatic manifestation of Hepatitis

Hep B	Hep C
• PAN • MGN	• Mixed cryoglobulinemia • Lichen Planus • MPGN

Table 25.1

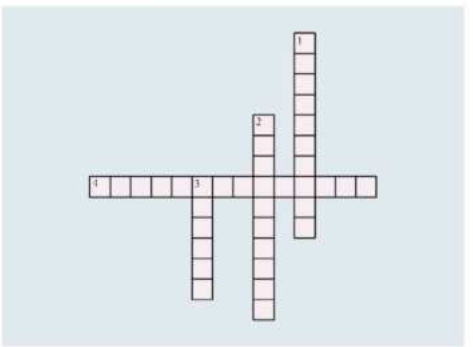
Mnemonic	Private	Hospitals	Favour	Rich	Clients
Feature (Virus)	HAV	HBA	HCV	HDV	HEV
Genome	RNA	DNA	RNA	RNA	RNA
Family	**P**icornaviridae Enterovirus	**He**padnaviridae	**F**laviviridae	**R**elies on HBV	**C**alicivirus Now Hepeviridae
Mode of infection	Feco-oral	Parenteral, Sexual, MTC	Parenteral		Feco oral route
IP	15-50 days	50-150 days	15-150 days	50-150 days	15-50 days
Envelope	-	+	+	+	-
Cultivable	Yes	No	No	No	No
Vaccine	Yes	yes	-	- Protection from B	yes
Fulminant	0.1%	0.1-1%	1%	5-20% A most common cause of pulmonary hepatitis	1-2%. Pregnancy 25%
Chronicity		+	+		
Carrier		1-30%			

Table 25.2

HbsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe	Interpretation
+	-	IgM	+	-	Acute Hepatitis B- High infectivity
+	-	IgG	+		Chronic Hepatitis B-High infectivity
-	+	+ (IgM + IgG)	-	+	Recovery
-	+	-	-	-	Immunity
-	-	+ (IgM)	-	-	Window period
Marker of winder = IgM anti HbC					

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Faeco-oral route

Down

1. Affects mononuclear cells
2. Incubation period: 15-50 days
4. Virological Marker In Blood

26 RNA VIRUS: MYXOVIRUS

Classification

- Orthomyxovirus
- Paramyxovirus

Orthomyxovirus	Features	Paramyxovirus
• Influenza A • Influenza B • Influenza C	**Members**	• M- measles • M- mumps • R - RSV • Parainfluenza • NIPAH virus
Segmented genome	**RNA**	Unsegmented genome
110 nm	**Size**	150 nm
Helical	**Nucleocapsid**	Helical
+	**Enveloped**	+
+	**Peplomers**	+
Negative sense	**RNA sense**	Negative sense

Important Information

- All RNA viruses are unsegmented but there are some exceptions - influenza is one such exception
- MR FAB - M for myxovirus
  - Helical symmetry
  - Negative sense RNA

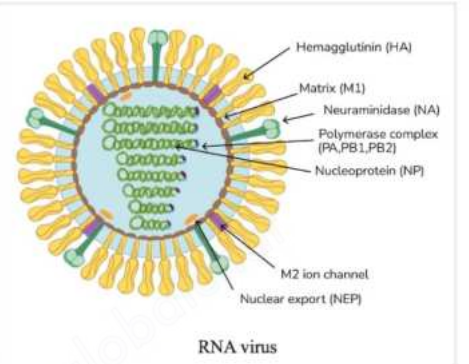
Segmented RNA - BIRA

- B - Bunya virus - 3 segments
- I - influenza - 8 segments
- R - reovirus - 8+3 = 11 segments
- A - Arenavirus - 1+1 = 2 segments

Orthomyxovirus family

- Influenza virus
- Negative sense RNA
- Segmented
- **Members**
  - Influenza A - 8 segments
  - Influenza B - 8 segments
  - Influenza C - 7 segments

Structure



RNA virus

- Outer layer
  - **Hemagglutinin - HA**
    - → More in number
    - → Triangular in shape
    - → Attach to the body - SIALIC ACID receptor
      - In respiratory epithelium
      - RBC
  - **Neuraminidase - NA**
    - → Less in number
    - → Mushroom shaped
    - → Helps in release of virus
    - → Also known as RDE - receptor destroying enzyme or sialidase
  - **M1 protein**
    - → Below the envelope
    - → Maintains structural integrity
  - **M2 protein**
    - → Ion channel
    - → Aid in uncoating of virus

Pathogenesis

Refer Image 26.1

1. **Attachment**
   - Influenza enters body
   - Hemagglutinin attaches to sialic acid receptor
2. **Endocytosis**
   - Internalization
   - Forming endosome
3. **Acidification of endosomes**

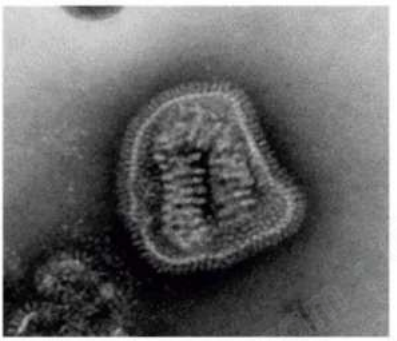
- For uncoating
- M2 ion channel allows entry of H+ ions - H+ influx

4. **Conversion of negative sense RNA into positive sense RNA**
- By RNA dependent RNA polymerase - RdRP

5. **Release**
- By neuraminidase
- Neuraminidase are sialidase
- They destroy the receptors

Treatment
- Amantadine and Rimantadine target M2 ion channels of influenza A

Electron microscopy



3 genera based on ribonucleoprotein - RNP
1. Influenza A
2. Influenza B
3. Influenza C

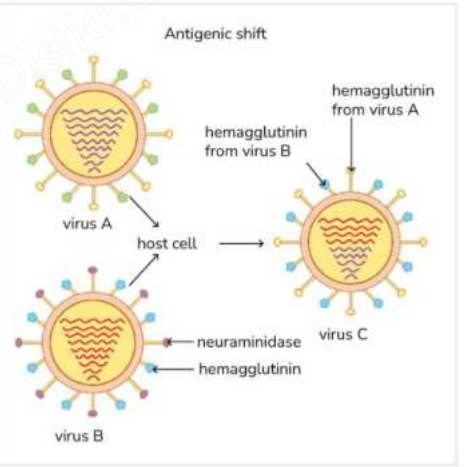
Differences

Influenza A	Influenza B	Influenza C
Humans and animals	Humans only	Non- pathogenic
Antigenic shift • More severe	No antigenic shift	
Epidemic and pandemic	Seasonal flu	

Antigenic shift vs antigenic drift

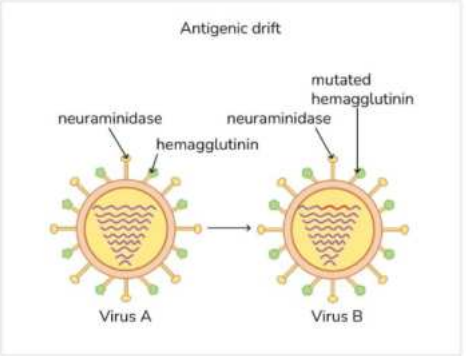
Antigenic shift	Antigenic drift
Sudden change	Gradual change
Exchange of RNA segments between influenza A Genetic reassortment	Mutation in H and N
Causes major epidemics and pandemics	Does not cause epidemics or pandemics
Seen in influenza A	Seen in influenza A and B

Antigenic shift



- Different type of influenza virus → Infect the same organism
- For example - pig
- Inside the pig → There is genetic reassortment → New hybrid will be formed
- Most common mixing vessels are pigs

Antigenic drift



- Minor mutations in hemagglutinin or neuraminidase
- Do not cause epidemic or pandemic

Type specific

- 3 genera based on H and N
- 18 on basis of H
- 11 based on N

Currently circulating strains

- Type A / H1N1 - WHO declared it pandemic on 11th June 2009
- Type A/H3N2
- Type A/ H5N1
- Type B
- There could be up and down between the cases every 10 years or so

Clinical features

- Incubation period - 18-72 hours
- Reservoir - human, animals,birds
- Mode - inhalation of droplets, fomites

Prodrome

- Fever, headache
- sore throat,cough
- Myalgia
- Running nose is usually absent
- Rarely GI symptoms
- Reye syndrome
  - Jamshedpur fever in kids
- Complications - pneumonia
- Most common extrapulmonary symptom - myalgia

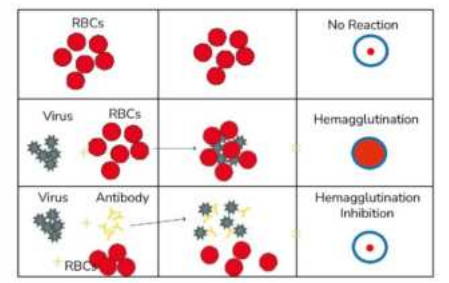
Lab diagnosis

- Specimen - Nasopharyngeal swab
- Swab made of dacron or polyester tips should be used
- Stored in VTM - viral transport medium
- Up to 4 days refrigerate at 4°C
- After 4 days- store at -70°C
  - The same protocol is followed for COVID as well.
- **Isolation**
  - Embryonated egg has
    - → Amniotic cavity
      - Grow influenza virus
    - → Allantoic cavity
      - For Influenza vaccine
- **Cell lines**
  - Primary monkey kidney cell line
- **Hemadsorption**
  - Influenza grown in cell lines
  - If there is growth, and animal RBCs are added - fowl RBCs
  - These RBCs are adsorbed by hemagglutinin
- Antigen detection - DIF
- Antibody detection - HAI, ELISA
- Reverse transcriptase PCR
- Real time PCR
  - PCR - sensitive and specific

ELISA

- Paired sera
- 2 samples taken 10-14 days apart
- ≥4 fold increase in antibody is seen

HAI - Hemagglutination inhibition test



1. If only RBCs are taken - they will form button - no reaction
2. If to the RBCs, influenza virus are added → forms a clump
   - Mat or carpet formation - hemagglutination
3. Now the RBCs and influenza virus and the patient sample is added (supposedly containing antibodies)
   - Now antigen antibody reacts and there are no viruses to attach to RBCs

- So RBCs form button
- This is hemagglutination inhibition

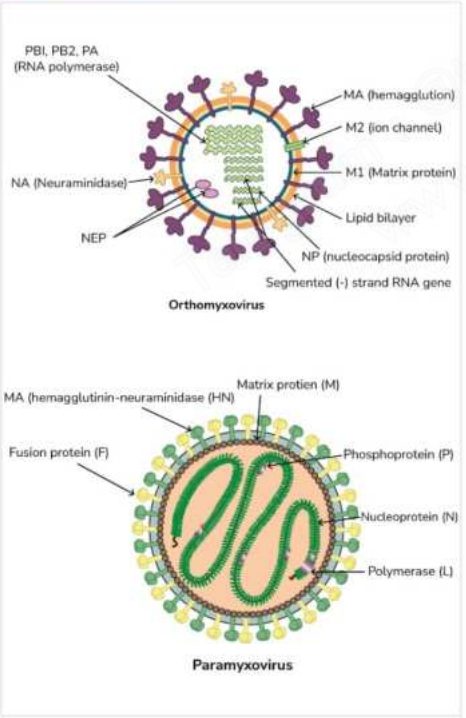
Treatment

- NA inhibitors
  - TAMIFLU / Oseltamivir- Oral route.
  - ZANAMIVIR/ RELENZA - nasal route
- M2 inhibitors
  - Amantadine and Rimantadine
  - Only for influenza A

Vaccine

- **Live nasal spray**
  - Trivalent vaccine - H1N1 (A), H3N2 (A), type B
  - Indicated - 2 - 49 years
  - Not indicated after 50 years
  - Live vaccine - so contraindicated in low immunity
- **Inactivated vaccine**
  - For healthcare workers
  - 2 doses given annually

Paramyxovirus family



- **Orthomyxovirus** - segmented RNA
  - Contains hemagglutinin and neuraminidase
- **Paramyxovirus** - single / unsegmented RNA
  - hemagglutinin and neuraminidase are on the same spike
  - F protein - fusion protein are present

1. Measles
2. Mumps
3. RSV
4. Parainfluenza
5. Nipah virus

Measles virus

- Also known as Rubeola

Important Information

- Measles - Rubeola
- Rubella - German measles

- Incubation period
  - 10 days in children
  - 21 days in adults

Important Information

Incubation periods

- Measles - 10 days
- Mumps - 19 days
- Rubella - 14 days

Transmission

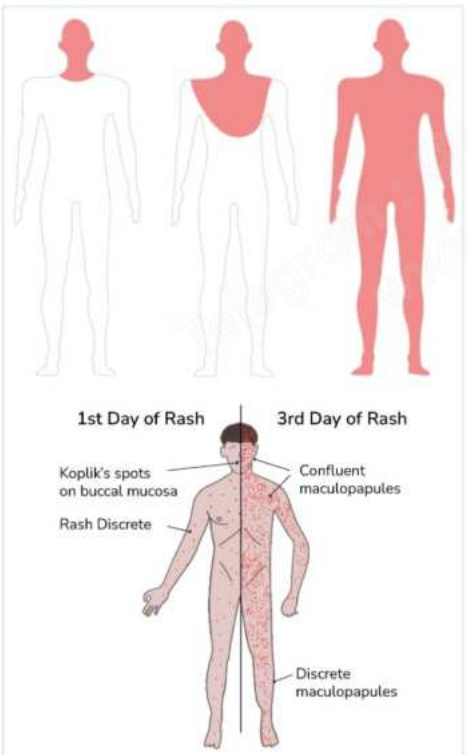
- Inhalation of respiratory droplets
- Source - cases
- No carrier
- Reservoir- humans
- **Period of communicability**
  - 4 days before rash and 4 days after rash
- Lifelong immunity because of single serotype

Prodromal symptoms

- Fever
- 3 Cs
  - Cough
  - Coryza
  - Conjunctivitis
- First manifestation - fever - 10th day
- Pathognomonic - Koplik spots - 12th day
- Rash - 14th day

- Near molars
- 2-3 days after fever
- Rash develops 2-3 days after Koplik spots
- White coloured dots on erythematous base

Rash



- Start from behind the ear
- To the face
- Follows rest of body
- Disappears in the same order
- Fever and Koplik spots disappear with the appearance of rash

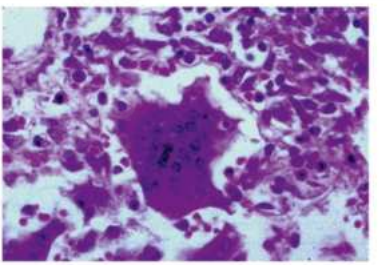
Reason for rash - theories

- Antibody mediated vasculitis
- T cell mediated damage

Complications

1. **Secondary bacterial infections**
- Otitis media
- Bronchopulmonary Pneumonia - due to bacteria

2. **Due to measles**
- GI - diarrhea
- Hecht's pneumonia - giant cell pneumonia - due to measles virus



- Warthin finkeldey giant cells
- Intracytoplasmic and intranuclear inclusions

3. **CNS manifestations**
- **Post measles encephalitis**
  - Antibody against myelin basic protein
- **SSPE - Subacute Sclerosing Panencephalitis**
  - After many years after measles
  - 7-13 years
  - But once it occurs, within 1-3 years, it becomes fatal
  - Deterioration of motor and cognitive functions
  - EEG - burst pattern

4. **In pregnancy**
- IUD

Lab diagnosis

- Sample - nasopharyngeal

- Cell line - Vero cell line
  - Vero - continuous cell line
  - Derived from african monkey
- Reverse transcriptase PCR
- **Cytopathic effect**
  - Warthin finkeldey giant cell
  - Intranuclear and intracytoplasmic inclusions

Vaccine
- **Strains**
  - Measles
  - MeaZles - Edmonston Zagreb Strain
- **Side effect**
  - Mild Measles like disease
  - **Toxic shock syndrome**
    → If measles vaccine vial gets contaminated with Staph.aureus

Mumps
- Source - cases
- Reservoir- humans
- Immunity - lifelong
- **Age** - 5-9 years age group

- **Incubation period** - 19 days
- **Transmission** - droplets / saliva
- Seen in winter and spring seasons
- **Period of communicability**
  - 1 week before to 1 week after the symptoms

Clinical features
- Most common - inapparent
- Most common manifestation - bilateral parotitis
- Second most common manifestation - unilateral orchitis
  - Does not lead to sterility
- Oophoritis in female
- Pancreatitis
- Aseptic meningitis
- Atypical mumps - directly meningitis
  - Without parotid involvement

- Mumps virus → multiply in the lungs → spin into blood and different organs
- One of the organ - parotid gland
- Pain increase on consuming citrus juices
- Bilateral parotid gland
  - Child - mumps
  - Adult smoker - warthin tumor

Diagnosis
- Antigen
- Antibody - ELISA
- RT PCR
- Culture / cell line

Vaccine
- Recommended Strains for mumps - Jeryl Lynn Strain

Important Information
- Strains
  - Measles - Edmonston Zagreb Strain
  - Mumps - Jeryl Lynn Strain
  - Rubella - RA 27/3

- MMR
- MMR-V - varicella

RSV - Respiratory syncytial virus
- RSV - A - more severe
- Rhinorrhea
- Microscopy - giant cell

Epidemiology
- Season - rainy
- Age group - 6w to 6 months
- Mode - inhalation, fomites
- Subgroups - RSV-A and RSV-B

Clinical features
- In infants - most common cause of LRTI
  - Fever
  - Rhinorrhea
  - Cough
  - Dyspnea
  - Wheezing
- In adults - causes URTI

Diagnosis
- Antigen
- Antibody
- RT PCR

- **Virus isolation**
  - HeLA - continuous cell line
  - Hep 2 - continuous cell line
- **Cytopathic effect**
  - Syncytium formation - Multinucleated giant cell
  - Cell with lots of nuclei

Treatment

- Newer drug - Palivizumab
  - Against fusion protein
- For severe infection - Ribavirin

Parainfluenza

- Winter season
- **Causes**
  - Most common - common cold
  - Child -Croup or laryngotracheal bronchitis
  - Severe cases - pneumonia
  - Most common complication - otitis media

NIPAH Virus

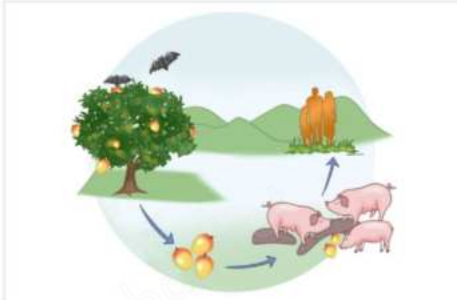
- Epidemiology - 2018 Kerala
- **NiPAH**
- N - Nervous system affected
  - 60-70 % mortality
- P - pigs are amplifiers
  - Fruit bats involved in life cycle
- A → H
  - Animal to humans

Clinical features

- Encephalitis
- Fever

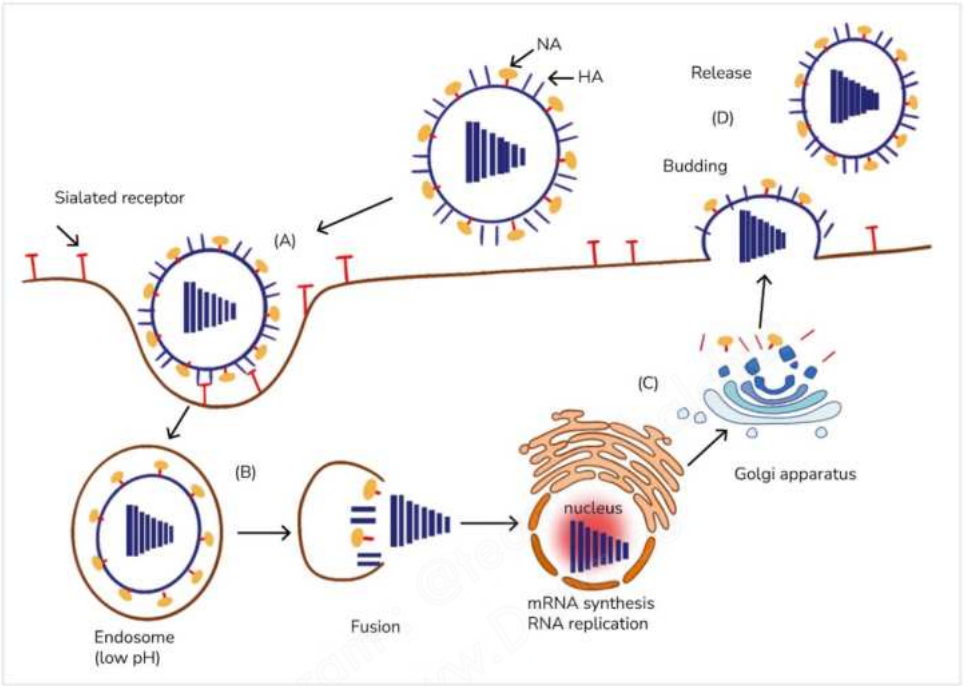
Diagnosis: RT PCR

Life cycle



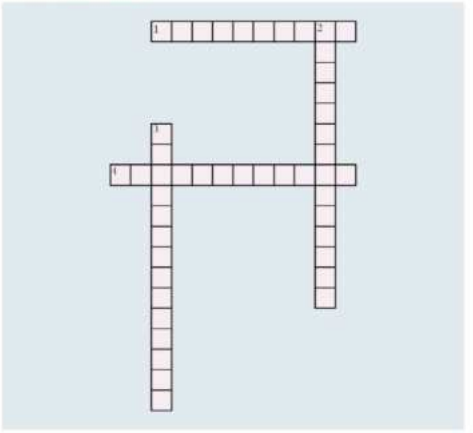
- Vector - fruit bat
- **Transmission**- bat's urine contaminating food and water
- Patient secretions

Image 26.1


NA
HA
Release
(D)
Budding
Sialated receptor
(A)
(C)
Golgi apparatus
(B)
nucleus
Endosome
(low pH)
Fusion
mRNA synthesis
RNA replication

CROSS WORD PUZZLES

Crossword Puzzle



Across

1. Non- pathogenic
4. Hemagglutinin attaches to sialyl acid receptor

Down

2. Do not cause epidemic or pandemic
3. Influenza A,B,C

27 RNA VIRUS : PICORNAVIRIDAE

Picornaviridae

- Smallest among RNA virus family (27-30 nm)
- 2 groups:
  - Enterovirus (Feco-oral transmission): polio, coxsackie, enterovirus, ECHOvirus
  - Rhinovirus (Inhalation)

Polio Virus

- Antigenic types are 1,2,3
- Type specific immunity

Type 1	Type 2	Type 3
Epidemic (most common cause)	Most antigenic No case since 1999	No case since 2013
	a/w VDPV (Vaccine derived polio virus)	a/w VAPP (Vaccine associated paralytic Polio)

- **Dense antigen:**
  - a/w whole virion
  - Type specific
  - Anti D antibodies are protective
  - Potency of injectable polio vaccine is measured using D antigen Unit
- **C antigen**
  - Non protective
- IP: 1-2 weeks
- Transmitted- Primarily Feco-oral or can be by inhalation
- Reservoir-Humans
- Communicability period is 1 week before and 2-3 after onset of signs & symptoms
- Carrier- None

Refer Image 27.1

Tonsillectomy should be avoided because virus can spread via glossopharyngeal nerve of tonsillar fossa

GI tract
↓
Lymphatics
↓
Blood (Viremia)
↓
8% "Abortive polio" fever/ headache / malaise / vomiting
CNS
↓
<1% "Non-paralytic polio virus in CSF, symptoms of meningitis"

- Motor neurons is the final site
- There is Nissl body degeneration (earliest change)
- There is a asymmetric descending flaccid Paralysis (ADFP):
  - Proximal > distal muscle
  - Leg muscle > arm muscle
  - Bigger hand muscle > smaller hand muscles
  - **Respiratory failure**
  - **Meningoencephalitis**
- **Diagnosis:**
  - Sample- Throat
  - Rectal / fecal swabs
  - Isolation- rare
  - Regular testing of sewage sample
  - **Transport**: specimen needs to be kept frozen
  - Cell line - Primary monkey kidney cell line
  - CPE (Cytopathic effect)- Shrinkage & degeneration of cell lines
  - Antibody detection- paired sera for 2 weeks with increasing titre
  - Molecular methods-
    → Real time Multiplex RT PCR
    → VP1 protein
    → Can differentiate between wild and vaccine strains

Coxsackie

- Coxsackie A causing Flaccid paralysis
- Coxsackie B causing Spastic paralysis
- Mode -Feco- oral
- Host - Human

Coxsackie A	Features	Coxsackie B
1-24	**Serotypes**	1-6
• Herpangina • Hand foot mouth disease • Acute Hemorrhagic conjunctivitis (coxsackie A24 or Enterovirus 70)		• Carditis • Bornholms disease • Pleurodynia / Devil's grip / Epidemic Myalgia • Hand foot mouth disease

- **Herpangina**: Vesicular Pharyngitis
- **HFMD**: Child with affected hand, foot, mouth (papulovesicular Lesions seen)
- **Carditis**- Dilated Cardiomyopathy
- **Pleurodynia**- infection of intercostal muscle resulting in Pain

Enterovirus

- 70- Acute Hemorrhagic Conjunctivitis
- 71- Hand foot mouth disease
- 72- Hepatitis A virus

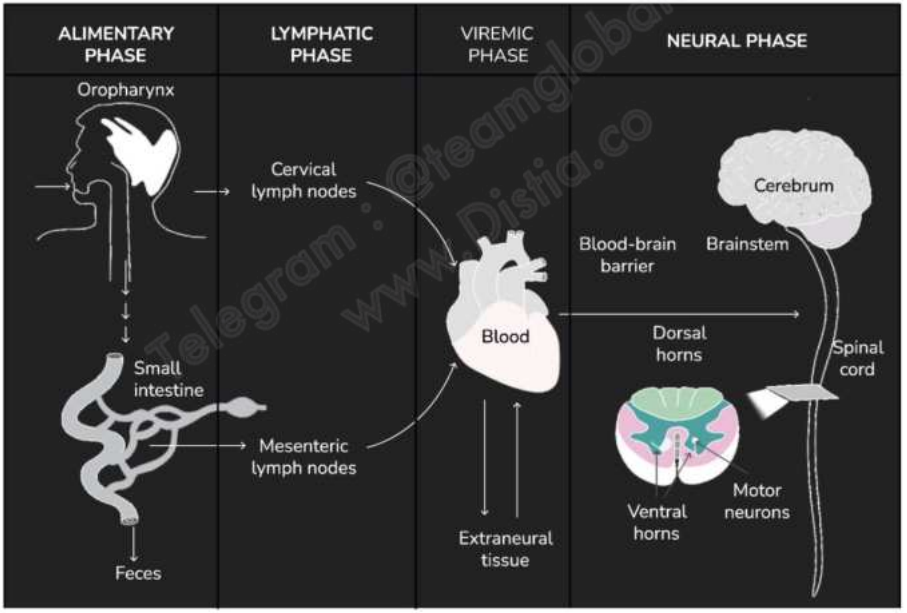
ECHO virus

- Enteric Cytopathogenic human orphan
- Serotypes 1-33 except 10,22,23,28
- Causes-
  - Aseptic Meningitis
  - Encephalitis
  - Common cold
  - Rash

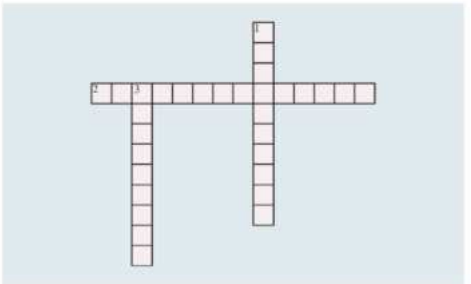
Rhinovirus

- Acid Labile
- 100 serotypes
- Immunity is type specific
- Inhalation mode
- Incubation period is 2-4 days
- Optimal temperature is 33° for growth
- Nasal igA (mucosal ab) gives immunity
- ICAM1 Receptor
  - Site of attachment of Rhinovirus
  - Site of attachment of PF in brain (cerebral malaria)
  - Site of attachment of Beta-2 integrin
- **C/f-** Common cold
- **Nasal IgA (mucosal Ab) gives immunity**

Image 27.1



Crossword Puzzzle



Across

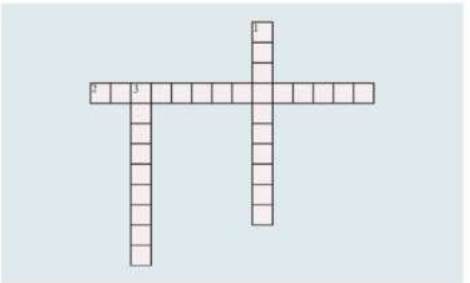
2. Smallest among RNA virus family (27-30 nm)

Down

1. Antigenic types are 1,2,3
3. Human host

CROSS WORD PUZZLES

Crossword Puzzzle



Across

2. Smallest among RNA virus family (27-30 nm)

Down

1. Antigenic types are 1,2,3

3. Human host

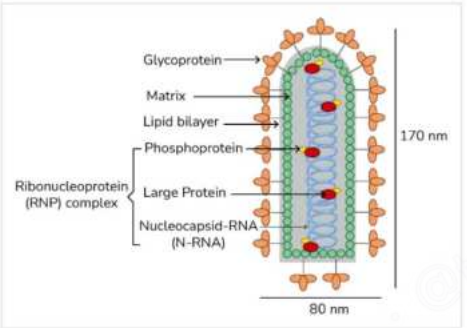
28 RNA VIRUS : RHABDOVIRIDAE

Rhabdoviridae family

- 2 genus
  - Lyssavirus
    → Rabies
  - Vesiculo virus
    → Vesicular stomatitis virus

Rabies

- 100 % fatal



- Electron microscopy- bullet shaped virus
  - Bullet kills, rabies also kills
- Enveloped virus
- Negative sense ssRNA

Important Information

Negative sense
MR FAB- negative sense

- M - myxovirus
- R - rhabdoviridae

Antigens

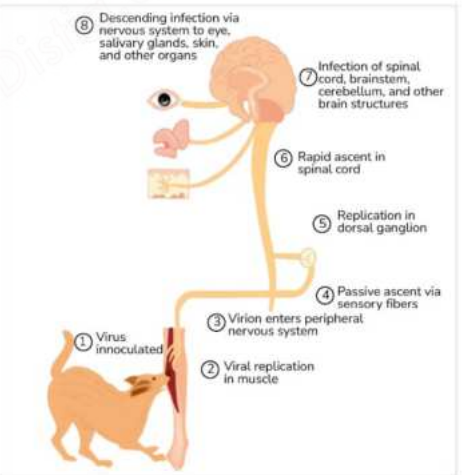
- Glycoproteins
  - Glycoprotein spikes help in pathogenesis and neuro invasiveness.
- Ribonucleo capsid

Glycoprotein G	Nucleoprotein
Present in envelope	Associated with viral RNA
Species specific	Group specific
Binds to acetylcholine receptor and induces neutralizing antibody	No role in binding and inducing antibody
Play role in vaccination	Cross react with other rabies related virus

Types

Street virus	Fixed virus
-	Produced when rabies is grown repeatedly in brain of rabbits
Furious rabies	Dumb rabies
-	Used in Vaccine preparation
Negri bodies seen	Not seen

Pathogenesis



- Animal - dog/ cat/ monkey/sheep/ goat/ bat
- Spread
  - Through bite
  - Lick on abrasion
  - Inhalation from infected Bat aerosol
  - Corneal transplantation

↓

In the muscle, virus replicates

↓

Virus binds to acetylcholine receptor

↓

Shows retrograde movement- centripetally

↓

Reaches spinal cord - dorsal root ganglion and brain

↓

In the brain, negri bodies are formed

↓

It can then spread centrifugally and go into

a. Cornea
b. Glands - salivary glands
   i. Submaxillary salivary glands - commonly affected
c. Skin

Clinical features

- Spread of rabies virus progress in axon at the speed of 3 mm/hour
- Time taken for Rabies to reach brain is different for different people
- Shorter for
  - Shorter height
  - Upper limb bite
  - Children

Clinical spectrum

- Short prodromal phase - 2-5 days
- Acute neurologic phase - 2-7 days
- Coma and death - 100 % fatal

Neurologic phase

- **Furious rabies/ encephalitis rabies**
  - 80 % patients
  - Encephalitis - Hyperexcitability
  - Phase of lucidity - slight improval
  - Stage of autonomic disturbances

  → Hypersalivation
  → Gooseflex
  → Arrhythmia
  → Hydrophobia
- **Dump paralytic rabies**
  - Paralytic symptoms present
  - Seen in

    → Partially vaccinated
    → Rat bite
  - Cardiac symptoms are absent

Lab diagnosis

- **Antigen detection**
  - Best specimen - from nape of neck

    → At least 10 hair follicles are taken
  - In later stages

    → Corneal impression smear
- Viral RNA - reverse transcriptase PCR - Most sensitive and specific assay
- **Viral isolation**

- **Animal inoculation**
  - Inoculated into Brain of suckling mice
  - Negri bodies

    → Intracytoplasmic bodies

- **Cell lines**
  - Chick embryo cell line - primary cell line
  - BHK cell line - continuous
  - WI38 - secondary

- **Antibody detection**
  - **CSF, blood**

    → Blood antibody appear late
    → Present in vaccinated persons also
    → CSF antibodies are more valuable
    → They are not seen in vaccinated people
  - **MNT** - Mouse Neutralization Test
  - **RFFIT** - Rapid Fluorescent Focus Inhibition Test
  - **FAVN** - Fluorescent Antibody Virus Neutralization test
  - **IFA** - Indirect Fluorescent Assay
  - **HAI** - Hemagglutination Inhibition
  - **CFT** - Complement Fixation Test

- **Autopsy**
  - If person dies, autopsy shows negri bodies in brain
  - Special stain - seller's stain
  - Immunoperoxidase stain

Treatment of dog bite

Categorization

1. Category I

- Intact skin
- Touching or feeding rabid animal
- Wound toilet only needed

2. Category II

- Minor scratches
- Abrasions without bleeding
- Wound toilet
- Vaccine needs to be given

3. Category III

- Transdermal scratch / bite

- Licks on abraded skin/ mucus
- Exposure to wild animals
- Wound toilet
- Vaccine
- Immunoglobulin

Wound toilet
- Wash with soap and water
- Duration - 15 to 20 minutes
- After that Topical iodine - povidone iodine is applied

Vaccine
- Type - now used human diploid cell culture vaccine
- Site
  - Adult - deltoid
  - Child - anterolateral thigh

Vaccine Regimes
- **IM- Intramuscular - Essen Regime**
  - 5 letters - 5 visits
  - 0, 3, 7, 14, 28
  - Total of 5 doses
- **ID - Intradermal - Thai red Regime**
  - Thai - 4 letters - 4 visits
  - 0, 3, 7, 28
  - On every visit - 2 doses are given
  - 8 doses are in total

Pre-exposure prophylaxis
- Veterinary worker
- Forest worker
- 3 visits
- 3 doses
- 0, 7, 21/28
- Can be given IM/ID

Re-exposure prophylaxis
- 2 days
- One dose each
- 0, 3
- Within 3 months of post exposure prophylaxis
  - Wound toilet only done
- After 3 months
  - Re exposure prophylaxis is given

Immunoglobulin
- For Category III
- **Sources**
  - Horse - equine
    → 40 IU/ kg
  - Human - lesser side effects
    → 20 IU/kg
- Infiltrated in or around the wound (maximum possible)

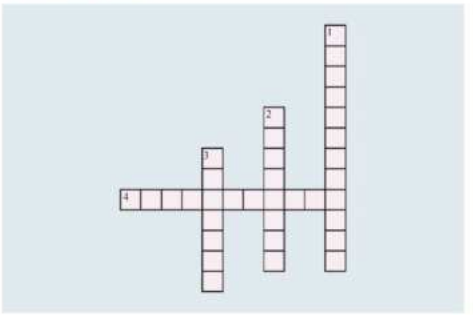
Vesiculo virus
- Vesicular stomatitis virus
- Vector - sandfly
- Reservoir - domestic animals
- Vesicles in and around the mouth

Important Information

Sandfly as a vector in
- Leishmania or kala azar
- Bartonella bacilliformis
  - bacilliFORmiS
  - OR - Oroya fever
  - FS - sandfly
- Vesicular stomatitis virus

CROSS WORD PUZZLES

Crossword Puzzle



Across

4. IM- Intramuscular

Down

1. Woundtoilet
2. Glycoproteins
3. Vesiculo virus

29 RNA VIRUS : ARBOVIRIDAE

ARB - Arthropod Borne

- 5 viruses
  - **Bunyaviridae**
  - **Togavirus (Mnemonic:** TONGA CAR, Horse equine)
    - → Rubella virus
    - → Chikungunya virus
    - → Eastern equine encephalitis
    - → Western equine encephalitis
  - **Flavivirus**
    - → Japanese encephalitis
    - → Yellow fever
    - → Zika
    - → Dengue
    - → West Nile fever
    - → Hepatitis C
  - **Reoviridae:** Colorado tick fever
  - **Rhabdoviridae:** Vesicular stomatitis virus (sandfly)

Bunyaviridae

- Bunya - 3 segments
- **Consists**
  - Bunyavirus - California encephalitis
  - Phlebovirus
  - → Sandfly fever
  - → Rift valley fever
  - Nairovirus
    - → Crimiam congo hemorrhagic fever - Gujarat
  - Hantavirus (rodents)
    - → Hantan renal syndrome
    - → Hanta pulmonary syndrome

Bunyavirus

- Disease - California Encephalitis
- Bunyavirus transmitted by Aedes mosquito

Phlebovirus

- Sandfly fever - Sandfly
- Rift valley fever - Aedes (Fever, Retinitis, Encephalitis)

Nairovirus

- Crimiam congo hemorrhagic fever - Gujarat
- Transmitted by Hard tick
- BSL IV
- Animal ⇌ Human

Hantavirus (rodents)

- Hantan renal syndrome - Interstitial nephritis
- Hanta pulmonary syndrome - Interstitial pneumonia
- Both are associated with Hemorrhages and Rodents

Togavirus

- Eastern equine encephalitis - Aedes
  - 50-70%
  - Seen in horses
- Western equine encephalitis - Culex
  - Seen in horses

Important Information

Incubation Periods of Diseases

- Dengue (5-6 days)
- Chikungunya (5-6 days)
- Japanese Encephalitis (5-15 days, 10 as median)
- Yellow fever (3-6 days)
- Kyasanur Forest Disease (4-8 days)

Chikungunya

- **Vector:** Aedes
- Fever (Saddle back fever-Biphasic fever)+Migratory arthritis (Small joints - Arthralgia)
- **IP:** 5-6 days
- Chik sign +ve (post chikungunya)

Chik sign

- Lymphadenopathy is also seen
- **Diagnosis**
  - Antibody
    - → Early - IgM (4 days - 2 months)
      - MAC ELISA
    - → Late - IgG (2 weeks - years)
  - RT PCR

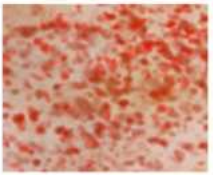
Rubella Virus

- Nowadays it is considered as Matonoviriade
- **Other names**
  - German measles
  - Three day measles

Important Information

- Measles - Rubeola
- German measles - Rubella

- **Mode**
  - Respiratory droplets
  - Trans-placental
- **CF**
  - **Respiratory droplets**
    - → **IP:** 14-21 days
    - → Upper RTI
    - → Posterior auricular Lymphadenopathy
    - → Rash
      - Lasts for 3 days
      - Thus 3 day measles
      - Similar rash



Rash

Important Information

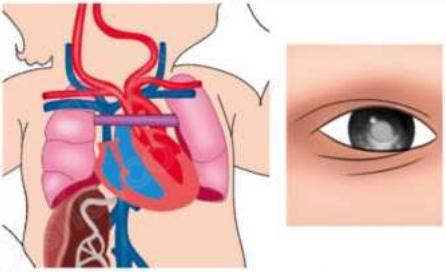
- Rubella
  - Forscheimer's spot on palate
  - Non specific
- Measles
  - Koplik spot near molars
  - Characteristic

  - Transplacental
    - → Teratogenic - Congenital rubella syndrome

Important Information

- Maximum congenital malformations occur in 1st trimester

    - → Patent Ductisartiriosis
    - → Sensorineural hearing loss
    - → Cataract
    - → Blueberry muffin syndrome (non specific)



Blueberry muffin syndrome or baby

- Congenital Rubella syndrome baby excrete the Rubella virus in Urine and Saliva.
- **Diagnosis**
  - Ag and Ab testing
  - Culture
    - → Cell lines
    - → No cell culture
  - RT PCR
  - **For Pregnant lady**
    - → **IgM -** Indicates acute or current infection (dangerous)
    - → **IgG > 1:8 -** Past infection (not dangerous)

- **Vaccine**
  - Live attenuated
  - Contraindicated in immunocompromised individuals.

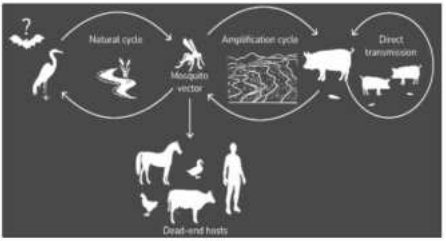
Important Information

- Measles MeasZles - Edmonston Zagreb strain
- Mumps (Kid name Jerry) - Jeryl Lynn strain
- Rubella - RA 27/3

Japanese Encephalitis

- Firstly seen in Gorakhpur, UP
- **Vector**
  - Culex tritaeniorhynchus (most common World)
  - Culex vishnui (most common in India)

- **Transmission cycle**
  - Life cycle



  - Vector: Culex
  - Host
    → Reservoir - Ardeid birds
    → AmPlifier - Pigs
    → Human - Dead end
- **CF**

Prodromal symptoms (Mild)
↓
Acute encephalitis
↓
Long term neurological deficit

- **Lab diagnosis**: RT PCR in CSF
- **Treatment**
  - Supportive measures
  - Symptomatic treatment

- **Vaccines**
  - **Live attenuated**
    → SA 14-14-2
    → Mostly used in India
  - **Inactivated**
    → Nakayama and Beijing P1 strain
    - Mouse brain derived
    - Formalin inactivated
    → Beijing P3 strain
    - Cell line derived

Important Information

- Nagayama spots are seen Roseola infantum.

Yellow Fever

- 7 genotypes
  - Epidemic in west Africa (5)
  - Endemic in south America (2)
- 1 serotype
- **Vector:** Aedes aegypti
- **2 major cycles**
  - **Jungle cycle:** Monkeys and forest mosquito
  - **Urban cycle:** Humans and urban mosquito
- **IP:** 5-7 days
- **CF**
  - **Mild**
    → Fever (fever + bradycardia = Faget's sign)
    → Chills and myalgia
  - **Severe**
    → Hemorrhagic manifestations (platelet dysfunction)
    → Hepatitis (Midzonal necrosis)

Important Information

- Faget's sign also seen in Typhoid fever

- **Indian status**
  - India is free from yellow fever because
    → Unvaccinated travelers coming from endemic zones to India are Quarantined for 6 days.
    → Aedes aegypti index is kept <1 near air and sea ports
- **Diagnosis**
  - Serology
    → IgM - 3 days of onset
    → IgG - Later
  - CPE - Cytopathic effect is seen
    → Liver - Midzonal necrosis
    → Body - Torres body

Important Information

Mnemonic

- Yellow has 6 letter - IP is 5-6 days
- YeLLow-ToRRes (concentrated on center-Midzonal necrosis)

- **Vaccine** - 17D vaccine
  - Live attenuated
  - Prepared from allantoic cavity
  - **Route** - Single dose subcutaneous
  - **Efficacy** - 7 to 35 years
  - Certificate of vaccination is obtained within 10 days and is valid life long
  - **Contraindications**
    → <9 months
    → Pregnancy
    → HIV/ Immunodeficiency
    → Egg allergy

Important Information

- Cholera and Yellow fever vaccines interact with each other, thus should not be given together
- So, there should be 3 weeks gap between both

Monkey Fever/ Kyasanur Forest Disease

- **Vector:** Hard tick
- **Host:** Monkey (amplifier)
- **IP:** 4-8 days (Mnemonic: Forest = Four-Eight)
- **CF**
  - Fever
  - Myalgia
  - Mucosal and cutaneous hemorrhages
- **Life cycle**

Refer Image 29.1

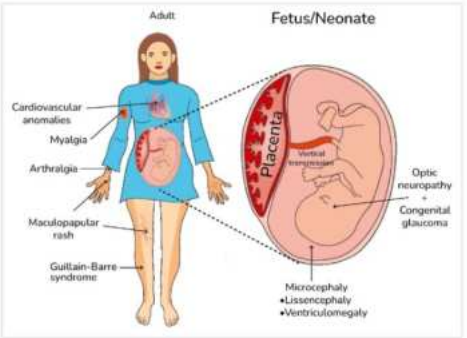
  - Tick bite (common)
  - Contact with animals
  - Person to person not known
- **Epidemiology:** January - June is the main season
- **Diagnosis:** RT PCR
- **Treatment:** Supportive

Zika Flavivirus

- **Vectors:** Aedes

Refer Image 29.2

- **Route and CF**
  - Sexual
    - → Fever, arthralgia, rash
    - → **Cardiac:** CVS defects
    - → **Neural:** GBS (rare)
  - Mother to child



    - → Horribeauer cells of placenta has deposition of Zika virus (PYQ)
    - → **Eyes:** Optic neuropathy and congenital glaucoma
    - → **Brain:** Microcephaly and ventriculomegaly
  - Blood transfusion
- **Diagnosis**
  - RT PCR (blood and urine)
  - IgM antibody testing

Important Information

- PRNT (Plaque Reduction Neutralization Test) is done for confirmation of the Ab for the particular organisms in all flaviviruses.

Dengue Virus

- MC mosquito borne infections
- Most rapidly spreading
- MC Arbovirus found in India
- **Types**
  - 4 serotypes (DENI-DEN 4)
  - Most dangerous is DEN 2
  - DEN 5 is recently reported in Bangkok
- **Vector:** Aedes

Important Information

- Type specific (Single serotype Ab can only protect to that serotype in future)
- Can be at a risk of ADE (Antibody Dependent Enhancement)
  - **Other names:** Original antigenic sin/ Immune enhancement hypothesis

    Primary dengue infection
    ↓
    Ab formed for primary infection serotype
    ↓
    Secondary dengue infection (more severe)
  - **Reason:** Ab from 1st time will bound with the 2nd time infected serotype

    ↓

    More severe (thus sin)
  - Transovarial transmission is seen in Aedes (Aedes gives it to Offsprings)

- **CF**
  - **Dengue fever**
    - → High fever - Break bone fever/ saddleback fever
    - → Watery eyes
    - → Frontal headache
    - → Rashes (few) – Maculopapular

- Upper limbs

→ Muscle and joint pains

- **Dengue hemorrhagic fever**
  - → All features of Dengue fever
  - → Platelet count <1 lac
  - → Platelet functional qualitative defects
  - → +ve tourniquet test (>20 spots/inch)
  - → HCT/ packed cell volume increased by 20%
    - Normal 35% in females
    - Normal 45% in males
- **Dengue shock syndrome**
  - → Dengue fever + Dengue hemorrhagic fever
  - → Endothelial damage
  - → Shock features are seen

WHO Grading Dengue Infections

Refer Table 29.3

- **Lab diagnosis**
  - **PCR:** +ve from day 1 to 7
  - NS1 antigen
  - IgM
  - Mac ELISA
- **Treatment**
  - Supportive
  - Platelet transfusion SDAP
  - Fluids (to correct hypovolemia)
- **Vaccine:** Dengraxia (DEN1-4)

AEDES

- Aedes aegypti
- Aedes albopictus
  - Tiger mosquito
  - Day time biters
  - Peak: down and dusk
  - Cigar shaped eggs
  - Transovarial transmission in mosquito

CULEX

- JE
- Arthritis like features
- West nile fever

Bancroftian filariasis

Image 29.1

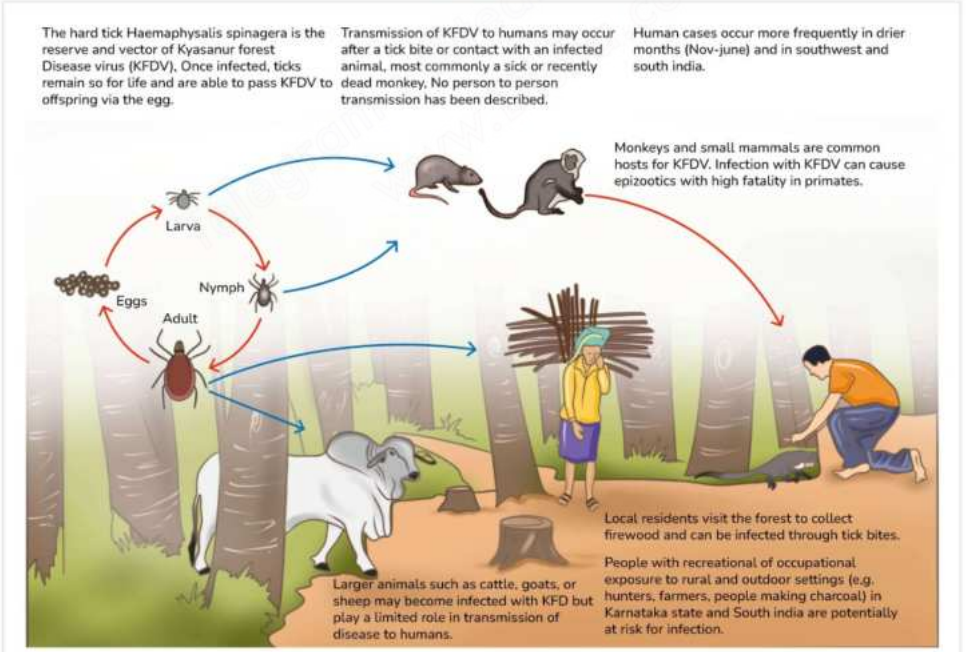


Image 29.2

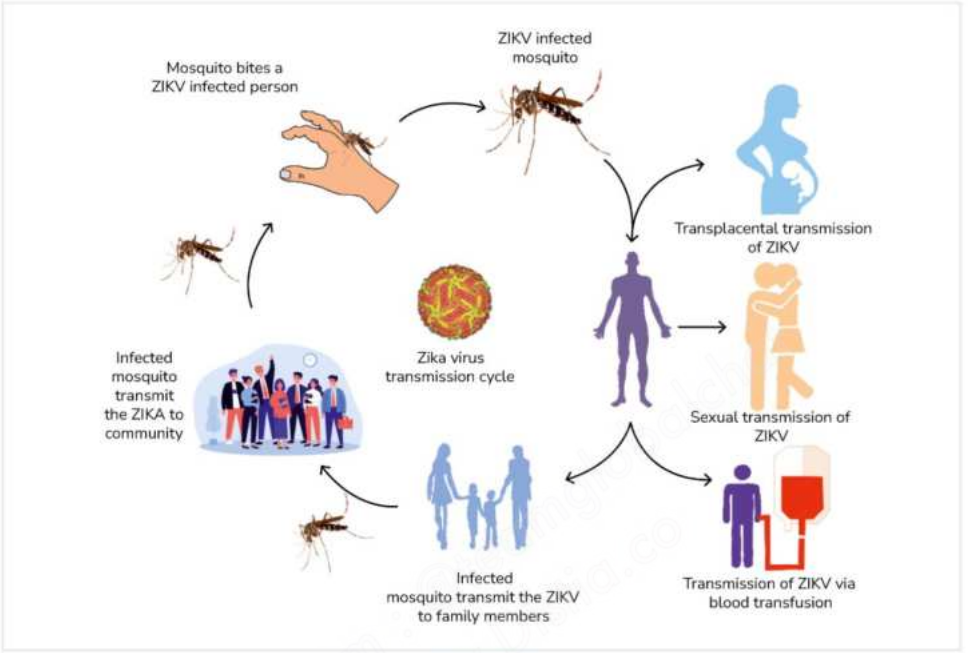
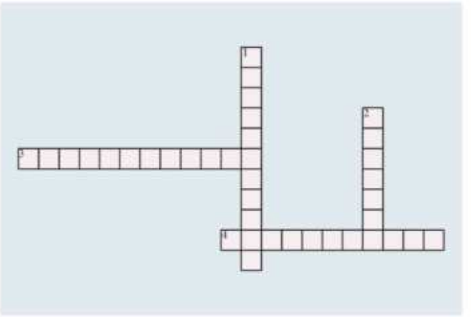


Table 29.3

DF/DHF	Grade	Symptoms	Labs
DF		• Fever with two or more the following features ○ Headache ○ Retro orbital pain ○ Myalgia ○ Arthralgia	• Leukopenia • Thrombocytopenia (100000) • No evidence of plasma loss
DHF	**I**	• Above signs plus positive tourniquet test	• Thrombocytopenia (< 100000) • HCT rises (>20%)
DHF	**II**	• Above signs plus Spontaneous bleeding	• Thrombocytopenia (< 100000) • HCT rises (>20%)
DHF	**III**	• Above sign plus circulatory failure (weak pulse, Hypotension restlessness)	• Thrombocytopenia (< 100000) • HCT rises >20%
DSS	**IV**	• Profound shock with undetectable blood pressure and pulse	• Thrombocytopenia (< 100000) • HCT rises (>20%)

Crossword Puzzle



Across

3. Japanese Encephalitis
4. Aedes aegypti

Down

1. Rashes (few) - Maculopapular
2. Live attenuated

30 RNA VIRUS : RETROVIRIDAE

Retroviridae

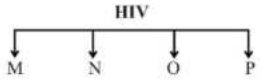
- Oncoviridae – HTLV 1, 2
- Lentiviridae – HIV 1, 2
  - HIV 1 >> HIV 2 (M/C in India)

HIV

- Reverse Transcriptase activity(seen in HIV and Hep b)
  - 100 – 120 nm
- Unsegmented ss RNA
  - 2 copies
  - ReTWO virus (pneumonic)

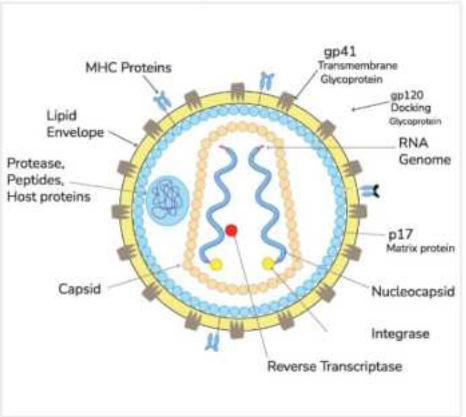
HIV SEROTYPING

- Sequence difference in Envelope (env) protein



- Serotype M is the Most common
- 10 subtypes [A-K]
- Circulating recombinant forms (CRF) CRF-AE can also be seen
  - HIV I Group M subtype B → most common worldwide
  - HIV I Group M subtype C → most common in India

Structure



Structural Genes

- HIV – 1 mature virion

Refer Image 30.1

- Envelope: gP 160
  - Surface: gP 120
  - Transmembrane: gP 41
- Gag
  - P6, P7, P17, P24
  - P24 is the most important [Needle stick injury]
- Pol
  - Reverse transcriptase: P 66

Non-Structural Genes

- Tat [transcriptional transactivator] – Speed up transcription
- Nef [Negative factor gene]
- Rev [Regulator of Virus]
- Vif [Viral infectivity factor]
- Vpu/ vpx (II) → unload/release from cell
- Vpr → transport from cytoplasm to nucleus
- LTR → Long Terminal Repeat (promotor & enhancer regions)

Pathogenesis:

- Attachment: mediated by envelope proteins P120 & GP41
  - Attachment to CD4/macrophages
  - Attachment to coreceptors

Coreceptors

- HIV strain
- R4 Strains
  - CXCR4 – on CD4 cells (4-4)
- R5 Strains
  - CCR5 – on macrophage (5 – phage)
  - CCR5 DELTA 32 MUTATIONS – no CCR5 – no entry

Refer Image 30.2

- Attachment (gP 120, 41) m CD 4 CCRS, C×CR4
- Endocytosis
- Uncoating
- RNA to DNA(by reverse transcriptase))
- Transport to nucleus (Vpr)
- Integration (Viral DNA & Host DNA)
- New virions release (By protease)

Transmission

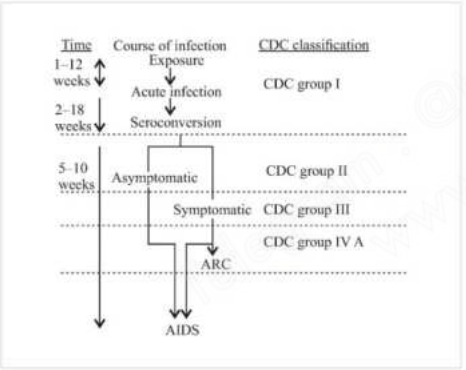
- Sexual (0.1-1%)

- Homosexual routes
- Heterosexual routes
- Unprotected receptor anal intercourse 1:30
- Unprotected vaginal intercourse 1:10000
- Blood transfusion (>90%)
  - Not commonly seen because of testing Done in blood bank
- Needle stick Injury
- Mother to child → Vertical (30%)
- Needle stick 1:300
- Needle sharing 1:150

Chances of getting HIV, Hep C, Hep B with Needle stick Injury

- HIV – 0.3%
- Hep (B) 30%
- HepC – 3%
- Hep (C) 3%
- HepB – 30% [only HBsAg]
- H (I) 0.3%

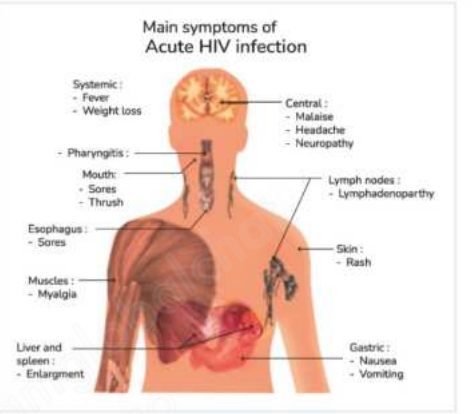
Clinical Features (CPC classification)



- CPC group I :
  - Initial infection (acute HIV syndrome)
  - Wide dissemination of virus
  - Seeding of lymphoid organs
- CPC group II
  - "CLINICAL LATENCY" not a microbiological latency
  - Asymptomatic
  - SEROCONVERSION(improvement of CD4 count)
- CDC group III
  - PGL (Persistent Generalised lymphadenopathy)
  - Enlarged LN
    → ≥2 non-contiguous extrainguinal sites
    → ≥3 months
- CDC group IV
  - ARC
  - AIDS Related Complex

Refer Image 30.3

Acute HIV Syndrome



CD4 count	Opportunistic infection
<500	Tuberculosis, Bacterial pneumonia, Herpes zoster, oropharyngeal, Candidiasis, non-Typhoid Salmonellosis, Kaposi sarcoma, Non- Hodgkin lymphoma
<200	Pneumocystis Jiroveci Pneumonia, Herpes Simplex Ulcer, Oesophogeal Candidiasis, Isospora Bell diarrhea, HIV wasting syndrome, HIV Associated dementia
<100	Cerebral Toxoplasmosis, Cryptococcal meningitis, Cryptosporidiosis, Microspordiosis, CMV, MAC, PMLE
<50	CMV, MAC, Toxoplasma gondi

CDC AIDS case definition

- Candidia – trachea esophagus bronchus
- Cryptococcus – meningitis
- Cryptosporidium – chronic diarrhoes >1 month
- CMV colitis –
- Cerebral toxoplasmosis – seizures
- CNS
- PMLE
- PCP CD <200
- HSV – punched out

- Kaposi
- MAC CD < 50
- Candida – trachea, esophagus, bronchus
- Cryptococcus – meningitis
- Cryptosporidium – chronic diarrhea > 1 month
- CMV colitis – serpiginous ulcers
- Cerebral toxoplasmosis – seizures
- CNS lymphoma – DUBCL
- PML
- PCP CD <200
- HSV – Punched out
- Kaposi sarcoma (HHV8)
- MAC CD 4<50

WHO AIDS definition

At least 2 major signs in combination with at least 1 minor sign

- Major Signs
  - Weight loss of at least 10% of body weight
  - Chronic diarrhoea for > 1 month
  - Prolonged fever for >1 month
- Minor signs
  - Persistent cough for > 1 month
  - Generalized pruritic dermatitis
  - History of herpes zoster
  - Oropharyngeal candidiasis
  - Chronic progressive or disseminated herpes virus infection
  - Generalized lymphadenopathy(PGL)

Important questions

Q. Most common opportunistic infection
Ans. TB

Q. Most common fungal infection
Ans. Candida

Q. Most common space occupying tumor
Ans. CNS Lymphoma

Q. Most common Glomerulonephritis
Ans. FSGS (HIV Associated nephropathy)

Q. Most common NHL
Ans. DLBCL

Q. Most common Hepatitis in HIV
Ans. Hepatitis B

Q. Most common skin symptom in HIV
Ans. Seborrheic Dermatitis

Q. Most common Endocrine symptom
Ans. Lipodystrophy

TB with HIV

- Treat TB first
  - Start patient on ATT → ART
- Immune reconstitution inflammatory syndrome [IRIS]
  - To present IRIS ATT is given first

Lung Infection In HIV

- Pneumococcus – lobe consolidation (CD4<500)
- TB – snow storm / hazy pattern(CD4<500)
- PCP – perihilar opacities (CD4<200)

Diagnosis

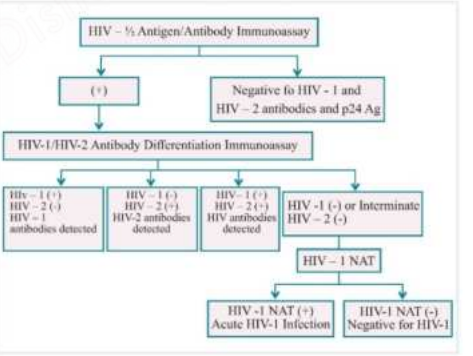
Testing

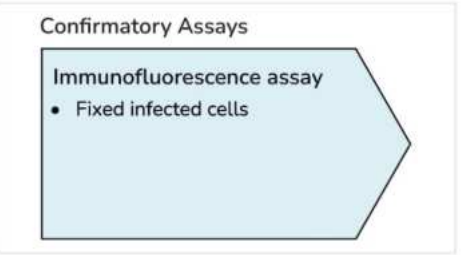
- CD4 COUNT
- Sensitive- ELISA 4th generation EIA

Refer Image 30.4

- WESTERN BLOT(confirmatory)
- RT PCR (even 40 copies detected)
- Paediatric HIV- DNA PCR

CDC Guidelines





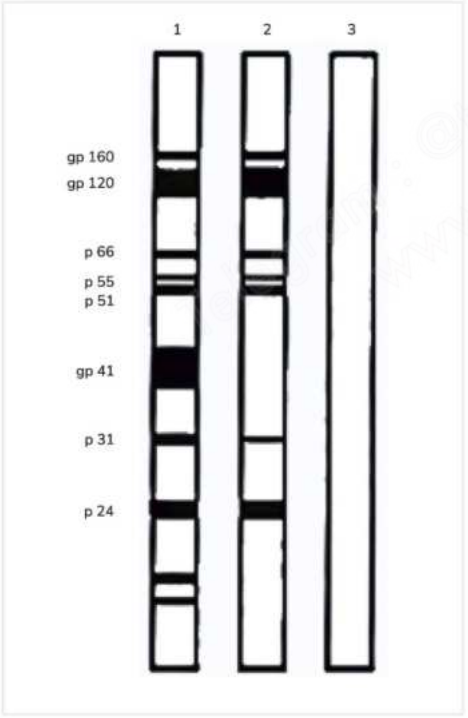
HIV-1 Western blot

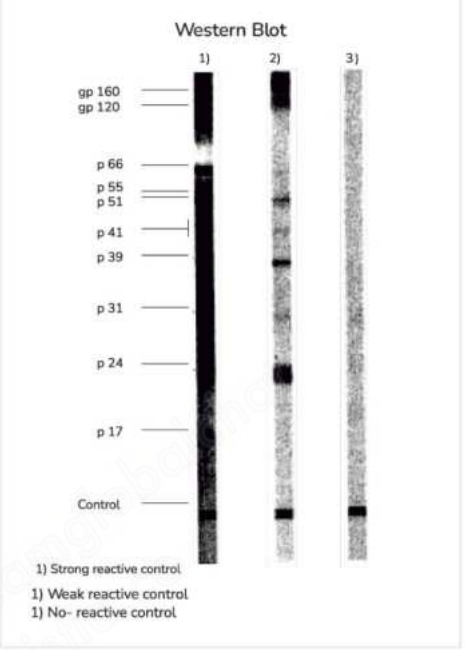
- Very specific
- Nitrocellulose strips with separated viral proteins
- Specific banding pattern indicative of HIV infection

Line or dot Immunoassays

- Recombinant peptides or protiens striped on membrane
- Include HIV-2 peptides
- Examples - InnoLIA, Multi-spot, Geenius

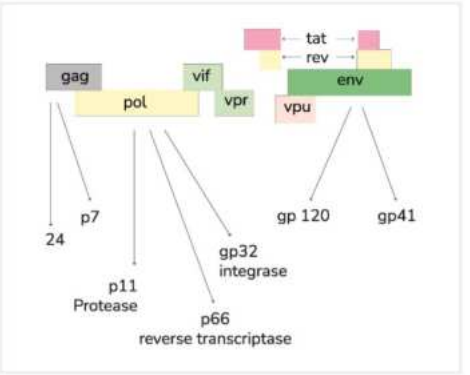
Western Blot





Western Blot Immunoblot Technique Antibodies Detected Against

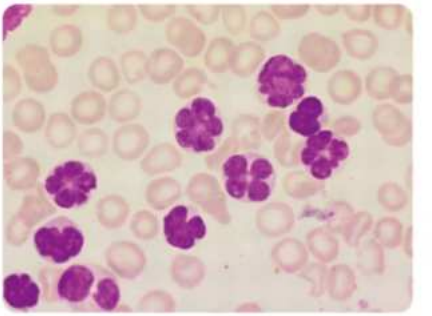
- WHO
  - Antibodies against 2 env bands With or without gag/pol bands
- CDC
  - Presence of any 2 bands (P24, gp160, gp120, gp41)



Serology

- HIV RNA
- p24

Refer Image 30.5



HTLV–1

- HTLV → **(Human T cell leukemia lymphoma virus)**
  ↓
  Tax gene
- Causes
  - Adult T cell Leukemia lymphoma (ATLL)
  - Tropical spastic paresis
- Microscopically
  - Clover leaf cells
- Mode of transmission
  - Blood
  - Body fluids (Breast milk)

Image 30.1

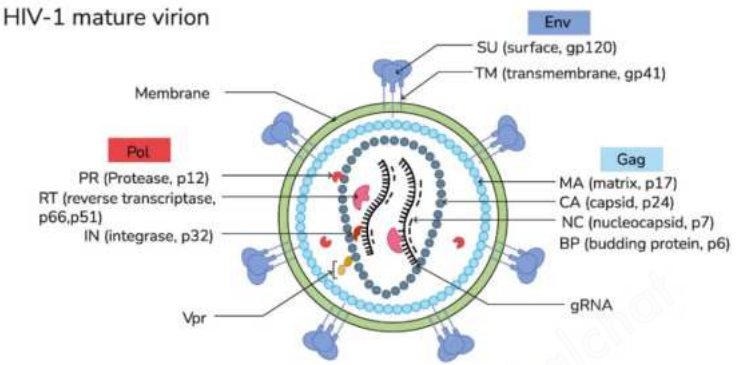


Image 30.2

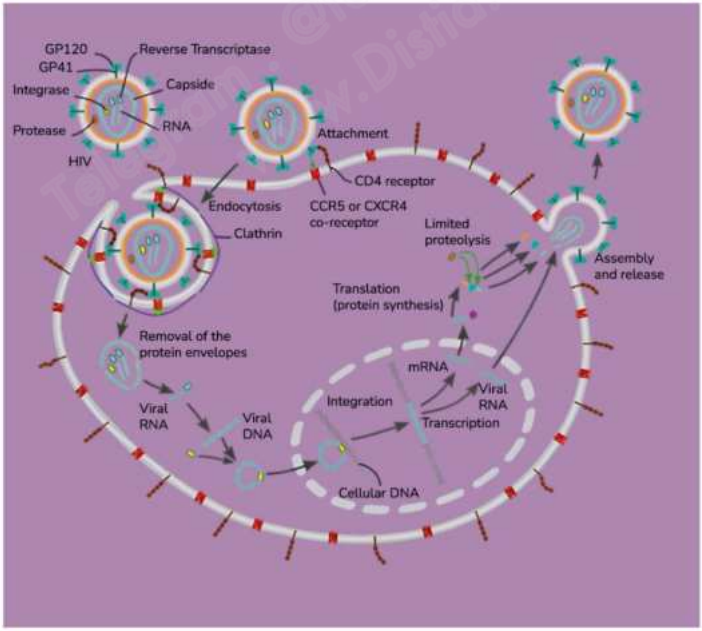


Image 30.3

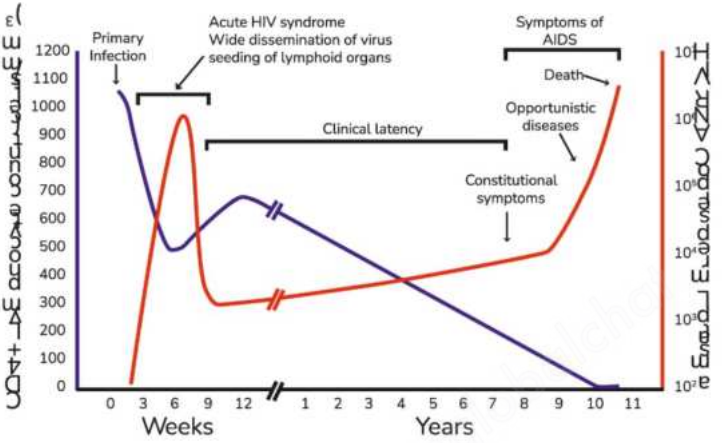


Image 30.4

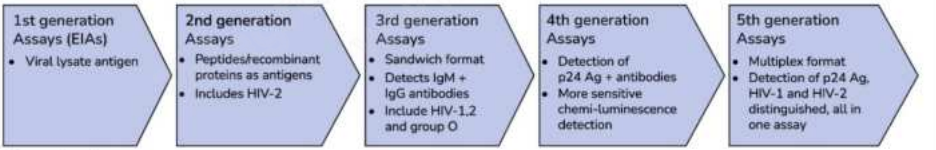
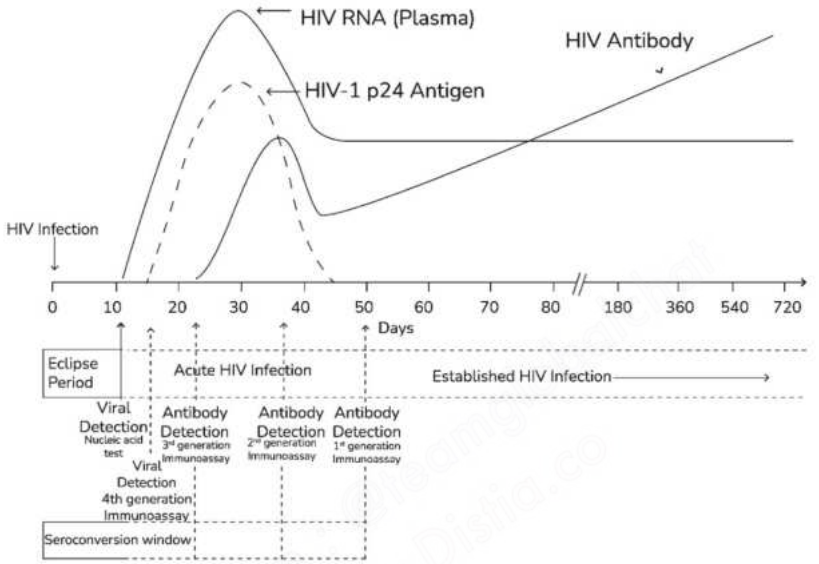


Image 30.5


HIV RNA (Plasma)
HIV Antibody
HIV-1 p24 Antigen
HIV Infection
0
10
20
30
40
50
60
70
80
180
360
540
720
Days
Eclipse Period
Acute HIV Infection
Established HIV Infection
Viral Detection
Nucleic acid test
Antibody Detection
3rd generation Immunoassay
Antibody Detection
2nd generation Immunoassay
Antibody Detection
1st generation Immunoassay
Viral Detection
4th generation Immunoassay
Seroconversion window

31 VIROLOGY- OTHER RNA VIRUS

Arenaviridae Family

- Negative Sense RNA
- Sand Sprinkled/Sandy appearance.
- Bioterrorism A agent
- Reservoir – rodents
- Transmission :Human to human
- Segmented RNA virus: 2 segments

Diagnosis

- RTPCR
- ELISA

Disease caused by arenaviridae virus.

- Lymphocytic choriomeningitis virus
- Lassa virus – Hemorrhagic Fever(HF)
- Junin virus - HF
- Machupo virus – HF

Reoviridae Family

Disease

- Colorado tick fever
- Rota virus

Rota virus

- Electron microscope: rotating wheel shape
- Only ds RNA virus
  - Segmented genome: 11 segments
- NSP4 (non structural protein)– ENTEROTOXIN
- Feco – oral (route)
- Small Infectivity Dose
- IP: 1-3 days
- M/C/C DIARRHOEA in children

Diagnosis

- Sample
  - Stool
- Antigen – ELISA
- RT PCR

Vaccine

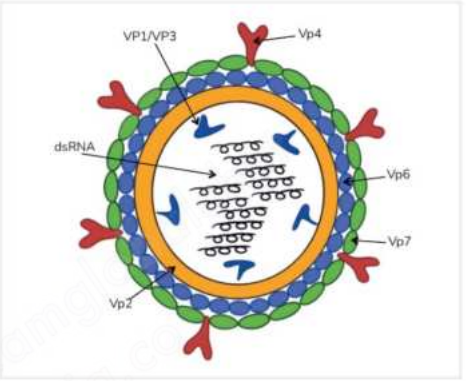
- Rotateq
- Rotarix

Side effect

- Intussusception

Structure

- VPI – 4,6,7
- NSPI 1– 6
- NSP4 – Enterotoxin – Diarrhoea
- Serogroups – A to I
- Serogroup A – Most common cause of diarrhoea in children <5 years



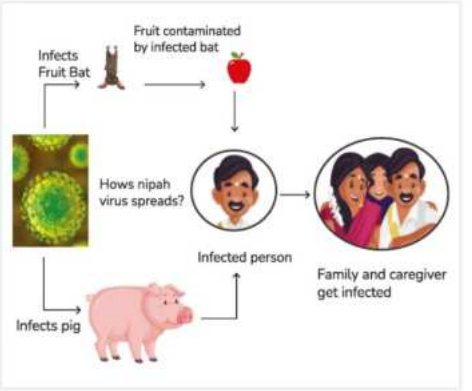
Filoviridae Family

Marburg virus – Hemorrhagic Fever

Ebola virus – common in Africa

Shape:Bowl of spaghetti / Filamentous organism

Spread: Secretion contact



- Reservoir: bats
- Spread: animal contact or human to human transmission

Types

- Zaire ebola is most virulent
- Ivory coast is least virulent
- Reston not known to cause disease

Features

- IP: 3-15 days(2days to 2 weeks)
- Biosafety level IV organism

Diagnosis

- Elisa
- IOC RT PCR

Treatment

- Inmazeb (atoltivimab, maltivimab, and odesivimab - ebgn) Injection

Vaccine

Hemorrhagic Fevers

- Arenavirus
- Bunyavirus
  - Crimian congo hemorrhagic fever
  - Rift valley fever
  - Hanta virus
- Flavivirus
  - Dengue
  - Yellow fever
  - KFD
- Filovirus
  - Ebola
  - Marbura virus
- Reovirus
  - Colorado tick fever

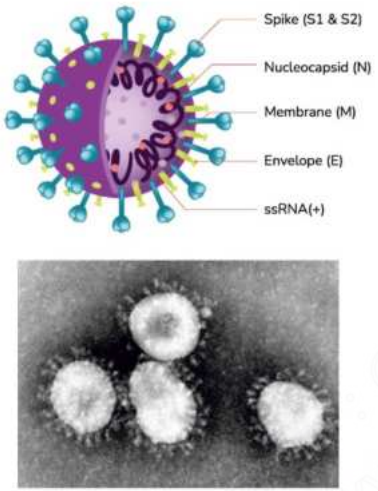
Viruses causing Gastroenteritis

- Adenovirus 40, 41
- Rotavirus
- Norwalk virus (Noroviridae)
- Calicivirus (Hep E)
- Astrovirus

32 RNA VIRUS : COVID 19

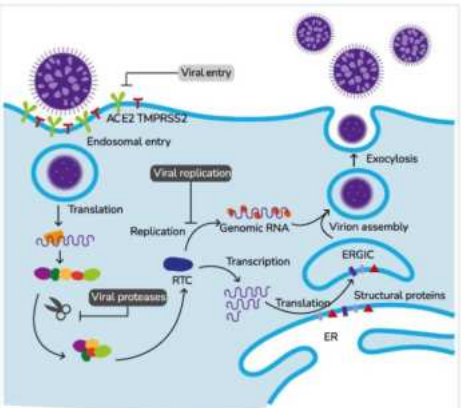
SARS CoV2/ Wuhan Virus

- Family
  - Coronaviridae
- Genus
  - Beta coronavirus

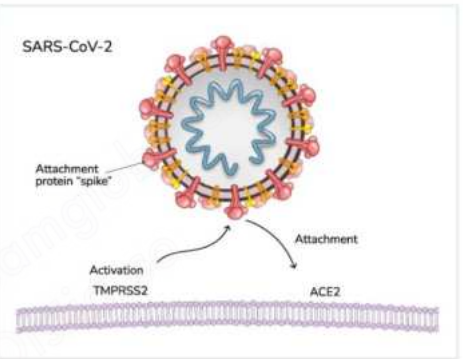


Electron microscope appearance

Structure and pathogenesis



- Spike S1 and S2 – help in attachment
- Non-segmented positive sense SSRNA
- Spike protein attach to ACE 2 receptors maximally present in
  - lungs (type 2 pneumocytes)
  - GIT
  - Heart
  - Kidney
- TMPRSS 2: Transmembrane serine protease 2



Important Information

- TMPRSS2: COVID 19 (SARS COV(2))
- TMPRSS6: IRIDA (Iron refractory IDA) mutation
- TMPRSS ERG fusion = Prostate Ca

- Transmission
  - Infected droplets
  - Aerosol
  - Contract transmission
- Maximum shedding occurs 5 – 8 hours before onset of symptoms
- Culturable virus: Isolated for next 6 – 8 days

Clinical features

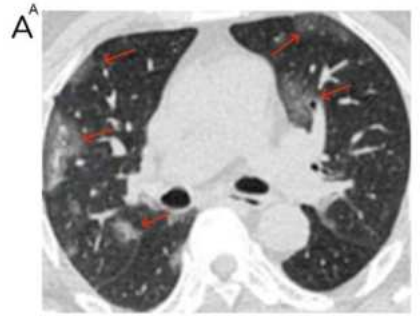
- Fever
- Dyspnea
- Dry cough
- Shortness of Breath
- Loss of smell
- Loss of taste
- GI complaints

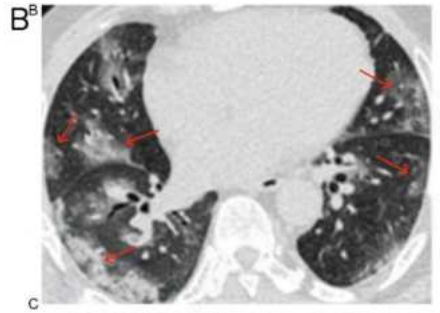
- Nausea, vomiting, pain, Diarrhoea
- Myalgia

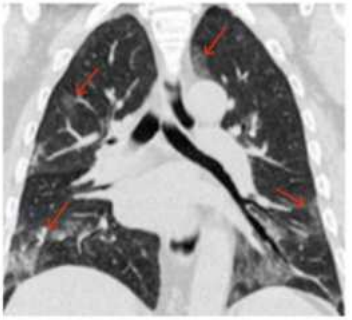
Diagnosis

Radiological findings

- Multifocal ground glass opacities (m/c)

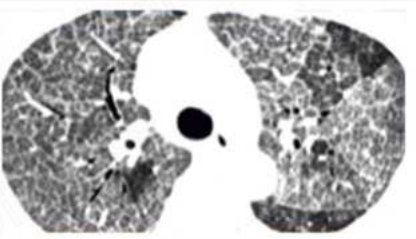




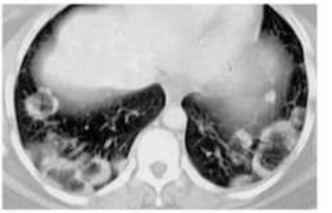


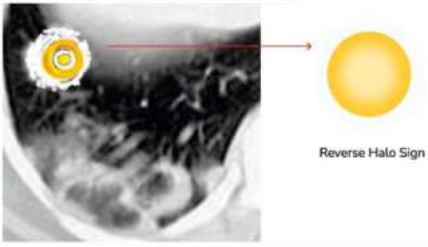
- Consolidation

- Pavement appearance: Multiple GGO with septal thickening



- Halo sign (centre is light and dense periphery)
- and reverse halo sign (centre is dark and periphery is light)





Pathological findings

- **Collection of sample**

UR specimens	LR specimens
• Nasopharyngeal swab • Oropharyngeal swab	• Atleast 2 – 3 ml ○ BAL ○ ET aspirate ○ Sputum

M/C: NP + OP sample
↓
Put in same Sterile Tube
↓
2 – 3 ml VTM

VTM

- Viral Transport Medium
  - HBSS with Ca, Mg IONS
  - Gentamycin, Amphotericin B
  - Fetal Bovine Serum
- Transport
  - 2-8 C upto 72 Hours
  - If delay storage -70 C (CDC) or -80 C (ICMR)

Blood Tests

CBC	
↓ Lymphocyte	ALC < 1000
↓ Platelets	
NLR (Poor Prognosis)	↑ > 3.1 (N/L ↓ ratio)
LFT → KFT	Organ damage
Troponin →	Cardiac damage
CRP, CK, LDH, Ferritin, IL6	↑
Albumin (-ve APR)	↓

Tests (Coagulopathy)

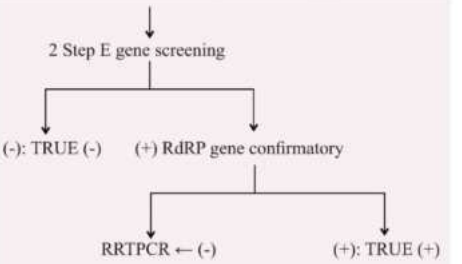
- PT, APTT ↑
- D-DIMER ↑ (D/C)
- Procalcitonin (n)/↓

RRTPCR (Quantitative PCR) Real Time Reverse Transcriptase PCR

- Gold standard
- Can even detect 10 copies/ml
- TAT: 4-6 hrs
- CT value (Cyclic Threshold is inversely propotional to load)
- **Semi confirmatory genes**
  - E. gene
  - N. gene
  - S. gene
- **Confirmatory genes**
  - RdRP gene
  - ORF1a gene

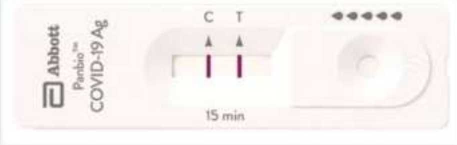
CBNAAT/TRUE NAAT

TRUE NAT/ CBNAAT/ Chip Based/ Cartridge Based Nucleic Acid Amplification Test	Portable PCR < 30 MIN



Rapid card test

- Principle: Immunochromatography



Antibody Testing

- ELISA

Feluda Test (Indian Scientists)

- FnCas9 Editor Linked Uniform Detection Assay
  - Principle: CRISPR – Cas 9 (Genome Editing Technology)
  - Paper Based Test Strip
  - Time: < 45 Minutes

Protection

N95 mask

- Not resistant to oil
- 95% air borne particles will be filtered

Q. In which of the following, viral load done by Real time PCR is of no role in investigation procedure? (AIIMS May 2018)

A. Person with hepatitis B on Tenofovir therapy
B. HSV causing temporal encephalitis
C. BK virus in patient of allograft renal transplant
D. CMV PCR in blood of patient of liver transplant

Q. A 5-year-old child came to OPD with fever, rashes on the body. There were rashes on axilla & flexor surface with various macule papule & vesicles. Most probable diagnosis is? (FMGE Aug 2020)

A. Measles
B. Chicken pox
C. Epstein Barr
D. Hand foot mouth disease

Q. Which vaccine is contraindicated in pregnancy? (NEET Jan 2019)

A. Hepatitis A
B. Hepatitis B
C. Rabies
D. Chicken pox

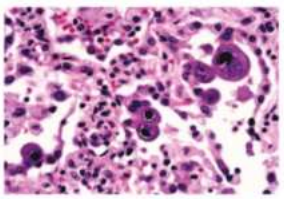
Q. Herpes Zoster multiply in? (NEET Jan 2019)

A. Peripheral nerve
B. Epithelium of skin
C. Dorsal root ganglion
D. Pharyngeal Epithelial cell

Q. Shingles is caused by which of the following? (NEET Jan 2018)

A. Varicella Zoster
B. Herpes Simplex
C. CMV
D. EBV

Q. Owl eye inclusion bodies seen in? (FMGE Dec 2020)



A. HSV
B. HHV
C. EBV
D. CMV

Q. After kidney transplantation which organism infection is more likely to happen? (NEET Jan 2018)

A. CMV
B. EBV
C. Hepatitis virus
D. Polyoma virus

Q. MC infection post solid organ transplantation? (AIIMS Nov 2019)

A. CMV
B. HSV
C. EBV
D. HPV

Q. Which of the following virus is from Herpes virus family? (FMGE Jun 2019)

A. Rubella
B. Measles
C. Rabies
D. EBV

Q. Nasopharyngeal carcinoma associated with which of the following? (FMGE Dec 2020)

A. HPV
B. HHV
C. EBV
D. HSV

Q. Oral hairy leucoplakia is caused by? (FMGE Aug 2020)

A. CMV
B. EBV
C. Kaposi Sarcoma
D. Varicella Zoster

Q. All of the following are associated with HHV 8 except? (AIIMS May 2018)

A. Kaposi Sarcoma
B. Primary effusion lymphoma
C. Castleman disease
D. T-cell leukaemia

Q. A 22-year-old college boy with history for sex with commercial workers came to Derma OPD with lesion in

genital region, tissue from this lesion was examined, which is the most likely finding? (AIIMS May 2019)

A. Intracytoplasmic Vacuolation
B. Multinucleated giant cell
C. Belongs to Herpes family
D. It's an RNA virus

Q. Molluscum Contagiosum is a? (FMGE June 2019)
A. Adeno virus
B. Flavi virus
C. Rubi virus
D. Pox virus

Q. Which sub-type of human papilloma virus has maximum chances of causing carcinoma cervix? (FMGE June 2018)
A. HPV 16 & 18
B. HPV 6 & 11
C. HPV 31 & 32
D. HPV 1&2

Q. Condyloma acuminatum is caused by? (FMGE Aug 2020)
A. Epstein bar virus
B. Varicella Zoster
C. Molluscum contagiosum
D. Human papilloma virus

Q. Maternal antibodies do not provide protective immunity to neonate in? (NEET Jan 2020)
A. Diphtheria
B. Pertussis
C. Tetanus
D. Polio

Q. An infant presented with the following lesions on his face & limbs. Which of the following is the most likely causative organism? (AIIMS Nov 2017)

A. HSV
B. HPV
C. Coxsackie
D. CMV

Q. Child present with oral & pharyngeal ulcerations & vesicular rashes of palms & soles which heal without Gusting. What could be the most probable diagnosis? (FMGE Aug 2020)

A. Measles
B. Hand foot mouth disease
C. Cutaneous drug reaction
D. Chickenpox

Q. Aseptic meningitis case fever of 4 days, with erythematous rash on chest with no h/o travel, tick bite. CSF findings - protein high, glucose normal, lymphocytosis monocytes 34%? (INICET Nov 2020)
A. Strep pneumoniae
B. Meningococci
C. West Nile
D. Enterovirus

Q. Recent influenza A pandemic was d/t? (NEET Jan 2020)
A. H1N1
B. H5N1
C. H7N7
D. H3N3

Q. Which vaccine strain is changed every yearly? (NEET Jan 2019)

A. Influenza
B. Rabies
C. Hepatitis
D. Ebola

Q. Warthin Finkeldey cells are seen in? (NEET Jan 2018)

A. Measles
B. Rubella
C. Rabies
D. Mumps

Q. SSPE is a complication of? (FMGE Jun 2018)

A. Measles
B. Mumps
C. Rubella
D. Rabies

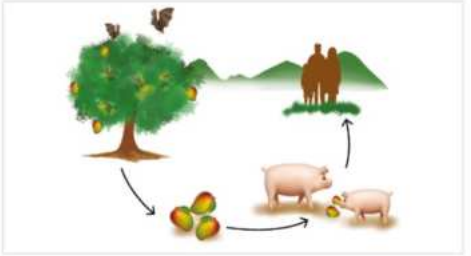
Q. A neonate was found to have cataract, deafness & cardiac defects. Which group of viruses does the mother was infected with? (NEET Jan 2019)

A. Togaviridae
B. Flaviviridae
C. Bunyaviridae
D. Arenaviridae

Q. True about congenital rubella syndrome is? (NEET Jan 2019)

A. Blueberry Muffin rash is seen
B. Virus can be isolated only upto 6 months after birth
C. Triad of CRS are cataract, cardiac defects, cerebral palsy
D. Infection is most serious after 5 months of pregnancy

Q. Which virus transmission shown in picture? (AIIMS Nov 2018)



A. Nipah Virus
B. Zika Virus
C. Ebola Virus
D. Corona Virus

Q. Virus with high mortality rate? (INICET Nov 2020)

A. Ebola (50%)
B. Influenza H1N1 (1-4%)
C. Rabies (100%)
D. Nipah (40-75%)

Q. Antemortem diagnosis of rabies is made with? (FMGE June 2019)

A. Rabies virus specific antibodies
B. Inoculation in culture media
C. Negri bodies in hippocampus
D. Corneal impression smear

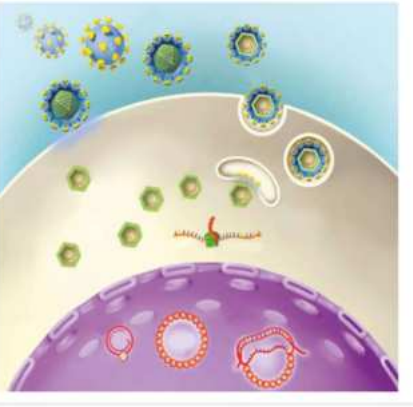
Q. Viral haemorrhagic fever in India caused by which virus? (AIIMS Nov 2017)

A. Ebola
B. Yellow virus
C. Crimean Congo
D. Marburg

Q. Which of the following is not true about JE? (NEET Jan 2020)

A. Human is reservoir
B. Pig is amplifier host
C. Transmitted by culex vishnui
D. Vaccination available

Q. The following diagram show life cycle of? (AIIMS Nov 2017)



A. HIV
B. Hep B
C. Influenza virus
D. Rabies

Q. Which is not true about hepatitis B viruses? (FMGE Aug 2020)

A. DNA viruses
B. Transmitted by focal-oral route
C. Can be transmitted from mother to child (Perinatal transmission)
D. Contains reverse transcriptase

Q. Cryoglobulinemia is associated with? (NEET Jan 2018)

A. Hepatitis A
B. Hepatitis B
C. Hepatitis C
D. Hepatitis D

Q. Shown in the figure is the HBV card test. Which of the following is the principle of the test? (NEET Jan 2020)

A. Immunochromatography
B. Chemiluminescence
C. ELISA
D. Immunofluorescence

Q. HIV patient presented with diarrhoea. On stool examination, following acid fast organism was seen. Is the drug of choice in this patient? (NEET Jan 2019)

A. TMP-SMX
B. Nitazoxanide
C. Primaquine
D. Niclosamide

Q. Approximate time interval between HIV infection CN manifestation of AIDS is? (NEET Jan 2018)

A. 7.5 years
B. 10 years
C. 12 years
D. 11 years

Q. Nurse got needle prick injury from HIV patient. What should be done at earliest? (FMGE Dec 2020)

A. ELISA
B. Blood culture
C. Western blot
D. P24 assay

Q. A 6-month child presented with diarrhoea and vomiting for three days. Which of the following enterotoxin is most likely responsible for this condition? (NEET Jan 2019)

A. NSP4
B. NSP6
C. VP3
D. VP7

Q. Vector for Zika virus? (FMGE Dec 2020)

A. Aedes
B. Culex
C. Anopheles
D. Louse

Q. What is not true regarding Zika Virus? (AIIMS Nov 2018)

A. Belong to family Flaviviridae
B. Transmission happens by mosquitoes
C. Not transmitted from mother to new-born
D. Possibility can cause microcephaly

Q. Which of the following statements is wrong about Ebola virus? (AIIMS June 2020)

A. It belongs to Filoviridae family
B. Transmitted by mosquito
C. It is type of nosocomial infection
D. Transmitted via direct contact

Q. Which of the following disease show deposition of PrP? (FMGE Dec 2020)

A. CJD
B. Rabies
C. Parkinson's disease
D. Alzheimer' diseases

33 MYCOLOGY PART-1

Structure of a Fungus

00:00:45

- Cell wall- Contains Chitin
- Cell Membrane- Contains Ergosterol
  - Exception- Pneumocystis carinii/jeroveci

Classification based on morphology

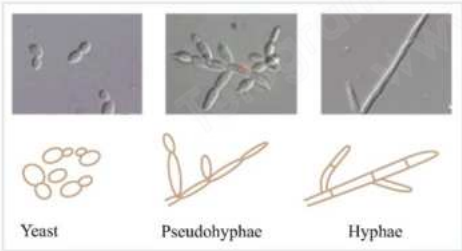
- Yeast
- Yeast like
- Mold/Hyphae
- Dimorphic fungal organisms

Yeast

- Shows presence of buds/ budding form
  - Cryptococcus
  - Saccharomyces cerevicae

Yeast like

- Element of yeast
- Elongated buds knowns as pseudohyphae
  - Candida
  - Malassezia
  - Trichosporon
  - Hortae



Important Information

- Saccharomyces cerevicae causing Crohn's Disease belongs to the yeast family.

Mold/Hyphae

- **Aseptate**
  - Hyphae is a single straight line without any segments
- **Septate**
  - Segmented hyphae
  - Phaeoid / Melanin- Pigmented
    → Piedra, alternaria, madurella, curvellaria
  - Hyaline
    → Fusarium, Acremonium, Aspergillus, Penicillium

Dimorphic Fungi

- Two morphologies are present:
  - Yeast is present at 37°C
  - Mold is present at 25° C

Mnemonic

- **Body** - Blastomyces
- **Heat** - Histoplasmosis
- **Probably** - Penicillium marnefii / Paracoccidioidomycosis
- **Changes** - Coccidioidomycosis
- **Shape** - Sporotrichosis

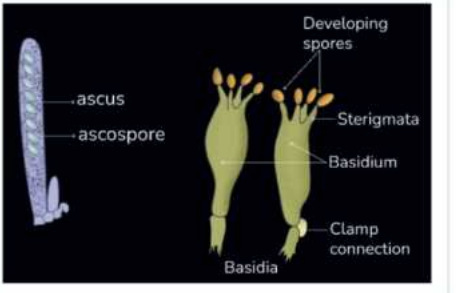
Classification based on Spores

00:12:28

- Sexual spores - ZABD or ZAB/D
- Asexual spores - ABSCC

Sexual Spores

- Zygospores
  - Zygomycetes
    → Rhizopus, Absidia, Mucor
- Ascospores
  - Aspergillus
- Basidiospores
  - Cryptococcus

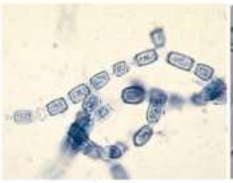


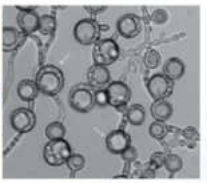
- Sexual spores are absent
- Also knowns as Fungi Imperfecta

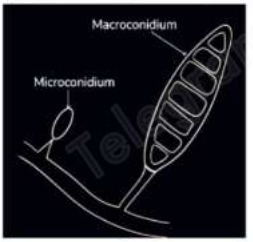
Asexual Spores

00:15:15

- Arthrospores - Characteristically seen in Coccidioidomycosis and Dermatophytes. It is the condensation of hyphal element .
- Blastospores
- Sporangiospores (zygomycetes)
  - Zygomycetes
    - → Family shows sexual as well as asexual spores
    - → Appears in Zygospores and Sporangiospores
- Chlamydospores
  - Double walled spores
  - Seen in candidal infection
- Conidia
  - Macroconidia - Large
  - Microconidia - Small







Q. How do you identify Arthrospores?
Ans: They are a condensation of a different fungal elements

Vegetative State

- Spiral
  - Trichophyton mentagrophytes
- Pectinate
- Favic Chandelier
  - Trichophyton schoenleinii
- Nodular organ
- Racquet hyphae
  - Epidermophyton floccosum

Classification based on location

00:22:12

- Superficial mycosis
  - Tinea Versicolor
  - Dermatophytes
- Subcutaneous mycosis
  - M = Mycetoma
  - R = Rhinosporidiosis
  - C = Chromoblastomycosis
  - S = Sporothrichosis
- Deep mycosis/ Systemic Mycosis
  - Dimorphic fungi
  - Opportunistic infections
    - → Candida
    - → Cryptococcus
    - → Aspergillus
    - → Mucormycosis

Laboratory Diagnosis

00:24:27

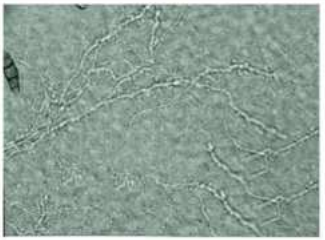
Sample

- Hair
- Skin
- Nail
- Pus
- Lungs
  - Sputum
  - Bronchial washings or Bronchoalveolar Lavage
    - → Presence of dry cough
    - → Caused by pneumocystis carinii
  - Lung biopsy
- Blood
- CSF
- Urine
- Body fluids

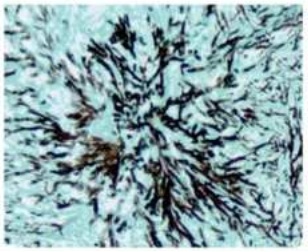
Detection

00:26:16

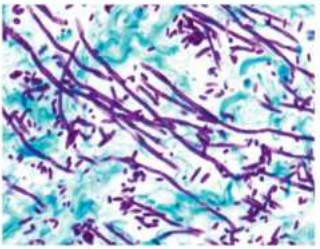
- **Direct examination**
  - Examine under a microscope directly
  - KOH Mount - 10-20% potassium hydroxide + Sample
  - **KOH mount -**
    - → To digest keratin from samples like hair, skin and nails
    - → After digestion of keratin Hyphae in visible under microscope



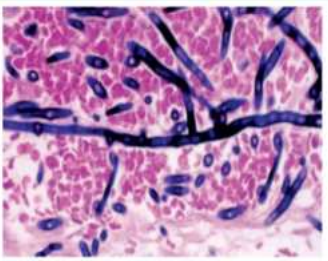
- **Stains**
  - Gomori / Grocott's Methenamine Silver Stain or GMS - Black color hyphae



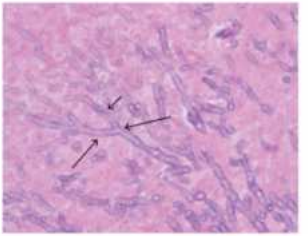
  - PAS or Per Iodic Acid Schiff - Pink color hyphae



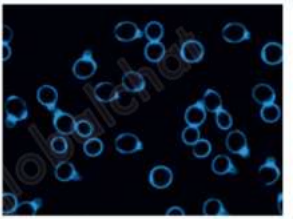
  - Gram stain - Purple color hyphae



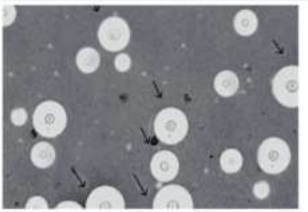
  - H&E - Pink and Blue hyphae



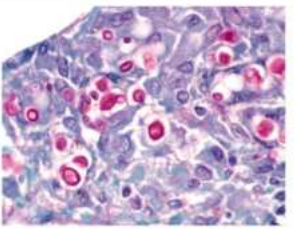
  - Calcofluor White - Fluorescent stain (Binds to Chitin)



  - **For CSF** - Negative Stain (India ink, Nigrosin) - Back ground stained giving a negative photo effect highlighting capsule of cryptococcus



  - **For Biopsy** - Mucicarmine - Bright Pink color hyphae

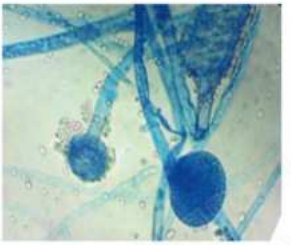


Culture

1. **Sabouraud Dextrose Agar**
   - 2% dextrose

- Antibiotics (gentamicin, chloramphenicol) - to kill bacteria
- Cycloheximide - to kill saprophytic fungi
- pH 5.6 - Acidic pH

- **Stains**
  - LPCB (lacto phenol cotton blue)
    - → Lactic acid - preserving morphology of the fungus
    - → Phenol - disinfectant
    - → Glycerol - prevent drying (hygroscopic)
    - → Cotton blue - dye

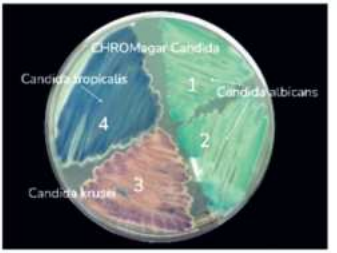


2. Corn meal agar

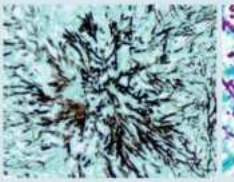
- To grow chlamydospores

3. Chrom agar

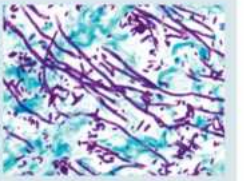
- To grow candida



Images with Modality



GMS

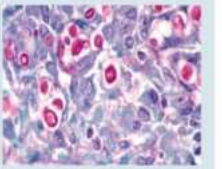


PAS

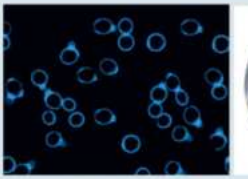
SDA

LPCB

-ve stain

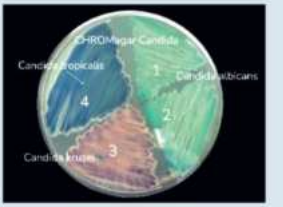


Mucicarmine



CFW

Bird seed agar



Chrom agar candida

Other Detection Methods

- **Serological testing -** Antibody Testing
- **Skin test -** Similar to skin testing for TB and Leprosy
- **PCR or Polymerase chain reaction**

MCQs

Q. A patient presented with scaly skin, redness, irritation and small blisters all over his skin. The doctor suspected fungal infection and sent his skin scraping to the laboratory. Which culture media is used for culturing the fungus?

A. Tellurite medium
B. NNN Medium
C. Chocolate Agar
D. SDA

Q. Which of the following is not dimorphic?

A. Talaromyces marneffei
B. Cryptococcus neoformans
C. Sporothrix schenckii
D. Histoplasma capsulatum

Mycology- Superficial Mycosis and Subcutaneous Mycosis

Tinea Versicolor

00:48:56

Tinea Versicolor- Stain and Culture

- Malassezia Furfur/ Tinea Versicolor / Pityriasis versicolor (Dermatology)
- C/F - Hypopigmented lesions (trunk, back)
- Wood lamp examination - UV light (yellow fluorescence)
  - Collecting skin scrapings
- Patient has hypopigmented lesions
- **10-20% KOH mount** - Spaghetti and Meatball like appearance / Banana and Grapes like appearances
- **Culture** - SDA with Olive Oil - Fried egg colonies
  - Culture morphology stain - LPCB - Spaghetti and Meatballs like appearance

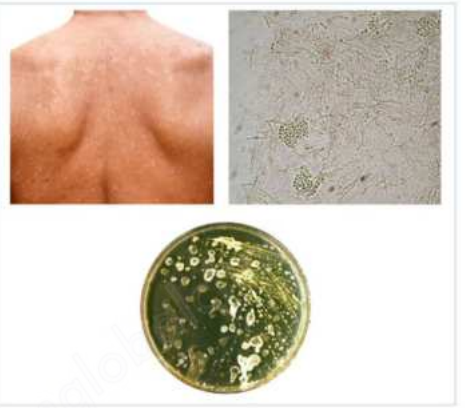
Spaghetti and Meatballs Appearance

- Malassezia Furfur
- Trichomonas
- Leptothrix

Fried Egg Appearance

- **Microbiology -** Malassezia Furfur, Mycoplasma
- **Pathology -** Oligodendroglioma, seminoma/dysgerminoma, Bone Marrow Biopsy- hairy cell leukemia

Classical Summary of Malassezia Furfur - Stain and Culture



Tinea Versicolor - Treatment

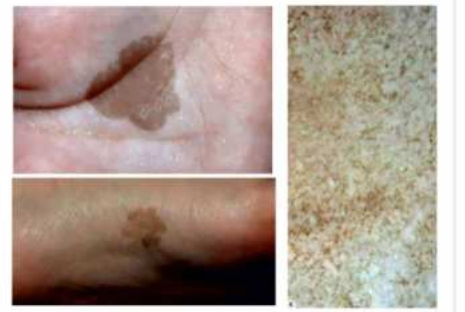
- **Topical**
  - 10% sulfur ointment
  - 1-2% imidazole creams
- **Systemic**
  - Itraconazole
  - Fluconazole

Important Information

- Usually one or both of them are given as a combination of topical treatments and systemic therapy.

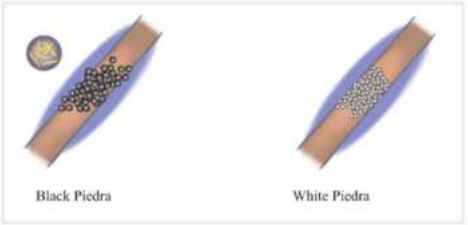
Tinea Nigra

00:58:26



- Brown or black color appears representing the word Nigra
- Organisms - Hortaea Werneckii
  - Halophilic (salt-loving)
  - Seen in fisherman
- Treatments
  - Rx- Topical Imidazole
- Location
  - Palm and sole

Piedra



Black Piedra

White Piedra

- **White Piedra** - White spots
  - Trichosporon Beigelii
- **Black Piedra** - Black spots
  - Piedraia Hortae

Treatment

- Cut / Shave hair
- Topical imidazole

Dermatophytosis

01:01:58

Tineasis

- T = Trichophyton
- M = Microsporum
- E = Epidermophyton

Classification

- **Anthropophilic**
  - T. Rubrum
  - T. Tonsurans
  - T. Schoenleinii
  - T. Violaceum
  - M. audounii
- **Zoophilic**
  - T. Equinum
  - M. Equinum
  - M. Canis
  - T. Verrucosum
- **Geophilic**
  - T. Terrestre
  - M. Gypseum

Q. Trichophyton Rubrum which is responsible for causing nail infection/ onychomycosis belongs to which category?

Ans: Anthropophilic

Tineasis - Clinical aspect

- **Infections caused in Head/Scalp**
  - **Tinea Capitis**
    → Scalp
  - **Tinea Favus (T. Schoenleinii)**
    → Crust (scutula)
  - **Kerion (T. Mentagrophytes, T. Verrucosum)**
    → Boggy Swelling

Face and body Infections	• Tinea Corporis - Trunk (non-hairy) • Tinea Barbae - Beard • Tinea Faciei - Face • Tinea Imbricata - Concentric lesions
Organisms	
Tinea Cruris	• Present on the groin • Dhobi/Jock Itch • Caused by constant moisture
Tinea Unguium	• Nail bed
Tinea Pedis	• Feet • Also knowns as athlete's foot
Tinea Corporis	• Presence of Classical ring like fungal infection • Occuring on the trunk
Tinea Imbricata	• Presence of Concentric circles

Arthrospores - Asexual Spores

01:11:49

Ectothrix

- Present outside the hair shaft
- Microsporum canis, microsporum audouinii

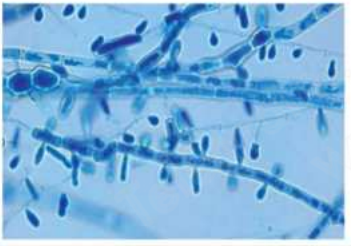
Endothrix

- Present inside the hair shaft
- **T** = Trichophyton tonsurans
- **V** = Trichophyton violaceum
- **S** = Trichophyton schoenleinii

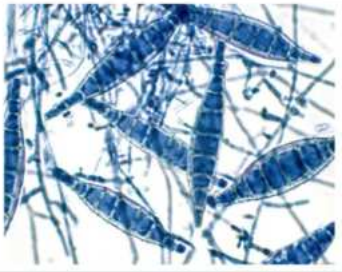
Types of organisms

Trichophyton - Skin hair , nails
Imicrosporum - Skin , hair
Epidermophyton - Skin , nails

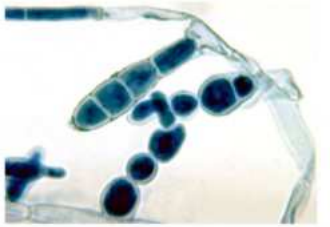
Organisms	Macroconidia	Microconidia
Trichophyton	Pencil	+++ (maximum)
Microsporum	Spindle	++
Epidermophyton	Club	+/- (least)



Microconidia representing Trichophyton (Abundant Microconidia)



Microconidia representing Microsporum (Lesser Microconidia)



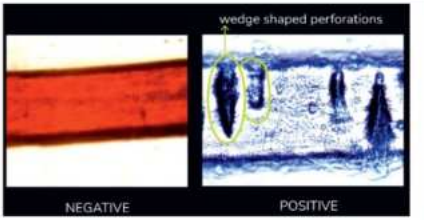
Microconidia representing Epidermophyton (Negligible Microconidia)

Hair Perforation Test

01:19:38

- Incubate hair with yeast and fungus for 2-3 weeks
- Perforation occurs means +ve test
- Perforation does not occurs means -ve test
- **Organisms**
  - T. Rubrum
  - T. Mentagrophytes

Organisms	Test result
T. Rubrum	Negative
T. Mentagrophytes	Positive



Dermatophytids / ID Reaction

- Satellite lesions away from original lesion
  - Probably due to fungal products
  - Can be following antifungal therapy also

Treatments

- **Tinea**
  - Topical antifungals (azole derivatives)
  - Itraconazole / Terbinafine (currently preferred)

- **Tinea Capitis**
  - Oral Griseofulvin

Subcutaneous Mycosis

01:23:48

- Mycetoma
- Rhinosporidiosis
- Chromoblastomycosis
- Sporotrichosis

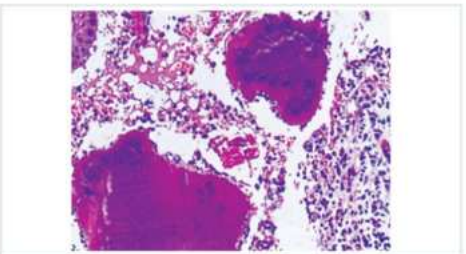
How to Remember: MRCS

Mycetoma

- Madura foot
- Multiple discharging sinuses

Actinomycetoma	Feature	Eumycetoma (Madura Foot)
Bacteria (gram +ve)	**Organism**	Fungus
Actinomadura madurae	**Examples**	Madurella mycetomatis
Red - Actinomadura pelletieri Off white / yellow - Nocardia Streptomyces Actinomadura	**Granules**	Brown (Melanin) - Madurella Exophiala White (Hyaline) - Acremonium Pseudallescheria boydii
Antibiotics Cotrimoxazole +/- Amikacin x 6-12 months	**Treatment**	Surgical amputation or resection

- Sunray appearance



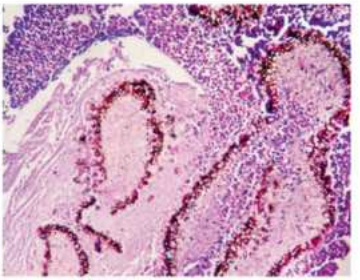
- Purple color organisms radiating
- Pink layer - Splendore Hoepplei Phenomenon

Important Information

- The most accepted theory regarding Splendore Hoepplei Phenomenon is the (Ag - Ab) antigen - antibody reaction.

Splendore Hoeppli Phenomenon

- Actinomyces
- Sporothrix schenckii



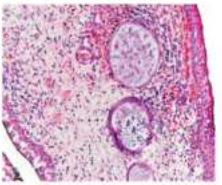
- Microscopic slide of fungal infection
- Brown color fungus
- Presence of melanin

Rhinosporidiosis

01:33:27

Organisms	Rhinosporidium Seeberi
Classified as	Aquatic Protozoa
Sites	Nasal Cavity - Most common Genitals and eyes - Rare
Gross	Strawberry polyp
Microscopic examination	Endospores
Culture	Non Cultivable
Treatment	Polypectomy

- Rhinosporidiosis
- Strawberry Nasal polyp
- Microscopic structure of rhinosporidiosis
- Sporangia are seen
- Tiny endospores appear inside sporangia

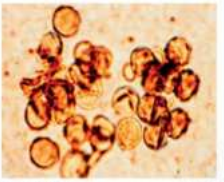



Important Information

- Rhinosporidiosis and Pneumocystis carinii cannot be grown in any culture.

Chromoblastomycosis 01:37:07

- Also knowns as Verrucous Dermatitis

Traumatic inoculation

- Commonly occurred in forest workers walking barefoot
- Warty cauliflower like growth
- Microscopic examination
  - Copper penny bodies

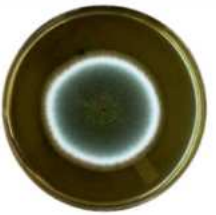
Important Information

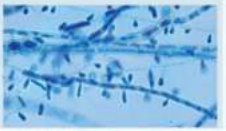
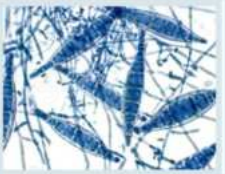
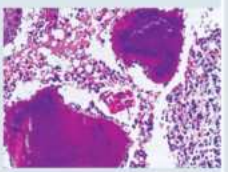
- Copper penny bodies also known as sclerotic body or medlar body or muriform body.

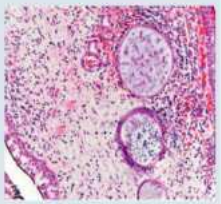
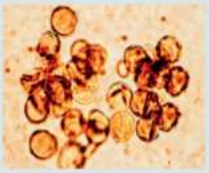
Organisms	• Fonsecaea pedrosi and F.compacta • Exophiala • Phialophora • Cladophialophora
Route	Traumatic (barefoot)
Treatment	• Amphotericin B, 5 - Fluorocytosine, voriconazole (recent) • Wide surgical resection • Laser if required

Q. Chromoblastomycosis which you see in the very famous forest worker are caused by which organisms?

Ans: The organisms include - Fonsecaea pedrosi and F.compacta, Exophiala, Phialophora and Cladophialophora.



Images	Diagnosis
	**T - P**encil macro
	**M - S**pindle macro
	**E - C**lub macro
	FOOT → ACTINO / EUMYCETOMA
	

Images	Diagnosis
 	Nasal RHINOSPORIDIOSIS (NO CULTURE)
 	COPPER PENNY CHROMOBLASTOMYCOSIS

MCQ

01:42:11

Q. A 28 years old man presented with a recent onset of rash that involved his upper arms. The patient was otherwise healthy, with no known allergies, medication or chemical exposure. The physical examination revealed multiple, hypopigmented, slightly scaly on both arms. The culture was isolated but the microbiologist could not get any results on regular SDA media. Which of the following fungi is difficult to isolate on Regular SDA media?

A. Candida
B. Dermatophytes
C. Cryptococcus
D. Malassezia Furfur

Q. A 30 years old male presented with discrete hypopigmented macules on his upper back, on examination of skin scrapings with 10% KOH under microscope spaghetti and meatball appearance was seen. What is the probable diagnosis?

A. Tinea versicolor
B. Tinea corporis
C. Mixed mycotic infection
D. candidiasis only

Q. Boggy swelling on scalp?

A. Tinea capitis
B. T. kerion
C. T. cruris
D. T. barbae

Answer: B (Boggy swelling on the scalp)

Q. Which of the following affects the hair, skin and nails?

A. Trichosporium
B. Trichophyton
C. Epidermophyton
D. Microsporum

Answer: B

Q. All are true regarding dermatophytes except?

A. They are differentiated based on macroconidia
B. Epidermophyton infects skin and nails
C. Microsporum has fusiform macroconidia
D. Epidermophyton has abundant microconidia

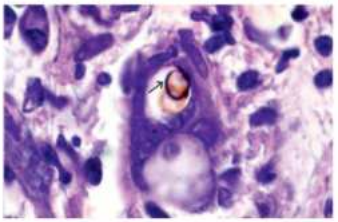
Q. Which of the following is used for cultivation of rhinosporidium?

A. Hela
B. Hep 2
C. SDA
D. None

Q. A 24 year old south Indian patient with a history of taking baths in ponds presented with unrelated nasal polyps. The patient was admitted and a polyp biopsy was taken which showed spherules with endospores. All of the following are true about the diagnosis of the given patient except?

A. Can be cultured an Sabouraud's
B. Spherules in biopsy
C. Bleeds to touch
D. Treatment is polypectomy

Q. H and E stain of nodular lesion on the arm of the forest worker is shown in the figure



01:51:10

Which of the following is the likely diagnosis?

A. Sporotrichosis
B. Mycetoma
C. Chromoblastomycosis
D. Botryomycosis

Q. A patient presented with a swelling in his right foot with multiple discharging sinuses. The lesions did not respond to antibiotics. The likely etiological agent would be?

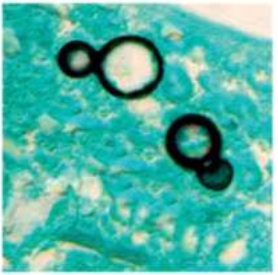
A. Actinomadura
B. Nocardia
C. Sporothrix
D. Madurella mycetoma

Mycology- Dimorphic Fungus

01:52:52

- Exists in two morphology
  - Yeast - 37°C (hot)
  - Mold/ Hyphae - 25°C (cold)

- **Body** = Blastomyces
- **Heat** = Histoplasmosis
- **Probably** = Penicillium Marneffei
- **Change** = Coccidioidomycosis
- **Shape** = Sporotrichosis



Blastomyces

- Also known as North American Blastomycosis, Gilchrist or Chicago disease
- **Organism** - Blastomyces Dermatitidis
- **Size** - 8-15 microns
- Broad Based budding
- Classical figure of 8 appearance
- GMS stain sowing black outline

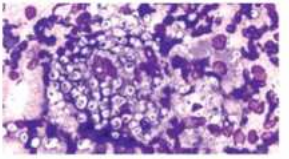
Treatment

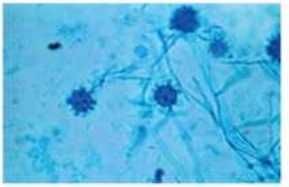
- **Mild disease**
  - Fluconazole/itraconazole
- **Severe or Disseminated**
  - Amphotericin B followed by itraconazole

Histoplasmosis

- Histoplasma Capsulatum var capsulatum - American
- Histoplasma Capsulatum var duboisi - African
- **Reservoir**
  - Caused by Bat or bird droppings (inhalation)
- Also knowns as Cave's disease/ Ohio disease/ Darling disease/ Reticuloendotheliosis
- **Organism**
  - Histoplasma Capsulatum

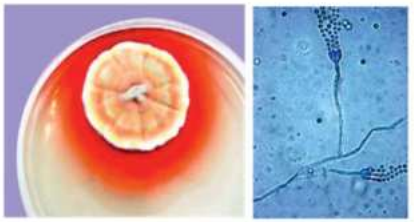
Q. Where does Histoplasma sit?
Ans: It sits in the Reticuloendothelial system





- Microscopic examination of Yeast
- Reticuloendothelial cell/ Macrophage
  - Nucleus is present in the center of the cell
  - Tiny spore like structures are Histoplasma
- LPCB stain
  - Tuberculate macroconidia
  - Fungus looks similar to TB

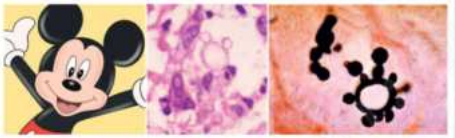
- Also knowns as Talaromyces Marneffei
- **Natural Reservoir**
  - Bamboo rats
- Brush / broom conidia morphology is seen



Q. What is the natural reservoir of Penicillium Marneffei?
Ans: Bamboo Rat

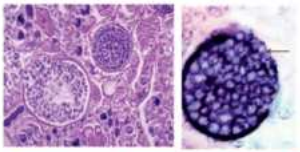
Q. What is the pigment that Penicillium Marneffei will show?
Ans: A red color pigment is formed.

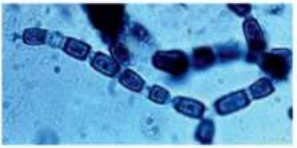
Paracoccidioidomycosis



- Also known as South American blastomycosis
- **Organism** - P. Brasiliensis
- **Microscopic Examination**
  - Pilot/ Mariner wheel appearance
  - Mickey mouse appearance

Coccidioidomycosis





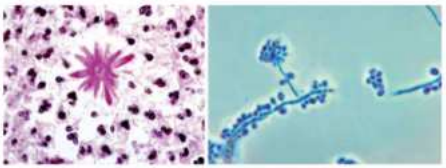
- Also knowns as Dessert rheumatism/ Valley fever/ California disease
  - Element of Joint involvement/ Migratory arthritis
- **Organism**
  - C. Immitis
- Barrel shaped arthrospores

Treatments
- **Mild disease**
  - Fluconazole/itraconazole
- **Severe or Disseminated**
  - Amphotericin B followed by itraconazole

Sporotrichosis

- Also known as Rose gardener's disease/ Lymphocutaneous disease
- **Organism**
  - Sporothrix Schenckii
- **Route**
  - Traumatic - Ulcer , pustule , nodule
  - Thorn prick + Nodules along lymphatic channels

Microscopic Examination of Sporotrichosis



- Star shaped body - Asteroid body
- Splendore Hoepplei Phenomenon - (Ag-Ab)
- Cigar shaped body - yeast
- **Under the LPCB stain**
  - Rosettes of conidia

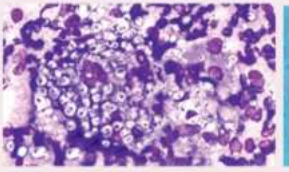
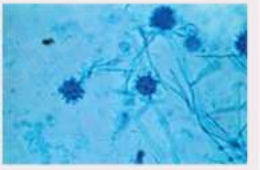
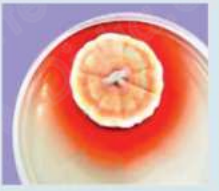
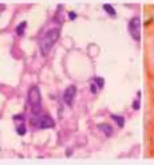
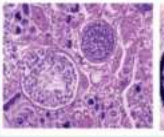
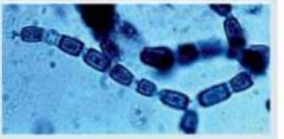
Treatment
- Itraconazole for 3-6 months

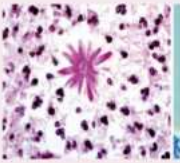
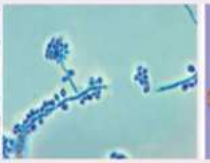
Important Information

- Asteroid body is seen in Sporothrix schenckii (extra cellular) and Sarcoidosis (intra cellular)

Classical summary of the Dimorphic fungal organisms

02:20:16

Organism	AKA	Images
Blastomyces	North American Blastomyces/ Chicago disease/ Disease	B - 8 
Histoplasmosis	Ohio / Darling disease/ cave's disease/ Reticuloendothelial disease	Tuberculate macrophage  
Penicillium marneffei	Talaromyces marneffei	Broom / brush appearance  
Paracoccidioidomycosis	South american blastomyces/	Pilot / Mariner wheel appearance   
Coccidioidomycosis	A B C D (Arthrospores - Barrel shaped Desert rheumatism)	Barrel shape   

Organism	AKA	Images
Sporothrix Schenckii	Rose gardener Asteroid body	Cigar body   

MCQ

Q. Which of the following fungi is most likely to be found in reticuloendothelial cells?

A. Histoplasma capsulatum
B. Sporothrix schenckii
C. Cryptococcus neoformans
D. Candida albicans

Q. A wedge-shaped perforation of the hair is found when a hair perforation test is done. Which of the following infections shows a positive hair perforation test?

A. Trichophyton
B. Exophiala werneckii
C. Epidermophyton
D. All of the above

Q. A girl who pricked her finger while pruning some rose bushes develops a local pustule that progresses to an ulcer. Several nodules then develop along the local lymphatic drainage. The most likely agent is?

A. Aspergillus fumigatus
B. Sporothrix schenckii
C. Cryptococcus neoformans
D. Candida albicans

Q. Which of the following statements is true for the direct examination of sputum or tissue sample is taken from coccidioidomycosis?

A. Identification of budding yeast with its pseudohyphae
B. The detection of spherules with endospores in sputum or tissue
C. The saprophytic phase is observed as septate mycelium with pyriform conidia
D. Tuberculate macroconidia are observed in culture

Q. A farmer in the Midwest of America developed flu-like symptoms with fever, headache, muscle pain, and cough for a week. Swabs specimens and the sputum sample were taken, radiography examination was also performed which showed diffuse bilateral pneumonia. After a week of culture, white mold colonies were observed with large and small conidia. Name the possible pathogen the farmer is exposed to?

A. Coccidioidomycosis
B. Sporotrichosis
C. Histoplasma
D. Blastomycosis

Q. Barrel-shaped arthrospores are seen in

A. Coccidioidomycosis
B. Paracoccidioidomycosis
C. Histoplasmosis
D. Candida

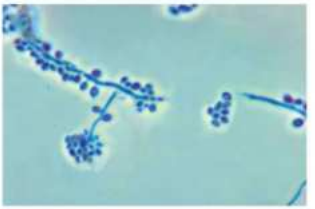
Q. Cigar body?

A. Sporotrichosis
B. Chromoblastomycosis
C. Histoplasmosis
D. Coccidioidomycosis

Q. Sclerotic Body?

A. Sporotrichosis
B. Chromoblastomycosis
C. Histoplasmosis
D. Coccidioidomycosis

Q. Which disease is associated with the LCB mount of the image given below?



A. Cryptococcus
B. Histoplasmosis
C. Sporotrichosis
D. Aspergillosis

Cryptococcus

00:00:40

- It is a true yeast.
- Has a polysaccharide capsule.
- Pathogenic: Cryptococcus neoformans
- Serotype: A, B, C, D, AD.
  - A, D, AD serotypes - Cryptococcus neoformans
  - B, C serotypes - Cryptococcus gattii

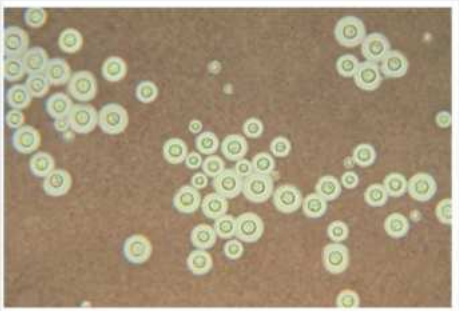
Cryptococcus Neoformans vs Cryptococcus Gattii

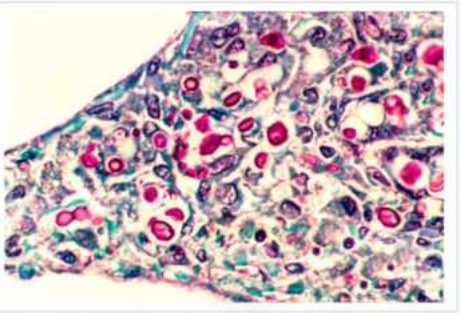
00:02:22

C. Neoformans	Features	C. Gattii
Whole world	**Distribution**	Tropical and subtropical parts
Soil with pigeon dropping	**Isolation**	Soil around eucalyptus tree
Immunocompromised patients	**Immunity**	Both immunocompromised and immunocompetent patients
Easy to treat	**Treatment**	Prolonged treatment

Cryptococcus Neoformans - Laboratory Diagnosis

- **Route :** Inhalation.
- **Entry:** Nose and Lungs.
- **Affect:** Usually whole body, but it affects the brain more (Meningitis).
  - Most cases have cryptococcal meningitis.
- **Stain Tests**
  - **CSF Sample:** Negative Stain (India Ink/Nigrosin).
  - **Biopsy:** Mucicarmine.





- **Culture Media Tests**
  - **SDA:** Shows mucoid colony (due to capsule).
  - **Niger or bird or caffeic seed agar:** Gives brown colour (due to melanin due to phenol oxidase /laccase).

- **Confirmatory Test**
  - LAT - Latex Agglutination Test.

Cryptococcus Neoformans – Treatment

00:09:42

- **Meningitis:** Liposomal amphotericin B (LAMB) + Flucytosine.

Candida

00:10:02

- Similar to yeast (Budding + Pseudohyphae).

Types of Organisms

- **Candida albicans** - Most common infection.
- **Candida dubliniensis** - Copy double to Candida albicans.
- **Candida glabrata** - No pseudohyphae.
- **Candida auris** - Resists azoles.
- **Candida krusei** - Resists azoles.
- **Candida kefyr** - Used as a control in susceptibility tests.

Candida - Infecting Sites

00:13:25

- GIT
- Genital tract
- Mucosa
- Skin
- Nails
- Internal organs

Types of Infections

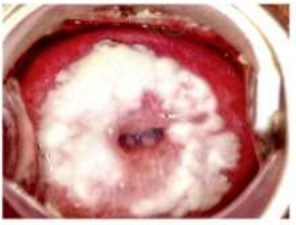
00:13:53

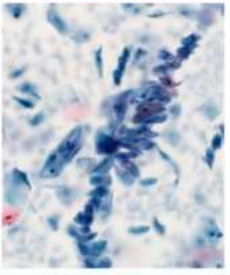
Muco-Cutaneous (Curdy white discharge)	• **Mouth:** Oral thrush • **Oesophagus:** Esophagitis • **Vagina:** Vulvovaginitis • **Penis:** Balanitis • **Eyes:** Ocular candidiasis
Nails	• Paronychia • Onychomycosis
Urinary tract	UTIs
Heart	Endocarditis
Brain	Meningitis
Lungs	Lis
In Kids	Diaper rashes

Candida - Vulvovaginitis

00:15:20

- Most common in women.
- Curdy white discharge is seen at the cervix.





- **Shish Kebab Effect:** Pseudohyphae is the stick and epithelial cells of the cervix are kebabs.

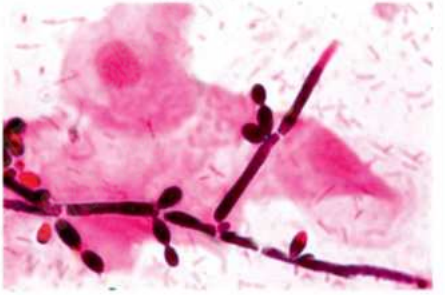
Candida - Predisposing Factors

- Old age
- Infancy
- DM
- Steroids
- Pregnancy
- HIV
- Immunosuppressives
- Immunodeficiency diseases
- Low Neutrophil count - Diaper rashes.

Candida Albicans - Laboratory Diagnosis

00:18:10

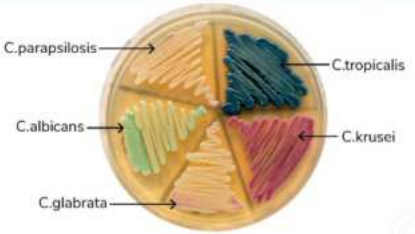
- Has both buds and pseudohyphae.
- Seems like yeast.



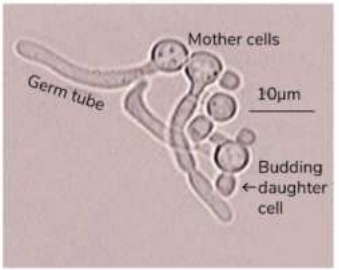
- **Culture media test - SDA**
  - Creamy - Cream like
  - Pasty - Paste
  - Yeasty - Odour

- **Corn meal agar test**
  - Candida + Corn meal agar = Chlamydospores
  - Identified by double wall configuration.

- **Chrom agar test**
  - Colour differentiation test
  - Each type of organism is seen differently.



- **Germ tube test**
  - Termed as Reynolds Braude Phenomenon
  - Candida + Human serum → Incubation → Big tube-like structure (Pseudohyphae).



Important Information

- C.albicans and C.dubliniensis have everything similar.

Candida Albicans vs Candida Dubliniensis 00:22:40

Candida Albicans	Feature	Candida Dubliniensis
+ve	Germ tube test	+ve
+ve	Chlamydospores	+ve
Yes	Growth above 45 degree	No
Yes	Growth in hypertonic saline	No

Candida - Treatment 00:23:50

- Thrush and Vulvovaginitis - Topical nystatin.
- Severe - Fluconazole/nystatin
- Deep (organ involvement) - LAMB.
- Azoles are commonly given (but not in auris or krusei as they are resistant).

Aspergillus - Asp 00:24:35

Types of Infection

- **ABPA**
  - Allergic BronchoPulmonary Aspergillosis (Combination of Type-1 and Type-3 Hypersensitivity).
  - Due to repeated and heavy exposure.
- **Aspergilloma**
  - A Lesion in lungs
  - Aspergillus grows in a pre-existing cavity (2° TB, Histoplasma, Sq cell carcinoma, Bronchiectasis).

Important Information

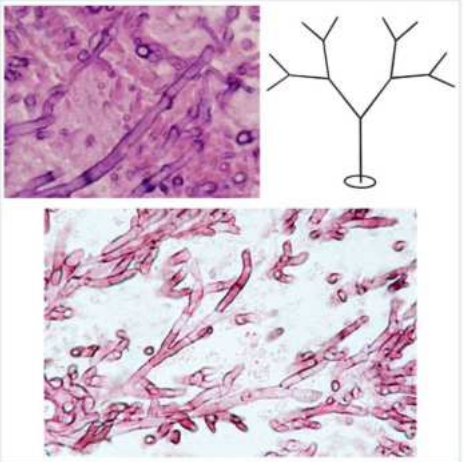
- Aspergillus does not cause cavities; rather it sits in the pre-existing cavity.

- **Invasive aspergillosis**
  - Disseminated
  - Everywhere in the body
- **Superficial**
  - Otomycosis - Ear

Aspergillus Under Microscope

00:28:43

- Pulmonary (**A SPE** rgillus)
  - Acute angle dichotomous branching (<45°, two branches like trees).



  - **SPE** (remember the septate hyphae).

Aspergillus Under Culture

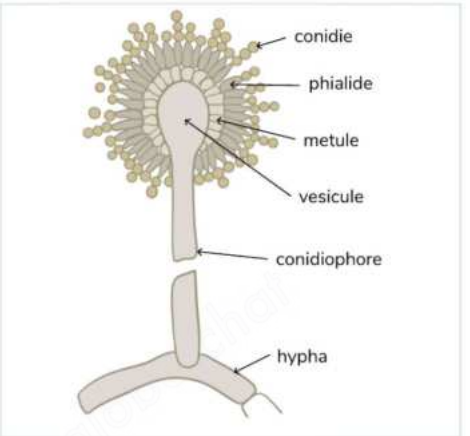
00:30:29

Major Types	Colony Colour
Aspergillus fumigatus	Smoky green (Fume - smoke)
Aspergillus flavus	Yellow green (Flavus - colours of flower)
Aspergillus niger	Black (Niger - black)

- Statin - Lactophenol cotton blue.

Culture Morphology

00:33:20



Refer Table 34.1

Important Information

Rare Type - Aspergillus Terreus

- Beige colour (cinnamon).
- Resistant to azoles.

Aflatoxin

00:36:38

- Aspergillus flavus releases toxin (Afla toxin).
- Cause mutation at codon 249 of p53 gene - leads to hepatocellular carcinoma (HCC).
- Groundnut contaminant - long term storage.

Aspergillus - Treatment

00:39:48

- **Aspergilloma** - Surgical resection needed.
- **Disseminated Aspergillosis** - Voriconazole.

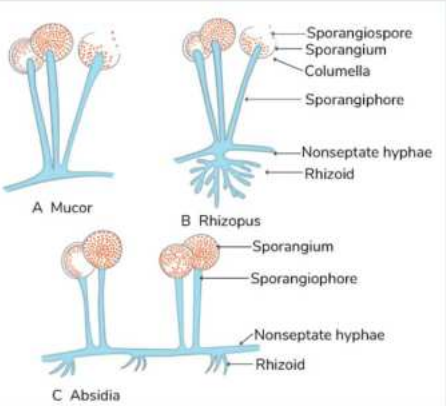
Important Information

- Voriconazole is used to treat disseminated aspergillosis.

Mucor / Zygomycetes / Glomeromycetes

00:39:39

- Has **RAM**
  - Rhizopus
  - Absidia/ Leichthemia
  - Mucor



Type	Hyphae	Root
Rhizopus	Aseptate	Below (Rhi - Root)
Absidia/ Leichthemia	Aseptate	In between
Mucor	Aseptate	No root

Clinical Symptoms

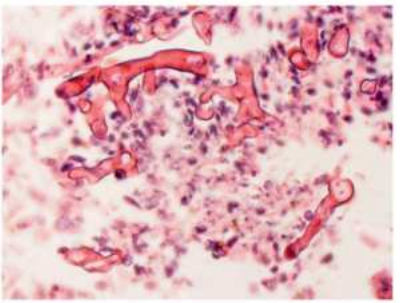
00:41:42

- **Rhizopus Oryzae**
  - Rhino-oculo-cerebral disease - Nasal discharge (Rhino), Proptosis (Oculo), Delirium (Cerebral - CNS).
- **Mucor**
  - Major
  - More concerned in Covid-19 due to use of steroids.
  - Lead to black colour lesions - Black fungus.

Mucor-Tissue Biopsy

00:43:17

- Opposite to Aspergillus.
- Obtuse angle (>120°) or 90° branching
- Aseptate hyphae



Aspergillus vs Mucor

00:44:00

Feature	Aspergillus	Mucor
Branching	<45°	>90° or 90°
Hyphae	Septate	Aseptate
Shape	Thin	Broad Ribbon

Mucor - SDA Culture

00:44:37

- Cotton wool growth.
- Salt and pepper look.
- Referred as lid lifters.

Mucor - Treatment

00:45:42

- **Black lesion** - Surgical resection
- Amphotericin-B
- Posaconazole (Alternative medicine)

Extra Info About Fungal Growth Testing

MARKER	BETA(1-3)D-GLUCAN test	ASPERGILLUS GALACTOMANNAN test	
WHAT IS IT?	Polysaccharide cell wall component of may fungi	Major polysaccharide constituent of Aspergillus cell walls	
PATHOGENS	ROUTINE USE	Candida, Aspergillus	Aspergillus
	Other +S	PJP, Histoplasma, Fusarium, Scedosparium, dermatophytes, Cocci	Fusarium, Pencillium, Histoplasma, Blastomyces, Paecilomyces, cryptococcus
	NEGATIVE	Cryptococcus, Mucorales, Blastomyces dermatidis	Mucarales

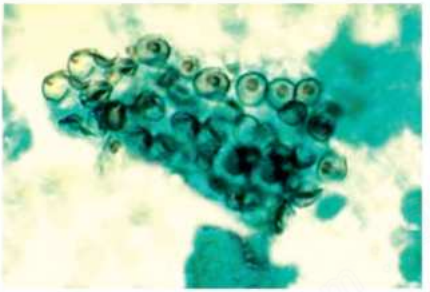
Important Information

- Beta-D-Glucan is negative for Blastomyces, Mucor, Cryptococcus (BMC)
- Aspergillus Galactomannan is negative for Mucor.

Pneumocystis Carinii

00:48:06

- Ergosterol is absent in cell membranes.
- **Disease -** Pneumocystis Carinii Pneumonia (PCP).
- **Feature -** Plasma Cell Pneumonia (PCP).
- **Appearance -** Crushed Ping Pong ball (PCP).
- **Clinical features -** Pneumonia features, Dry cough.
- **Sample -** BronchoAlveolar Lavage (BAL) is taken.
- **Stain -** GMS (Gomori Methenamine Silver).
- **Microscopy -** Crushed Ping Pong ball (also called Hat or Cup and Saucer appearance).



Important Information

- Pneumocystis carinii and Rhinosporidiosis are non-cultivable.

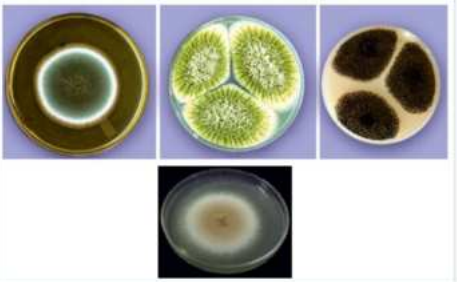
Treatment

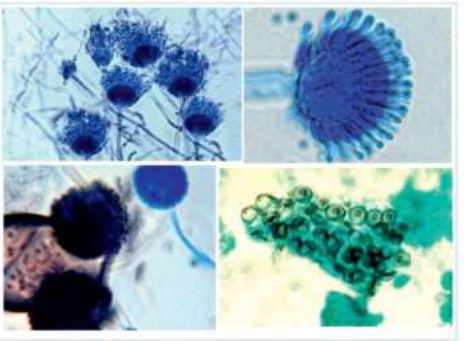
00:53:07

- Pneumocystis - Cotrimoxazole and Pentamidine (PCP).

Summary

00:54:02





MCQ

00:58:50

Q. Reynolds braude phenomenon is seen with?

A. Candida albicans

B. Candida dubliniensis

C. Gardnerella

D. Histoplasma

Answer: Both A and B (Reynolds braude phenomenon is seen in Germ tube test, which is positive for both Candida albicans and dubliniensis).

Q. Morphological feature of pneumocystis carinii infection is?

A. Haemorrhage

B. Intra-alveolar exudate with plasma cells infiltrate

C. Abscess

D. ARDS

Q. A vitreous aspirate from a case of metastatic endophthalmitis on culture yields Gram-positive round to oval cell, 12-14 micron in size. The aspirate on Gram stating shows the presence of pseudohyphae. Which of the following is the most likely etiological agent?

A. Aspergillus

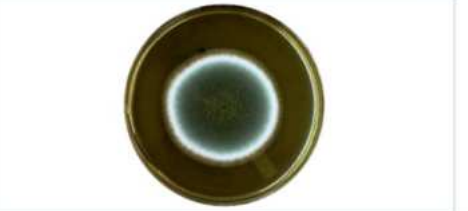
B. Rhizopus

C. Candida

D. Fusarium

Q. Colony of aspergillus shown in the image is?

01:00:18

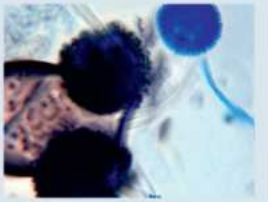


A. Aspergillus fumigatus
B. Aspergillus flavus
C. Aspergillus niger
D. Aspergillus nidulans

Q. BETA (1-3) D glucan testing is done for?
A. Candida
B. Fusarium
C. Aspergillus
D. Pneumocystis
E. All of the above

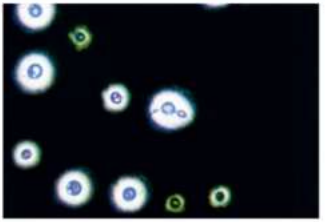
Q. LPCB statined periorbital exudates in severe panophthalmitis with cellulitis in an elderly diabetic shows obtuse angle branching aseptate and broad hyphae. Which of the following is the most likely diagnosis?
A. Candida
B. Aspergillus
C. Penicillium
D. Rhizopus species

Table 34.1

Type	Conidia Position	Image
Aspergillus fumigatus	Along the upper part of vesicles (fume - present at upper part)	
Aspergillus flavus	All around the vesicles (flower has petals all around)	
Aspergillus niger	Blackish colour	

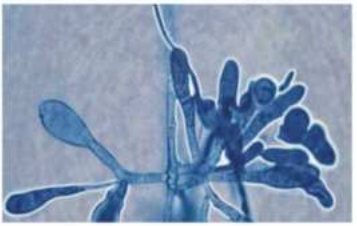
Q. Which is not a dimorphic fungus? (AIIMS Nov 2017)
A. P. Marneffei
B. Histoplasma Capsulatum
C. Blastomyces Dermatitidis
D. Pneumocystis Jirovecii

Q. Identify organism stained by India ink dye & shown below? (FMGE Dec 2020)



A. Cryptococcus
B. Chlamydia
C. Histoplasma
D. Aspergillus

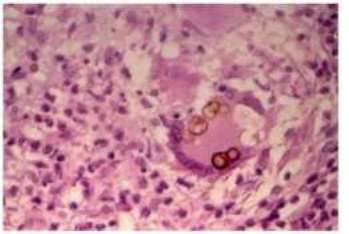
Q. The LPCB mount shown below is of? (FMGE Aug 2020)



A. Aspergillus Niger
B. Epidermophyton
C. Rhizopus
D. None of the above

Q. A patient presented with some unknown fungal infection. Microscopic examination revealed brown coloured spherical fungi with septate hyphae. Possible conditions? (FMGE June 2019)
A. Histoplasmosis
B. Chromoblastomycosis
C. Coccidioidomycosis
D. Candida albicans

Q. A 40-year-old farmer was struck with a wooden stick while working in the forest of Himachal Pradesh. The wound later became deep infection & involved the surrounding lymphatics too? (AIIMS Nov 2017)



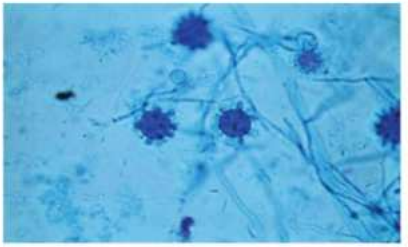
A. Chromoblastomycosis
B. Histoplasmosis
C. Sporotrichosis
D. Penicillium Marneffei

Q. Which of the following is most likely to be acquired by traumatic inoculation? (NEET Jan 2020)
A. Blastomyces Dermatitidis
B. Sporothrix Schenekii
C. Coccidioides immitis
D. Paracoccidioides Brasiliensis

Q. Gardner noticed multiple vesicles on his right leg along the lymphatics. What is the probable cause for this? (AIIMS June 2020)

A. Sporothrix schenekii
B. Histoplasma capsulatum
C. Candida albicans
D. Rhinosporidium Seeberi

Q. Patient with history of travel to Ohio shows the following culture smear characteristic with lactophenol cotton blue staining? (FMGE Dec 2020)



A. Coccidiomycosis
B. Histoplasmosis
C. Cryptococcus
D. Blastomycosis

Q. What could be most probable diagnosis for the image given below? (FMGE Aug 2020)

A. Leucoplakia
B. Oral cavity CA
C. Oral thrush by candida
D. Oral lichen planus

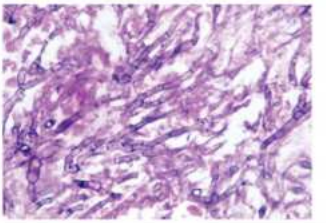
Q. An HIV positive patient with CD4 count of 300 / Wm presents with mucosal lesions in the mouth. On microscopy budding yeast & pseudo-hyphae are seen. What is the likely diagnosis? (NEET Jan 2020)
A. Candidiasis
B. Oral hairy leucoplakia
C. Lichen planus
D. Oral cavity CA

Q. 1-3 Beta d-glucan assay is done for which infection? (AIIMS Nov 2017)
A. Invasive candidiasis
B. Cryptococcus
C. Invasive Rhinosporidiosis
D. Rhinocerebral Mucormycosis

Q. HIV positive patient develop Cryptococcal meningitis which rapid test will be used? (FMGE Aug 2020)
A. Indian Ink Test
B. Latex card agglutination test (Antigen based test)
C. Culture
D. PCR

Q. Beta 3 glucan assay testing done for? (AIIMS Nov 2019)
A. Invasive candidiasis
B. Aspergillosis
C. Pneumocystis Carinii
D. Mucormycosis

Q. Fungal Hyphae with acute angle branching & septate hyphae. Identify? (FMGE Dec 2019)



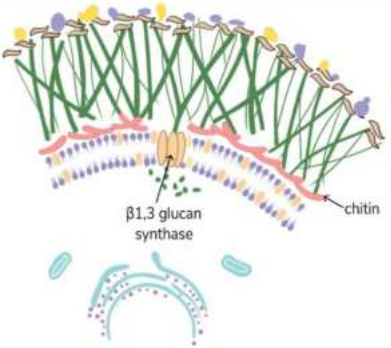
A. Aspergillosis
B. Mucor
C. Histoplasma
D. Sporothrix

Q. Following figure showing slide of organism stained with Gommori methenamine silver. What features & which organism is it? (AIIMS Nov 2017)

A. Fungi is showing acute angle branching with septate filament & is aspergillus
B. Fungi is showing non septate acute angle branching &is candida

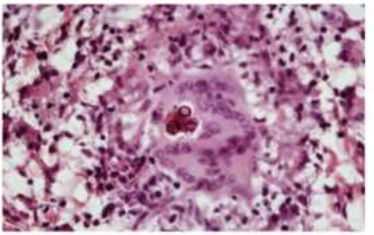
C. Fungi is showing right acute angle branching with septa and is mucor
D. Fungi is showing non septate acute angle branching &is aspergillus

Q. Identify the pathogen (INI CET May 2022)



A. Aspergillus
B. Mycoplasma
C. Mycobacterium
D. Rhinosporidium

Q. Biopsy from warty lesions of foot shown on the image which of the following is shown below? (INI CET May 2022)



A. Budding yeast
B. Negri body
C. Sclerotic body
D. Asteriod body

Q. A patient presented with severe itching in the axilla. A sample is taken. When the organisms are isolated, they shows red color on SDA. Which organism is this? (NEET PG May 2022)

A. T. Rubrum
B. T. Violecum
C. T. Schoneii
D. None of the above

35 PARASITOLOGY : CLASSIFICATION OF AMOEBA

Classification 00:00:47

1. **Amoeba**
- **Intestine**
  - Entamoeba Histolytica
  - Entamoeba coli
- **Free living**
  - Naegleria fowleri
  - Acanthamoeba

2. **Ciliated**
- Balantidium coli

3. **Flagellated**
- **Intestine:** Giardia lamblia
- **Genitalia:** Trichomonas vaginalis
- **Blood and tissue**
  - Leishmania
  - Trypanosoma

4. **Sporozoa**
- Malaria
- Babesia
- Toxoplasma
- Coccidian parasites

Intestinal Amoeba - Entamoeba Histolytica 00:03:33

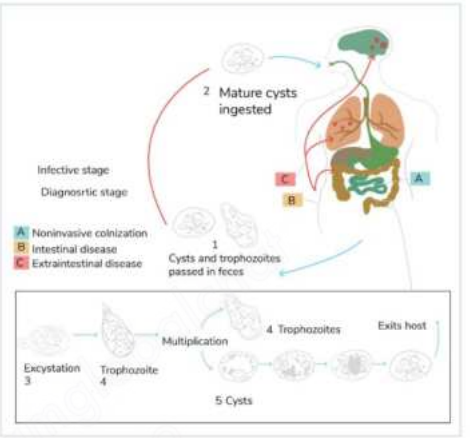
- 3 stages
  - Trophozoite
  - Pre-cyst
  - Cyst
- Host: Humans
- Infective form:
  - Cyst form
  - Quadrinucleate cyst
- Infective dose: Small <100 cysts

Important Information

Organisms with Small Infectivity Dose
Mnemonic: CHHOTA SMALL
- **C** – Cryptosporidium
- **H** - E.Histolytica
- **H** - Enterohemorrhagic coli
- **S**-Shigella
- **LLAM** - Giardia lamblia

- Cysts survive chlorination of water.
- Mode of transmission-
  - Most common: Faeco-oral route
  - Rarely: Homosexual

Life Cycle of Entamoeba Histolytica 00:07:15



- Human host
- Quadrinucleate cyst
- Ingestion (Faeco-oral route)

Quadrinucleate cyst
↓
Ingestion
↓
Excystation in Small intestine
↓
Trophozoites
↓
Encystation in Large intestine
↓
Cysts
↓
Both Trophozoites and Cysts come out via fecal matter
↓
Cysts survive in environment as it is strong.
↓
Cysts
↓
Ingestion
↓
Repeat

Virulence Factors 00:10:58

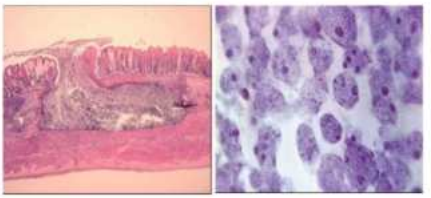
- Gal/ GalNac Lectin antigen
- Enzymes
  - Metallo-collagenase
  - Neuraminidase

Organs Affected

00:11:53

01. Intestine

- Causes traveler's diarrhea
- Flask shaped ulcer are seen in histopath.



Why Flask Shaped Ulcer?

- Damages the mucosa and submucosa.
- Can't damage the muscularis as it is difficult to break.
- Thus, it starts breaking submucosa on left and right.
- Resulting in flask shaped ulcer

- Inside flask shaped ulcers- Trophozoites are seen.
  - 1 big nucleus\
  - Engulfed RBCs (Erythrophagocytosis)
- Clinical Features-
  - Most common: Asymptomatic intraluminal amebiasis (50-80%)
  - Amoebic colitis (Large intestine)
  - Appendicitis (Appendix)
  - Bloody diarrhea
- Complications-
  - Perforation
  - Peritonitis
- Chronic cases
  - Ameboma/ Amoebic granuloma at Rectosigmoid region

02.Liver

00:16:40

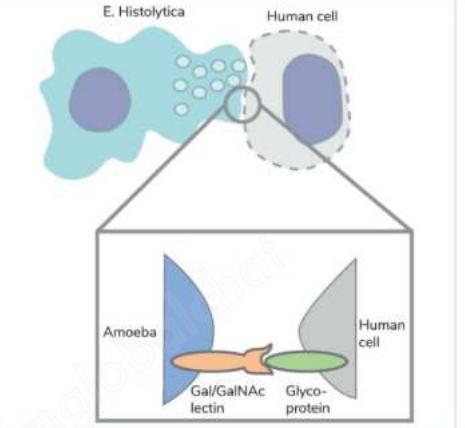
- The posterior superior surface of the right lobe is affected.
- **Gross:** Anchovy sauce appearance
- **Microscopy:** Trophozoites in Pus

To Remember

Trophozoites are seen in microscopy for both liver and intestine.

Trogocytosis

00:18:08



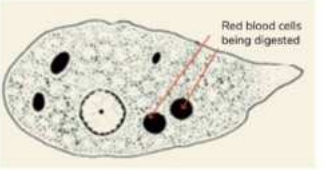
- Attached to cell with the help of Gal/ GalNac Lectin antigen
- Leads to Nibbling of cells (forming borrow like rat) which is called as Trogocytosis

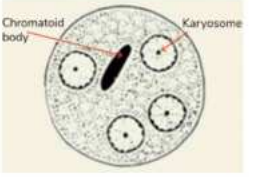
Diagnosis of Entamoeba Histolytica

00:19:26

- 3 stool samples (consecutive days)
  - Intermittent shedding
  - **Trophozoites** - Active disease
  - **Cyst** - Carrier

Trophozoite





- Pointed pseudopodia
- 1 nucleus
- Central karyosome
- Cartwheel nucleus
- Erythrophagocytosis

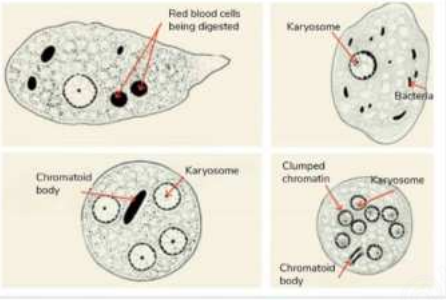
Cyst

- 1 to 4 nucleus-Quadrinucleate
- Thick Chromatoid body/ bar

Chromatoid Bar

- **Composition:** Ribonucleoprotein (RNP)
- **Stain:** Iron hematoxylin

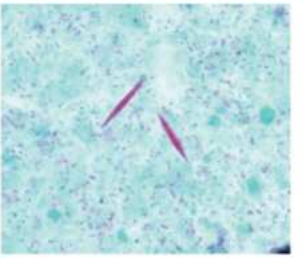
Entamoeba Histolytica vs Entamoeba Coli 00:24:01



Entamoeba Histolytica	Entamoeba Coli
• **Trophozoite** o Pointed pseudopodia o 1 nucleus o Central karyosome o Cartwheel nucleus o Erythrophagocytosis • **Cyst** o 1 to 4 nucleus - Quadrinucleate o Thick Chromatoid body/ bar	• **Trophozoite** o Blunt pseudopodia o 1 nucleus o Eccentric karyosome o No Cartwheel nucleus o No Erythrophagocytosis • **Cyst** o 1 to 8 nucleus - Octanucleate o Thin Chromatoid body/ bar

- **Culture Tests - Stool culture**
  - Polyxenic medium-Supplemented with bacteria (low parasite load)
    → Nelson culture
    → Balamuth medium
    → Boeck and DrBohlav medium
  - Axenic culture medium - Pure, no bacterial supplement
    → Diamond medium (pure as diamond)
- **Other**
  - Antigen/ Antibody testing (ELISA)
- Miscellaneous finding on stool microscopy- Charcot Leyden crystal

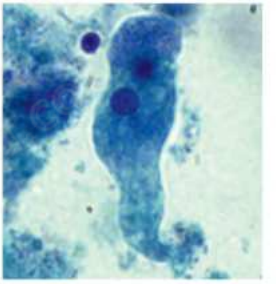
- **Composition:** Galectin 10 from eosinophils



Eosinophil Elevation

- Allergy
- Parasitic infections

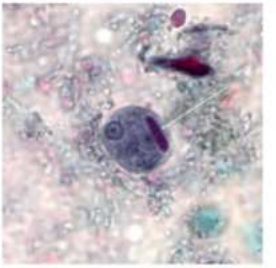
Image Based Testing



Q.

Answer: Entamoeba Histolytica trophozoite

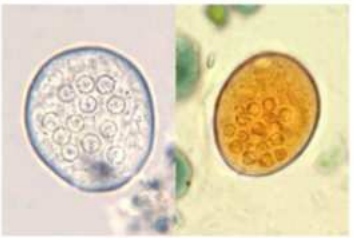
- Has pointed pseudopodia
- 1 nucleus
- Central karyosome
- Cartwheel nucleus
- Erythrophagocytosis



Q.

Answer: Entamoeba Histolytica cyst

- 1 to 4 nucleus – Quadrinucleate
- Thick Chromatoid body/ bar



Q.

Answer: Entamoeba Coli cyst (non pathogenic)

1 to 8 nucleus - Octanucleate

Treatment

00:31:10

- **Active**
  - Metronidazole x 10 days
  - Followed by luminal amebicide - Paromomycin.
- **Asymptomatic**
  - Paromomycin - For prevention if patient is carrier.

Other Members of the Entamoeba Family

00:32:18

- Entamoeba dispar-same size of trophozoit
- Entamoeba moshkovskii - same trophozoit
- Entamoeba bangladeshi-same trophozoit
- Entamoeba hartmanni - small size trophozoit (half the size)

To Remember

PCR and Enzyme studies (Zymodeme studies) are done to differentiate the Entamoeba members.

MCQs

Q. Diagnostic test for amoebic hepatitis

A. Cysts in sterile pus
B. Trophozoites in the pus
C. CYst in the intestine
D. Trophozoites in the feces

Answer: Trophozoites in the pus

Q. Number of nuclei in mature cyst of entamoeba coli?

A. 1
B. 2
C. 4
D. 8

Answer: 8

Q. What is the composition of amoebic chromatoidal bars?

A. Ribonucleoprotein
B. Glycogen
C. Glycoprotein
D. Lipoprotein

Answer: Ribonucleoprotein (RNP)

Q. The single characteristic feature that is most Diagnostic in differentiating entamoeba histolytica from the other intestinal amoebae is the?

A. Size of the cyst
B. Structure of the nucleus
C. Presence of chromatoid bodies
D. Number of nuclei

Answer: Structure of the nucleus

Free Living Amoeba

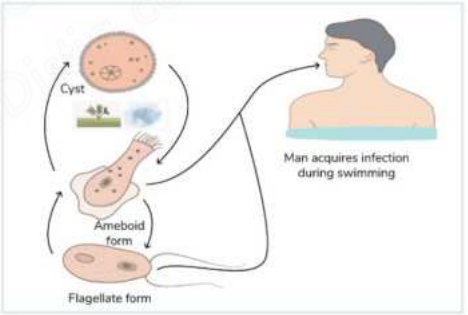
00:36:25

1. Naegleria fowleri
2. Acanthamoeba
3. Balamuthia

01. Naegleria Fowleri

Life Cycle

00:36:41



- Cyst- round
- Trophozoite - amoeboflagella
  - Sometimes it can have flagella and other times it can be in amoeboid form.
  - Infective form.

Trophozoite enters via swimming

↓

Water with Trophozoite enters nose

↓

Reaches brain (via cribriform plate)

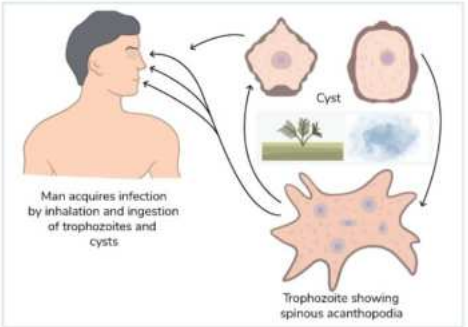
↓

PAME (Primary Amoebic Meningoencephalitis)

02. Acanthamoeba

00:39:34

Life Cycle



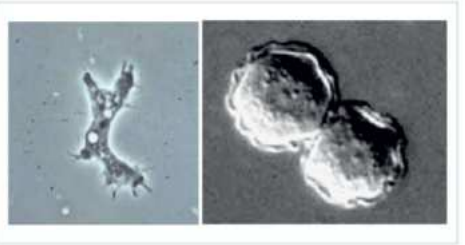
- Trophozoites show spikes (Acantho - Spikes)
- Cyst is present.
- Both Cyst and Trophozoites can cause disease.
- Entry through-
  - Eyes
  - Nose
  - Mouth
  - Cutaneous
- **Disease caused:** GAME-Granulomatous Amoebic Encephalitis

Naegleria Fowleri vs Acanthamoeba

00:41:36

Features	Naegleria Fowleri	Acanthamoeba
Disease	PAME	GAME
Risk factor	Swimming	Immunodeficiency
Infective form	Trophozoite	Both Cyst and Trophozoite
Clinical course	Acute (fatal <5 days)	Chronic (late)
Spread	Neural	Blood
Diagnosis	CSF • Trophozoites • Increased polymorphs	Brain biopsy • Trophozoites • Cysts
Culture	NNA (Non-Nutrient Agar) - lawn culture of Entamoeba coli	NNA (Non-Nutrient Agar) -lawn culture of Entamoeba coli
Gold standard	NAAT (Nucleic Acid Amplification Testing)	NAAT (Nucleic Acid Amplification Testing)
Treatment	Amphotericin B	Pentamidine + Azole + Sulfonamide + Flucytosine

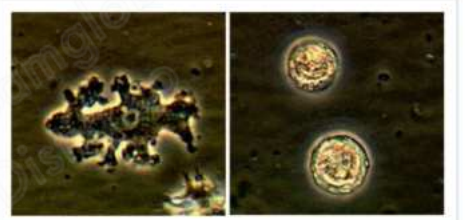
Acanthamoeba



- **Image A**
  - Trophozoite
  - Spikes
- **Image B**
  - Cyst
  - Outer Wrinkled Wall

03. Balamuthia Mandrillaris

00:47:16



- **Similarities with Acanthamoeba**
  - Disease: GAME
  - Causative: Trophozoite and Cyst
- **Difference from Acanthamoeba**
  - Trophozoite is fish shaped.
  - Cyst is smoother.
  - Can occur in both normal and Immunodeficiency.

To Remember

- Acanthamoeba can infect the eyes via contact lens and lead to Amoebic Keratitis
- Not seen in Balamuthia Mandrillaris

MCQs

Q. A patient with contact lenses developed corneal ulcers and symptoms of conjunctivitis. Saline Mount preparation of corneal scraping shows double walled cyst with irregular outer wall and smooth inner wall. Probable diagnosis?

A. Acanthamoeba
B. Naegleria
C. Entamoeba
D. Giardia

Answer: Acanthamoeba

Q. A 15 year old girl residing in a village recently returned from a vacation visiting her friends in another village. She complained of severe headache and fever and was diagnosed as a case of pyogenic meningitis and admitted to the hospital. She died 5 days later. Which of the following organisms should be considered in the diagnosis?

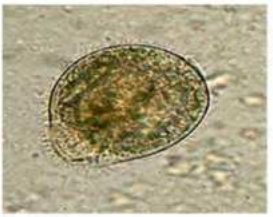
A. Entamoeba histolytica
B. Naegleria fowleri
C. Toxoplasma gondii
D. Falciparum malaria

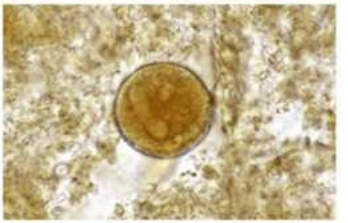
Answer: Naegleria fowleri

Ciliate-Balantidium Coli

00:51:16

- Only ciliated parasite of humans.
- Largest Protozoa invading human intestine.
  - Large intestine involvement
- Infective form - Cyst
- Both trophozoite and cyst are binucleated
  - 1 big bean shaped nucleus
  - 1 small nucleus
- It shows Rotatory motility with the help of cilia.
- Reservoir- pigs
- Treatment- Doxycycline





MCQs

Q. All are true about B.coli except?

A. It is a protozoa.
B. Cyst is the infective stage.
C. Usually invades liver.
D. It is the largest protozoan parasite inhabiting large intestine in a man.

Correct Answer: C

Q. There is no change in the number of nuclei of cyst and of trophozoite of ?

A. Balantidium coli
B. Entamoeba coli
C. Entamoeba histolytica
D. Giardia lamblia

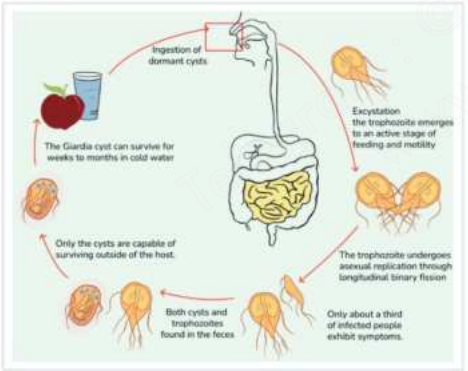
Correct Answer: a

Giardia lamblia

00:00:36

- Most common parasitic infection in the world
- Most common parasite found in stool
- Habitat - Mucosa of duodenum and upper ileum
- Infective stage - cyst
  - Most have this infective stage except Naegleria fowleri, where foul play occurs with trophozoites.
- Infective dose
  - 10-25 cysts can cause infection
  - Have a small infectivity dose
- Mnemonic of small infectivity dose
  - Chhota small
  - C - Cryptosporidium
  - H -Entamoeba Histolytica
  - H - Enterohemorrhagic E. coli
  - S - Shigella
  - Mall/ laam- Lamblia
- Route of infection
  - Feco-oral route

Life cycle of Giardia lamblia



- Host is only human
- Contaminated food and water is consumed
- Ingested form - cyst
- Excystation occur
  - The cyst will change into a trophozoite

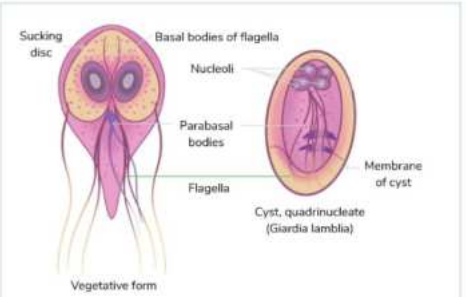
Q. How does Giardia lamblia attach to the duodenum?
Ans. By
- Suckling disc/sucking disc
- Lectin antigen

- Trophozoite will divide
- And after attachment to the duodenum of the intestine, it will cause the **blunting of the villi**, and iron deficiency anemia will be there.
  - Because Duodenum is affected and iron will not be absorbed
- The patient will also get Disaccharidase enzyme deficiency because disaccharidase is not absorbed
- The patient will get classical malabsorption because
  - Fat, B12, folic acid deficiency, and protein deficiency also follows
- The hallmark of malabsorption is that the fat which does not get absorbed, will go out into the stool
  - Steatorrhea
    - → Bulky, frothy, foul smelling, and difficult to flush
- Giardia lamblia leads to fatty diarrhea
- Stool sample will have cyst (can survive in the environment) and trophozoite

Lab diagnosis

00:07:51

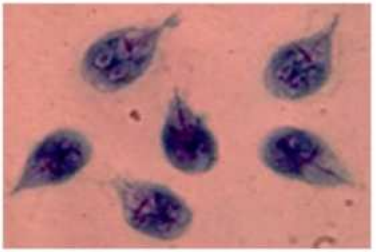
- **Sample** - stool
  - May or may not show cyst and trophozoite
  - Take 3 consecutive day stool sample
- **Microscopy**
  - Cyst and trophozoite are seen



Trophozoite of Giardia lamblia and cyst

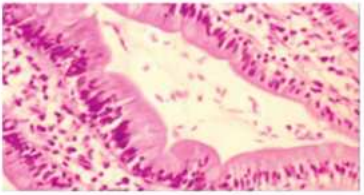
- **String test**- done when trophozoites are not seen in stool sample
  - The Duodenal content is taken
  - The string is put into the intestine; that's why it's called enterotest
  - Also done in
    - → Vibrio cholera
    - → Klebsiella

- A capsule will have a string and the capsule is swallowed
  - → It goes in the duodenum and it dissolves
  - → The string is pulled out and it has duodenal contents
- A microscopic examination is then done



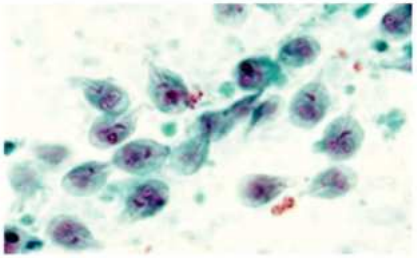
Trophozoite of Giardia lamblia

- When stool and string test fail then **duodenal biopsy** is done
  - Giardia lamblia is a luminal organism
    - → Mnemonic-not only angry, but he is a girta hua man (falling in the lumen)



Duodenal Biopsy

  - Sickle shape organism is seen in the lumen
  - Falling leaf motility is shown by the trophozoite
- **Antibody and antigen test is done**
- **Gold standard -**
  - Polymerase chain reaction



Trophozoite of Giardia lamblia

Cyst of Giardia lamblia

Treatment

- Drug of choice
  - Metronidazole
    - → People showing resistance to it because its a longer therapy
  - Tinidazole
    - → Single dose

Trichomonas Vaginalis

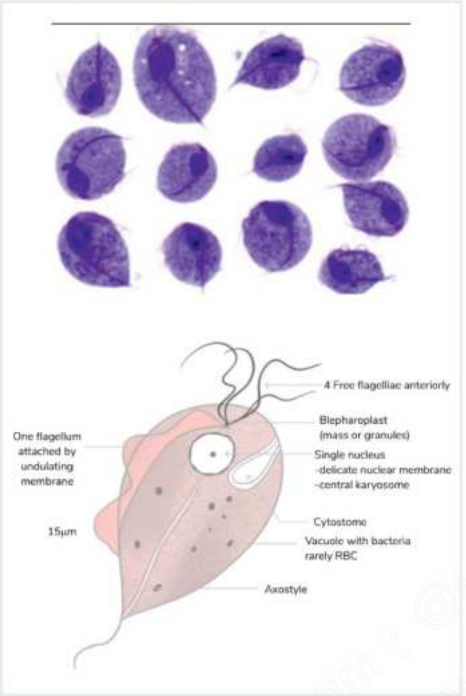
00:17:08

- Most common cause of STD and NGU
- Vagina indicates sexually transmitted disease and non gonococcal urethritis
- Habitat - one of the genital
  - Urethra, vagina, prostate
  - Occur in both male and female
    - → Treatment is of both the partners
- Mode of transmission
  - Sexually transmitted disease
- Reservoir
  - Females
- Infective & Diagnosis stage-trophozoite
- Incubation period
  - 4-28 days
- In male
  - Urethritis
  - Bladder involvement (cystitis)
  - Prostate involvement (Prostatitis)
- In Female
  - Strawberry cervix / colpitis macularis
    - → Red dots are seen on cervix
    - → A greenish discharge is seen

Diagnosis

- Sample - discharge sample
  - PAP smear is done from the cervix

Microscopic examination-



- Trophozoite is seen only
- On pap smear
  - A pear shape is seen, and one nucleus

Motility-

- Shows twitching motility
- Jerky/wobbling/ rotatory

Culture

- Lash cysteine hydrolysate serum
- Diamond medium
- Trussell and Johnson medium
- Feinberg Whittington medium

Treatment

- Metronidazole given to both the partners

Important Differences between Giardia Lamblia and Trichomonas Vaginalis

00:24:03

Category	Giardia Lamblia	Trichomonas Vaginalis
Motility	Falling leaf	Twitching
Site	Duodenum	Genitals
Trophozoite	Two nuclei	One nucleus
Flagella	4 pairs (8)	Four anterior and one posterior = total five

MCQ

Q. Which of the following statements is true about Trichomonas?

A. It has 5 flagella at the posterior end
B. Cysts form is infective
C. Has a suckling disc for attachment
D. Survives well in hot and cold water

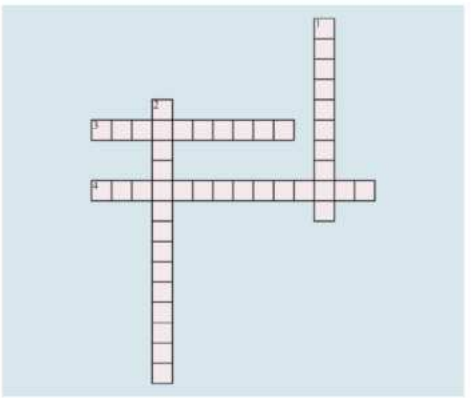
Ans. d

Explanation –

- Only one flagella at the posterior end
- No cyst
- Suckling disc is there in giardia lamblia

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. The string is put into the intestine; that's why it's called Enterotest
4. Organisms hanging in the lumen

Down

1. cyst and trophozoite are seen
2. Most common parasitic infection in the world

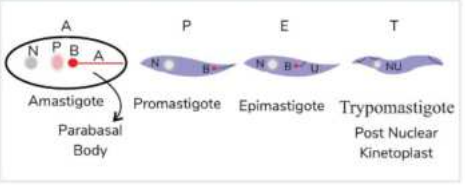
37 PARASITOLOGY: HEMOFLAGELLATES

Hemoflagellates 00:00:06

- Present in blood and tissues.
- E.g.-
  - Leishmania
  - Trypanosoma

APET 00:00:32

- A -Amastigote
- P -Promastigote
- E -Epimastigote
- T -Trypomastigote



- In Amastigote- Flagella A is trying to come out but could not.
- In rest all three (Promastigote, Epimastigote, Trypomastigote- Flagella A is coming out from Kinetoplast
- P in the image refers to Parabasal body & B in image refers to Blepharoplast- together they are known as Kinetoplast
- Flagellar end is the anterior end in all & the nuclear end is posterior end.
- Kinetoplast is always anterior except in Trypomastigote in which there is Post Nuclear Kinetoplast

Mnemonics 00:03:31

- BET
  - B- Trypanosoma brucei
  - E- Epimastigote
  - T- Trypomastigote are seen
- CATE
  - C- Trypanosoma Cruzi
  - A- Amastigote
  - T- Trypomastigote
  - E- Epimastigote are seen.
- LAP
  - L- Leishmania
  - A- Amastigote
  - P- Promastigote are seen.

Trypanosomes 00:05:10

2 groups

1. Salivaria
   - Comes from saliva when insects bite
   - Species of Trypanosoma brucei
2. Stercoraria
   - Insect passes the organism in feces
   - Trypanosoma Cruzi

Trypanosoma Cruzi / South American Trypanosomiasis 00:06:30

- Definitive host: Man
- Intermediate host: Reduviid bug / Triatomine / Kissing Bug (nocturnal)
- Reservoir: Many animals such as Armadillo, Cat, Dog, Pig

Life cycle 00:09:04

Refer Images 37.1

- Amastigote, Trypomastigote, Epimastigote will be seen.

Reduviid bug→Trypomastigote (infective form)→Bites Human → Amastigote form → Trypomastigote → Enters blood → Bug will come and bite again taking away Trypomastigote → Epimastigote → Trypomastigote

- In insects- Epimastigote and Trypomastigote are seen
- In Adults- Amastigote and Trypomastigote are seen

Chagas Disease 00:12:41

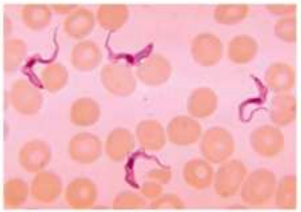
Acute Chagas Disease	Chronic Chagas Disease

Chagoma (entry site edema)
• Lymphadenopathy
• Eyes big (Romana Sign- Periorbital edema
• Hepatosplenomegaly
	• Heart –
	  ○ Myocarditis
	  ○ Dilated cardiomyopathy.
	• Megaesophagus (Achalasia cardia)
	• Megacolon
	• Brain (Meningoencephalitis)

Romana Sign

Diagnosis 00:14:53

- Sample-
  - Blood sample
  - Buffy Coat (WBCs between RBCs & Plasma)



- Trypomastigotes are seen
  - Nucleus at center
  - Kinetoplast from where Flagella is coming out from anterior end
  - Post-nuclear Kinetoplast
- Culture- NNN (Novy McNeal Nicolle) used in
  - T. Cruzi
  - Leishmania

Treatment 00:17:09

- Drug of choice is Benznidazole.
- Symptomatic treatment

Trypanosoma Brucei 00:18:13

- Causes Sleeping Sickness.

Features	T.B Gambiense	T.B Rhodesiense
Aka	West African sleeping sickness	East African sleeping sickness
Vector	TseTse fly	TseTse fly
Primary reservoir	Humans	Animals
Clinical features	Winterbottom sign - posterior cervical LN	-
Parasitemia, Virulence and Resistance	Less	More
Treatment	Pentamidine	Suramine

Diagnosis 00:21:56

- Sample:
  - Blood
  - CSF
- Trypomastigote is seen
- Culture: Weinmann's medium

Parasites Causing Myocarditis 00:23:30

- Trichinella Spiralis
- Trypanosoma Cruzi
- TB rhodesiense
- Toxoplasma Gondii
- Echinococcus

Leishmania Donovani 00:24:38

- Vector: Sandfly
- Infective Form: Promastigote
- Diagnostic Form: Amastigote
  - L.D bodies seen by microbiologists are Amastigote.
- Disease: Kala Azar

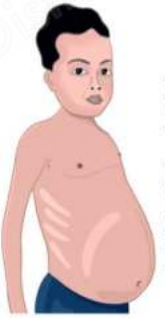
T. CRUZi TRIATOMINE BUG ~	T. BRUCEi (S.S) Tse Tse fly	LEISHMANIA Sandfly

Life cycle 00:26:40

Refer Image 37.2

Sandfly → Promastigote → Human being → Amastigote (LD bodies) → Sandfly → Promastigote

Kala Azar 00:27:54



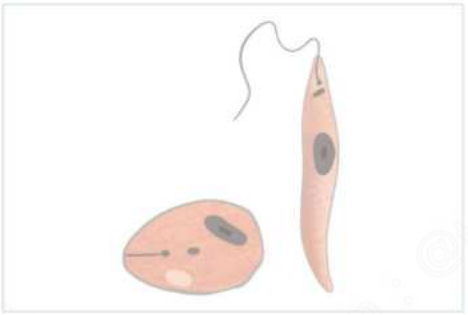
- Enlargement of the spleen
- Enlargement of the liver
- Night sweats
- Severe temperature or irregular bouts of fever that can last for weeks
- Bleeding
- Blackening of the skin
- Scaly skin
- Dark and ashen skin
- Cough
- Weakness
- Substantial weight loss

- Enlargement of the spleen (Protuberant abdomen)
- Enlargement of the liver
- Night sweats
- Severe temperature or irregular bouts of fever that can last for weeks
- Bleeding
- Blackening of the skin (Hence named - Kala azar)
- Scaly skin
- Dark and ashen skin
- Cough
- Weakness

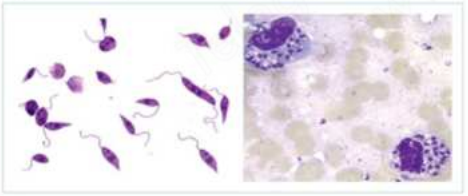
- Substancial weight loss
- If a patient is HIV+ & ends up having Kala Azar- Hepatosplenomegaly is not seen

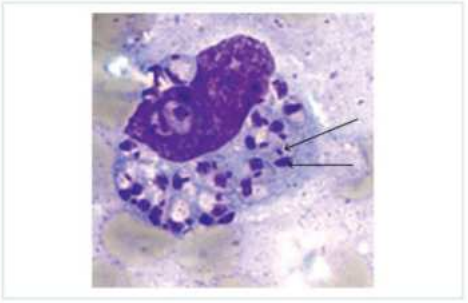
Diagnosis 00:29:20

- Sample-
  - Spleen (most sensitive)
    → Spleen is big and is extremely vascular organ & can result in bleeding
  - BM (most preferred)
  - Blood
  - BAL (HIV)
    → It is because in HIV+ patients spleen and liver are not very commonly involved, so lung might be involved
- Amastigotes (absent Flagella) aka LD bodies are diagnostic.

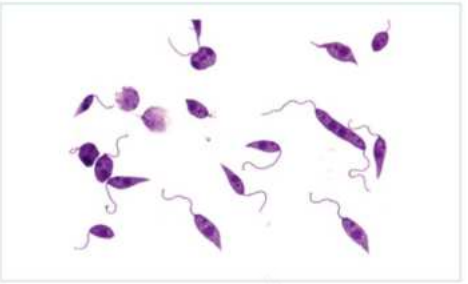


Left- Amastigote
Right – Promastigote





- LD bodies/ Amastigote forms are engulfed/ eaten by Macrophages (Reticuloendothelial cells)
- The organisms has Nucleus & Kinetoplast



It is Promastigote forms because Flagella is coming out

- Blood test: Pancytopenia
- Bone Marrow analysis-Macrophages engulfing the LD bodies
- Hypergammaglobulinemia (Antibody)
  - Napier's Aldehyde Test
  - Chopra's Antimony Test
- ELISA
- Media- NNN
- PCR
- Skin Test (Type IV hypersensitivity): Montenegro Test

Treatment 00:35:27

- Drug of choice: Liposomal Amphotericin B.

Post Kala Azar Dermal Leishmaniasis- PKDL 00:35:43

- Treated patient of Kala Azar (with Liposomal Amphotericin B) develops Hypopigmented nodules after 1-2 years.
- Treatment with oral Miltefosine is given.

Cutaneous Leishmaniasis

00:36:56

- Caused by- L. Tropica
- Oriental Sore / Delhi Boil/ Baghdad Boil/ Chiclero Ulcer / Aleppo button
  - Erythematous border
  - In center of border, a lot of yellow color exudative discharge present

- Treatment: Sodium stibogluconate (SSG)

Mucocutaneous Leishmaniasis / Espundia

00:38:06

- Caused by L. Brasiliensis
- It is associated with Buccal and skin
- Treatment: Sodium stibogluconate (SSG)

Leishmaniasis Recidivans

00:39:14

- Aka Relapsing Leishmaniasis
- Often due to inadequate treatment
- Nodular lesions or rash around central healing
- There is a central healed portion with nodular lesions

MCQ's

Q. Trypanosoma cruzi initially penetrated through the mucosa and then multiplies in a lesion Chagoma. In the chronic stage of this disease, where are the main lesions often observed?

a. GIT and Respiratory
b. Heart and GIT
c. Heart and liver
d. Spleen and pancreas

Q. NNN medium is used for isolation of

1. Leishmania donovani
2. Giardia
3. Trichomonas
4. Trypanosoma cruzi
5. Trypanosoma brucei

A. 1,2,3
B. 1,4,5
C. 2,3,5
D. 1,4

Q. Romana sign is seen in:

a. Chagas disease
b. Sleeping sickness
c. Kala azar
d. Fasciola

Q. In Chagas disease which stage is found in the muscle tissue?

a. Amastigote
b. Promastigote
c. Epimastigote
d. Trypomastigote

Q. Tsetse fly is a vector for:

a. Japanese encephalitis
b. Chagas disease
c. Dengue fever
d. Sleeping sickness

Q. Winterbottom sign is seen in:

a. Toxocariasis
b. Leishmaniasis
c. African trypanosomiasis
d. Toxoplasmosis

Image 37.1

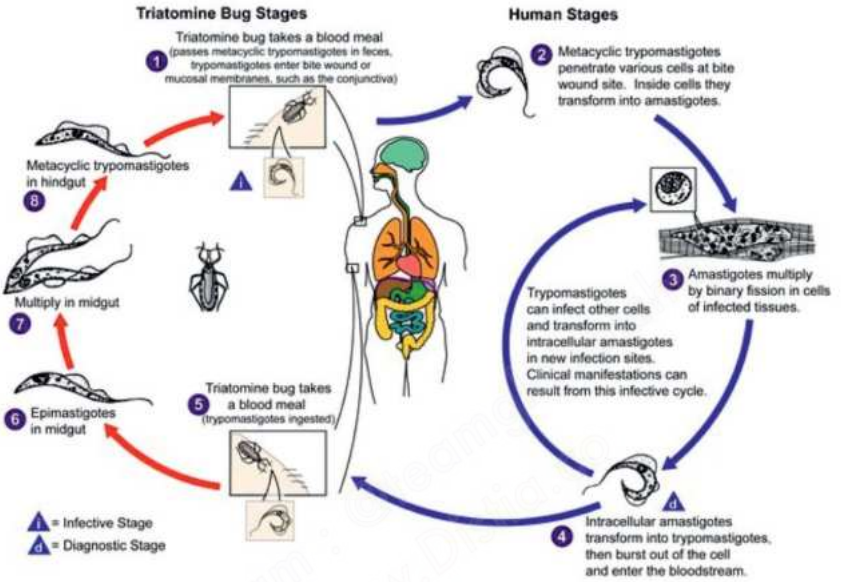
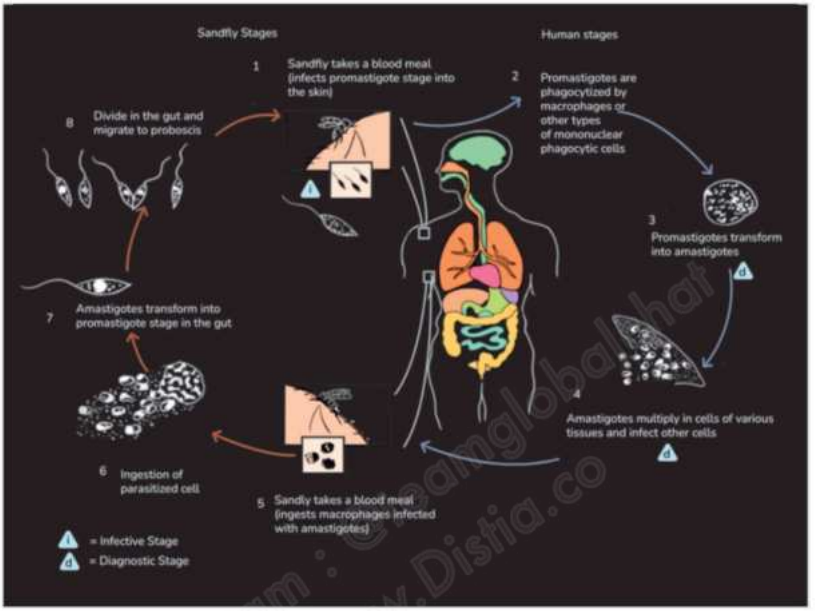
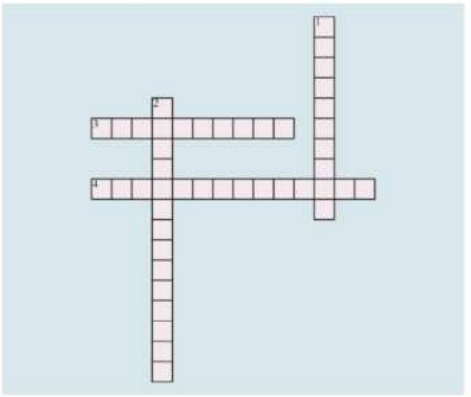

Triatomine Bug Stages
Human Stages
Triatomine bug takes a blood meal
(passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
1
Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes.
2
Metacyclic trypomastigotes in hindgut
8
Amastigotes multiply by binary fission in cells of infected tissues.
3
Multiply in midgut
7
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle.
Triatomine bug takes a blood meal
(trypomastigotes ingested)
5
Epimastigotes in midgut
6
= Infective Stage
= Diagnostic Stage
Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream.
4

Image 37.2



CROSS WORD PUZZLES

Crossword Puzzle



Across

3. The string is put into the intestine; that's why it's called Enterotest
4. Organisms hanging in the lumen

Down

1. cyst and trophozoite are seen
2. Most common parasitic infection in the world

38 PARASITOLOGY : COCCIDIAN PARASITES

List of Coccidian Parasites:
- Toxoplasma Gondii
- Cryptosporidium
- Cyclospora
- Isospora
- Sarcocystis

Toxoplasma Gondii 00:00:22

- Definitive Host-Cats (The oocyst/ Sexual Reproduction occurs)
- Intermediate Host-Humans (Other animals include sheep, rodent, pigs etc)
- Infective Forms
  - Sporulated oocyst
  - Tachyzoites
  - Bradyzoites
- Mode of Transmission
  - Blood Transfusion: Tachyzoites
  - Ingestion: Sporulated oocyst via ingestion of contaminated soil, food or water. Bradyzoites occur via undercooked meat.

Note to Remember:
Mnemonic- BTBT
- **B** can stand for Blood transfusion which would mean **T**achyzoites are the form of parasites that can enter the body.
- **T** will stand for **T**issue (ingestion of undercooked tissues or meat) this would mean the parasite that enters the body are **B**radyzoites.)
- Tachyzoites multiply actively, and Bradyzoites multiply slowly.

Definitive Host
↓
Oocyst in feces (Fresh)
↓
Old (Sporulated)

Extra Information:
Q. What type of Toxoplasma gondii transfer via organ transplant?
Answer. Bradyzoite, since they are a tissue.

- **Congenital toxoplasmosis:**
  - More **severe:** Trimester I (organogenesis)
  - More **common**: Trimester III (during vaginal delivery)
  - Conditions experienced by the patients:
    - → **C**horioretinitis
    - → **C**erebral Calcification
    - → **C**onvulsions
    - → **M**icrocephaly,Hydrocephalus
    - → **M**ental Retardation
- Similarities between CMV (Cytomegalovirus) and Congenital Toxoplasmosis include:
  - Chorioretinitis
  - Microcephaly
  - Mental Retardation.
- The major difference between CMV & Congenital Toxoplasmosis
  - CMV possesses Periventricular Calcification and
  - Congenital Toxoplasmosis possesses Cerebral Calcification and Convulsions.
- **Adult Toxoplasmosis** (immunocompetent)
- Mostly Asymptomatic
- Painless cervical LN.
- For immunocompromised adults (HIV+, Bone Marrow Transplant or BMT.)

In the case of an **HIV+** individual,
- Encephalitis,
- Brain Stem (m/c affected).

In case of **BMT** patients
- Pneumonia
- Chest X-ray of the patient shows a Bilateral Ground Glass Opacities

For confirmation-Giemsa stain shows Tachyzoites in CSF or BAL sample

Diagnosis 00:13:05
- IgM- acute infection (they fall down slow)
- IgA/E- acute infection (they tend to fall down faster than IgM)
- IgG- chronic infection (life-long antibody)

IgG
↓
Early↔Late
(low avidity) (high avidity)

Antibodies	Interpretation
IgM+ IgG-	Acute Infection
IgM+ IgG+ (low avidity)	Acute Infection
IgG+ (strong avidity)	Chronic Infection
IgM- IgG+	Remote Infection
IgM- IgG-	No Infection

- Non-cultivable on cell free media
- Intraperitoneal injection (mice)
- The gold standard for Toxoplasmosis is the Sabin Feldman test. It is a type of a complement fixation test (CFT).

Patient Serum (Ab+)
+
Tachyzoites (Ag+)
↓
Activates complement system (destroys the Tachyzoites)
+
Add methylene blue
↓
Tachyzoites distorted colorless

- Antibody present- The colour would be absent
- Antibody absent -The colour would be present.

Treatment

- Pyrimethamine + sulfadiazine
  (Folinic acid)
- **Diarrhoea in Immunocompromised Host**
  - Cryptosporidium: Types- Hominis, Parvum
  - Cyclospora: Type- Cayetanensis
  - Isospora: Type- Belli

Cryptosporidium 00:20:05

- Has small infectivity dose
- Cysts can survive chlorination of H_2O.
- Associated with auto infection.

Lifecycle

- Transmission of Cryptosporidium parvum occur through exposure to infected animals or exposure to water contaminated by feces of infected animals.
- Following ingestion excystation occurs. The sporozoites are released and hides in the tunnel,which is known as Parasitophorous vacuoles which hides is the brush border cells of GIT
- In these cells, the parasites develop that sporulated oocyst in the infected host.
- Two different types of oocysts are produced, the thick-walled, which is commonly excreted from the host, and the thin-walled oocyst, which is primarily involved in autoinfection.

Refer Image 38.1

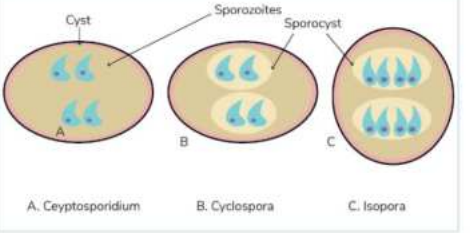
Cyclospora and Isospora 00:24:20

Refer Image 38.2

Refer Image 38.3

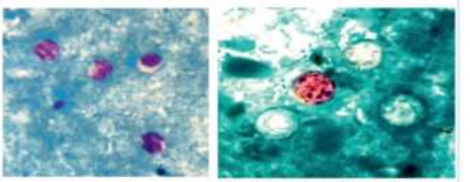
- They have unsporulated oocysts in feces which is why it does not cause autoinfection.
- Cyclospora is associated with raspberry consumption.

Difference between Cryptosporidium, Cyclospora and Isospora

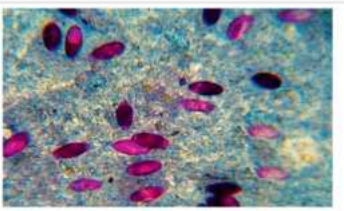


Cryptosporidium	Cyclospora	Isospora/Cysto Isospora
4 sporozoites	2-2 sporozoites in each wheel.	8 sporozoites

Feature	Cryptosporidium	Cyclospora	Isospora
Size	4-6micron	8-12micron	24-36micron
Shape	Round	Round	Oval
Acid Fast Cold ZN stain	+	Variable +/-	+
Autoflorescence	-	+	+
Treatment	Nitazoxanide	Cotrimoxazole	Cotrimoxazole



Cryptosporidium Cyclospora Variable acid Fast



Isospora

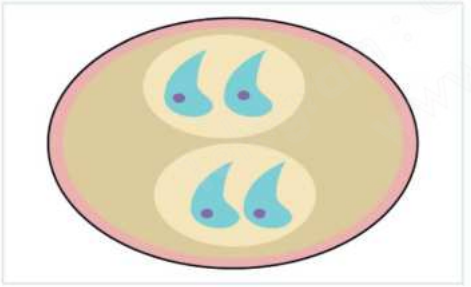
MCQs

Q. HIV positive person presented with diarrhoea. Stool examination revealed round acid fast cyst with 8-12 microns diameter, diagnosis is?
A. E coli
B. E Histolytica
C. Cryptosporidium
D. Cyclospora
Ans. d. Cyclospora

Q. Parasitophorous vacuole is suggestive of?
A. Cryptosporidium
B. Isospora
C. Cyclospora
D. Giardia
Ans. a. Cryptosporidium

Q. The life cycle shown below best describes which of the following pathogens?
A. Cryptosporidium
B. Isospora
C. Cyclospora
D. Giardia
Ans. a. Cryptosporidium

Q. The oocyst shown below is depictive of?



A. Cryptosporidium
B. Isospora
C. Cyclospora
D. Giardia
Ans. c. Cyclospora

Q. A person who has recently consumed a box of raspberries came down with severe watery diarhhoea. What is the most likely diagnosis?
A. Cyclospora
B. Cryptosporidium
C. Isospora
D. Vibrio
Ans. a. Cyclospora

Q. Sabin Feldman test is used for?
A. Leishmania
B. Malaria
C. Toxoplasma
D. Trypanosoma
Ans. c. Toxoplasma

Q. Which of the following is not a coccidean?
A. Cyclospora
B. Isospora
C. Cryptosporidium
D. Entamoeba coli
Ans. d. Entamoeba coli.

Image 38.1

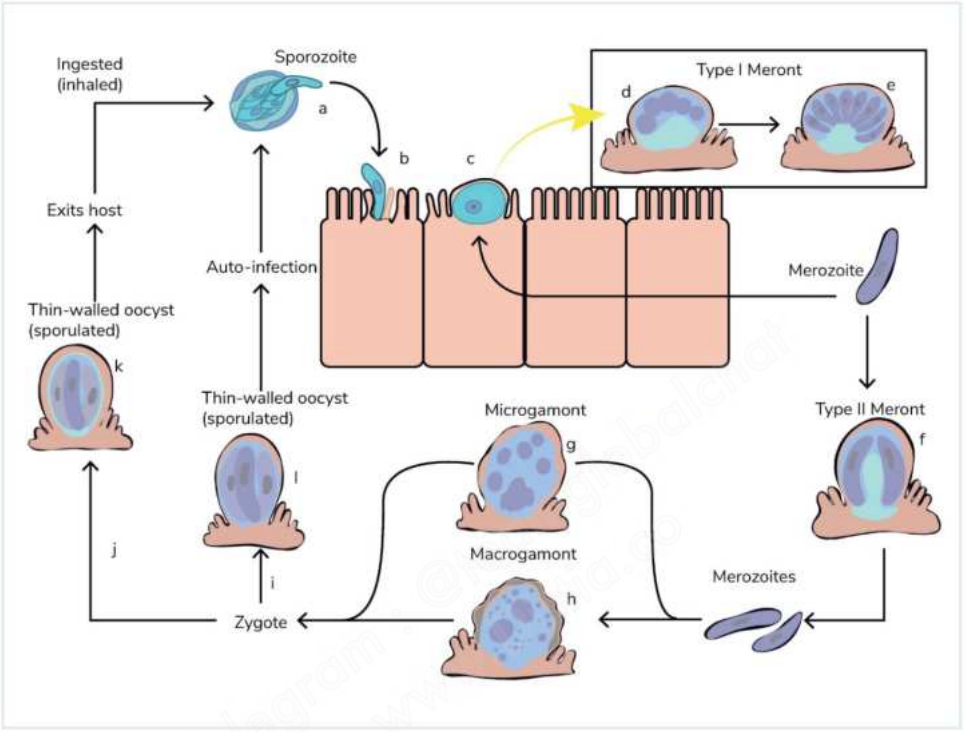


Image 38.2

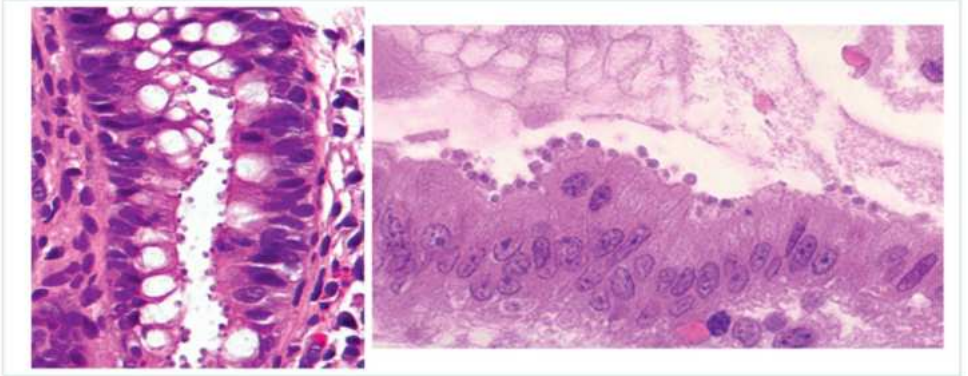
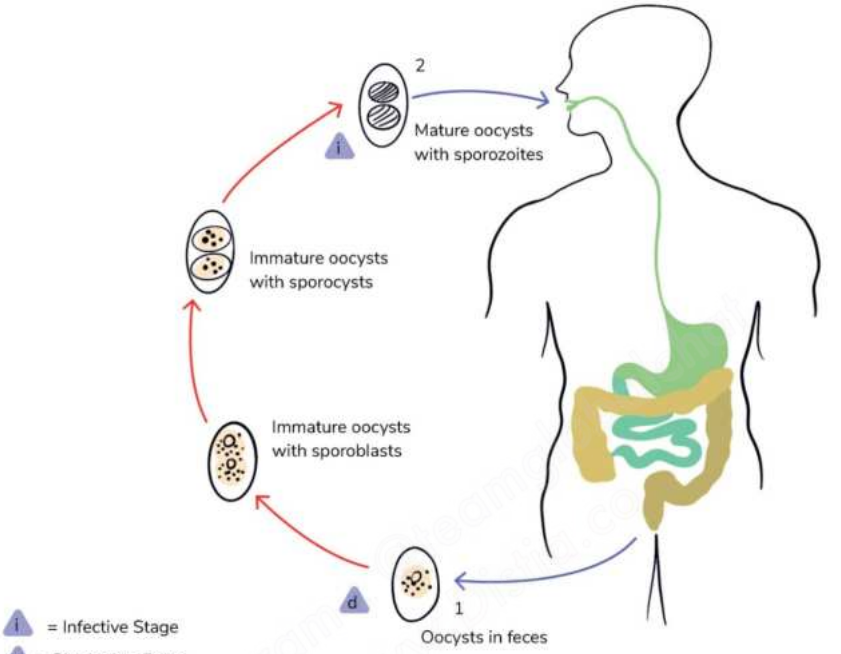
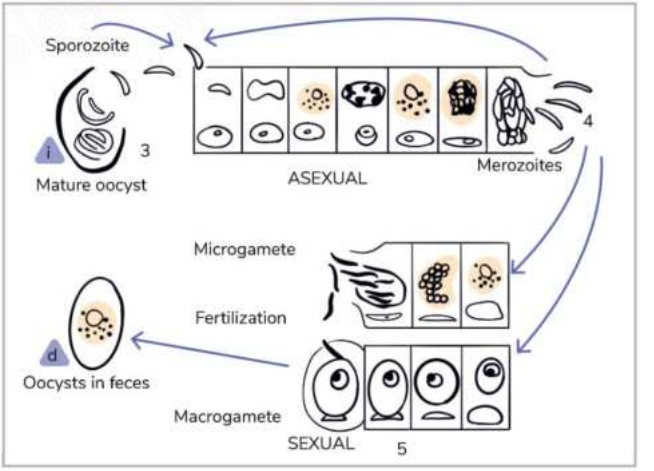
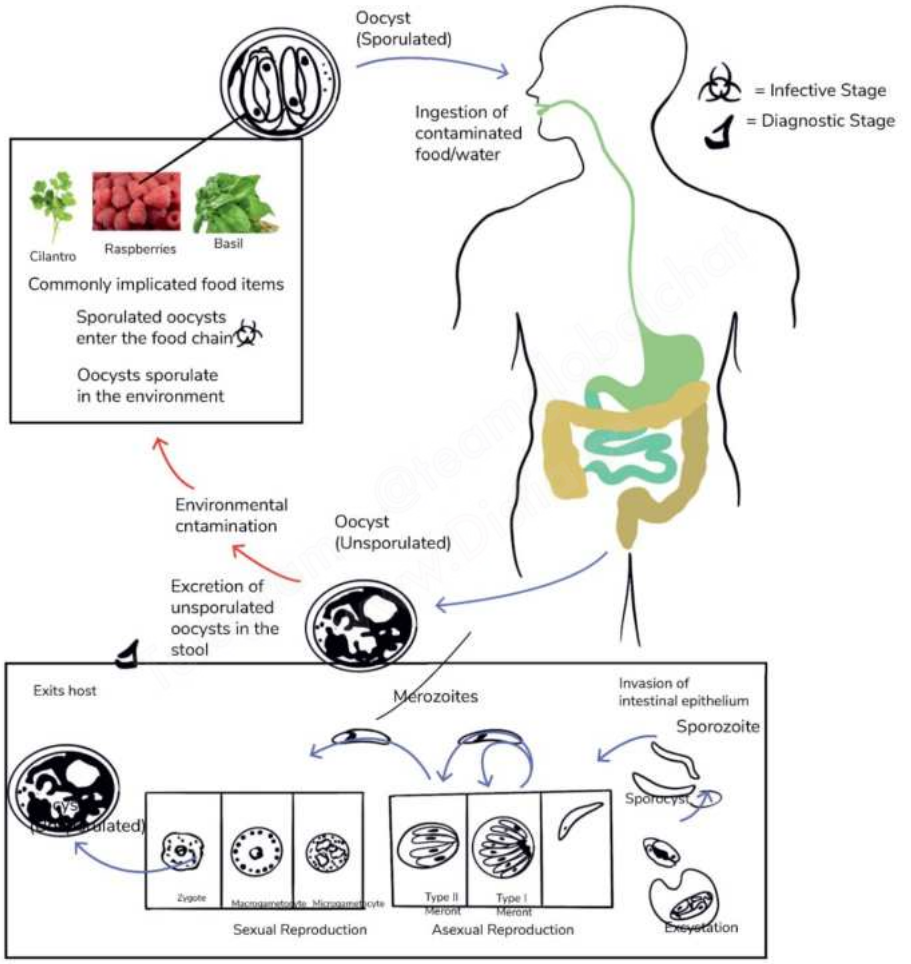


Image 38.3


2
Mature oocysts
with sporozoites
i
Immature oocysts
with sporocysts
Immature oocysts
with sporoblasts
d
1
Oocysts in feces
i = Infective Stage
d = Diagnostic Stage


Sporozoite
i
3
Mature oocyst
4
ASEXUAL
Merozoites
Microgamete
Fertilization
d
Oocysts in feces
Macrogamete
SEXUAL
5


Oocyst
(Sporulated)
= Infective Stage
= Diagnostic Stage
Ingestion of
contaminated
food/water
Cilantro
Raspberries
Basil
Commonly implicated food items
Sporulated oocysts
enter the food chain
Oocysts sporulate
in the environment
Environmental
cntamination
Oocyst
(Unsporulated)
Excretion of
unsporulated
oocysts in the
stool
Exits host
Merozoites
Invasion of
intestinal epithelium
Sporozoite
Sporocyst
Zygote
Type II
Meront
Type I
Meront
Sexual Reproduction
Asexual Reproduction
Excystation

39 HAEMATOZOA (PLASMODIUM AND BABESIA)

Sporozoites:
- Malaria
- Babesia

Malaria 00:00.16
- World Malaria day - April 25th
- Malaria Month in India - June

Malaria Species 00:00.31

P. Vivax	• Benign • Tertian (fever comes every 3rd day)
P. Falciparum	• Malignant • Tertian • Aggressive course - cerebral malaria
P. malariae	• Benign • Quartan (fever comes on 4th day)
P. ovale	• Benign • Tertian
P. knowlesi	• Benign • Quotidian (daily) - 24 hours • Not in India

Definitive host	**Female Anopheles mosquito**
Intermediate host	**Humans**
Infective form to humans	
From Saliva of mosquito	**Sporozoites**
From blood and Mother to child transmission	**Trophozoites**
Infective form to mosquito	**Give the Gametocytes**

Life Cycle 00:03.33

Refer Image 39.1

- Mosquito bites → Saliva → Sporozoites → Exoerythrocytic cycle (outside RBCs) → Liver → Schizonts formation and rupture → Erythrocytic cycle → Gets deposited in RBCs → Trophozoites → Gametocytes (male and female) → Mosquito → Sporogenic cycle → Ookinete (motile) → Oocyst (non-motile) → Ruptures → Release sporozoites → Stay in saliva.
- P.vivax and P. falciparum bind with RBCs by
  - Duffy antigen - P.vivax bind to this antigen
  - P.falciparum will bind to Basigin.
- Sporogenic cycle is extrinsic and takes 10-14 days to happen.
- Atleast 12 gametocytes must be present per microlitre of blood - to initiate infection.

Incubation period 00:10.15
- Exo-erythrocytic schizogony + Erythrocytic schizogony

P. vivax	13-17 days
P. falciparum	12 days
P.malariae	1 month (28-30 days)
P.ovale	13-17 days
P.knowlesi	8-10 days

Cytoadherence, Rosetting, Agglutination 00:11.36
- Seen with P. falciparum.

Red blood cell
↓
Infected by p.falciparum (infects brain)
↓
P.falciparum starts making knobs – PF-Erythrocyte Membrane Protein
↓
These knobs will bind to ICAM present in endothelial cells
↓
The cell will get adhered – Cytoadherence

If all the parasitized red blood cells agglutinated or clumped– Auto agglutinated

Some normal RBCs combine with parasitized RBCs or get clumped with parasitized RBCs- Rosetting

Blood Transfusion Malaria 00:14.32
- Blood transfusion– Trophozoites are seen.
- Pre- erythrocytic schizogony not seen.
- IP is very short.
- Relapse not seen

Features	P. vivax	P. falciparum	P. malariae	P. ovale
Relapse	Yes	No	No	Yes
Recrudescence	No	Yes	Yes	No

Prepatent phase	• The interval between the entry of sporozoites into the body to the first parasite in the blood
Relapse	• In the liver – hypnozoites activated from time to time. • Absent in blood transfusion.
Recrudescence	• Inadequate treatment • Resistance to treatment

Clinical Features 00:18.49

Benign malaria - Febrile paroxysms
- Cold phase – chills and shivering
- Hot phase- fever
- Sweating
- Whenever in RBCs schizoids will burst, that is when there is going to be fever.
- Most important cytokine for a fever - TNF α

Malignant malaria 00:20.03
- Cerebral malaria
- Fever, headache, nausea, HSM
- Confusion
- Paralysis
- Coma
- Durck Granuloma - P.falciparum

Black water malaria 00:20.39
- RBC breakdown-Intravascular hemolysis → Hemoglobinuria.

Renal manifestation
- Nephrotic syndrome (Membranous glomerulonephritis)
- Most common with plasmodium malariae

Tropical Splenomegaly 00:21.33
- Repeated infection
- Most common in tropical countries
- It is chronic malaria.
- Reticuloendothelial (macrophage) hyperplasia

Algid malaria
- Malaria with circulatory failure

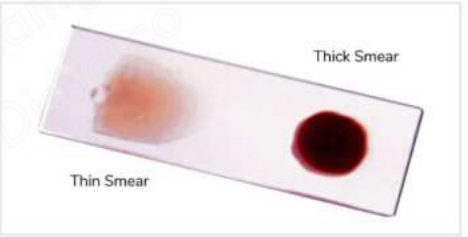
P. knowlesi 00:22.40
- Quotidian
- IP- 8-10 days
- Vector- Anopheles leucosphyrus
- Found in Malaysia
- Not seen in India.
- Caused by monkeys.

Immunity Against Malaria 00:23.10
- Humans who don't have Duffy antigens are immune to malaria.
- Sickle cell trait
- Thalassemia trait
- Fetal Hb
- G6PD deficiency
- Ovalocytosis

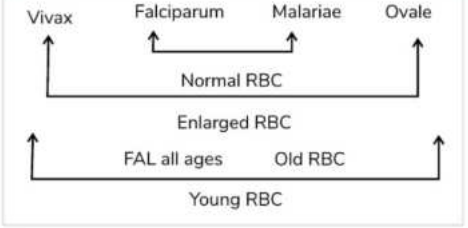
Diagnosis 00:24.47

1. Light microscopy	Gold standard
2. Fluorescent microscopy	Rapid screening modality-Expensive Kawamoto technique– Acridine orange
3. Microhematocrit method	Also known as QBC (Quantitative buffy coat)
4. RDT	Rapid diagnostic test / card test
5. PCR	Most sensitive test

Light Microscopy 00:25.25



- Gold standard
- **2 smears**:
  - Thick smear (just be able to read the words behind the slide)
    → Quantification
    → Sensitivity- 5 parasites/microlitre
  - Thin smear (tongue shaped)
    → Identification of species.
    → Sensitivity - 200 parasites/ microlitre
- 200-300 OIF (oil emergent field– 100x) examined before reporting negative
- **Stains used**
  - Romanowsky stain
  - Geimsa
  - Leishman
  - Wright
  - Jenner
  - FIELD (for P. Falciparum)
  - JSB- Jaswant Singh & Bhattacharya staining



- Vivax and ovale affects young RBCs and causes enlargement.
- All the other plasmodium will have normal size of RBCs.
- P. falciparum affects old RBCs

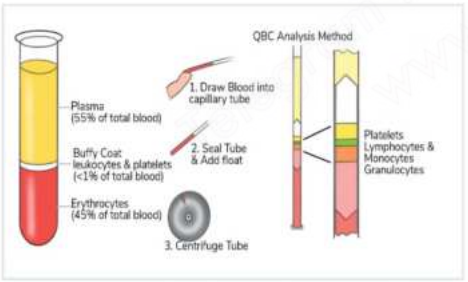
	P. vivax	P. falciparum	P. malariae	P. ovale
Medical examination	Schaffner dots	Maurer's dots	Ziemann's dots	James dots

Refer Image 39.2

- Malarial pigment –Hemozoin (Yellow-brown pigment)

Refer Image 39.3

QBC- Quantitative Buffy Coat 00:44.14



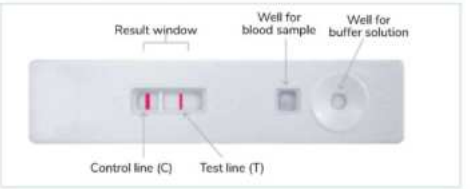
- Blood is taken → Centrifuged → RBC at the bottom and plasma at the top
- Tiny little area in middle - called as BUFFY COAT
  - All the organisms are seen here.

Refer Image 39.4

- In the tube there is a fluorescent dye- Acridine orange stain
  - Malaria will shine out.
- Sensitivity is equal to 2 parasites / microlitre blood.

Rapid diagnostic test (RDT) 00:47.25

- Immunochromatographic test– principle
- Sensitivity = 50-100 parasites/microlitre



- Nitrocellulose membrane
- Control line– validation line
- Circle (hole) - Buffer
- Square- where blood sample is placed

- All the malaria species have aldolase and LDH.
- P.falciparum has histidine rich protein 2– HRP2
- Less reliable

Card test 1	Card test 2	Card test 3	Card test 4
• Control line - present • Pan malaria- absent • P. falciparum- absent	• Control - present • Pan malaria - present • P. falciparum - absent	• Control line - present • Pan malaria - present • P. falciparum- present (It can be mixed infection or p.falciparum)	INVALID

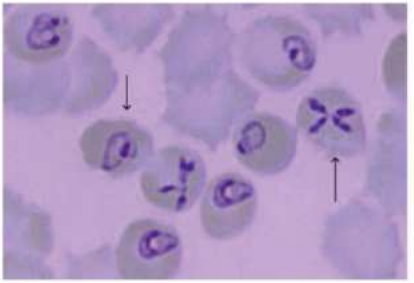
Babesia

00:52:48

- Intraerythrocytic protozoa
- No hemozoin pigment
- **Definitive hosts**: Hard ixodid tick
- **Intermediate hosts**: Rodent/mammal
- **Accidental dead-end hosts** – Human

Maltese Cross

00:52:23



- Rings are making **a tetrad of ring.**
- **Maltese cross of babesia**
- Can also be seen in:
  - Glove powder / starch
  - Nephrotic syndrome (Lipid urine)
  - Fabry disease (urine)
  - Cryptococcus (CSF)

Types of Babesia

00:53:34

Features	B. microti	B. divergens
Host	Rodent	Cattle
Spleen	Normal	Splenectomised
Clinical features	Mild	Severe

Clinical features

00:54:42

- Fever
- Malaise
- Chills
- Sweating

Treatment

00:54:49

- Azithromycin + Atovaquone
- **Severe cases**
  - >10% parasitemia
  - Clindamycin + Quinine

MCQs

Q. 22-year-old male patient with fever and chills. His blood counts show HB12gm/dl, TLC 13000/mm3, DLC N50/L29/M20/E1. Peripheral smear examination shows a presence of RBCs and pressure of applique forms. Few of the RBCs show the presence of the band. What would be your diagnosis?

A. P. falciparum
B. P. Vivax
C. P. malariae
D. Mixed infection

Q. What is the diagnosis?

A. Positive for plasmodium falciparum
B. Positive for plasmodium Vivax
C. Positive for no falciparum malaria
D. Mixed infection
E. A and D

Q. Which of the following is an incorrect antibody detection test for diagnosis of plasmodium falciparum?

A. It is based on the principle of ICT
B. Detects aldolase antigen
C. Detects LDH antigen
D. Detects HRP1 antigen

Image 39.1

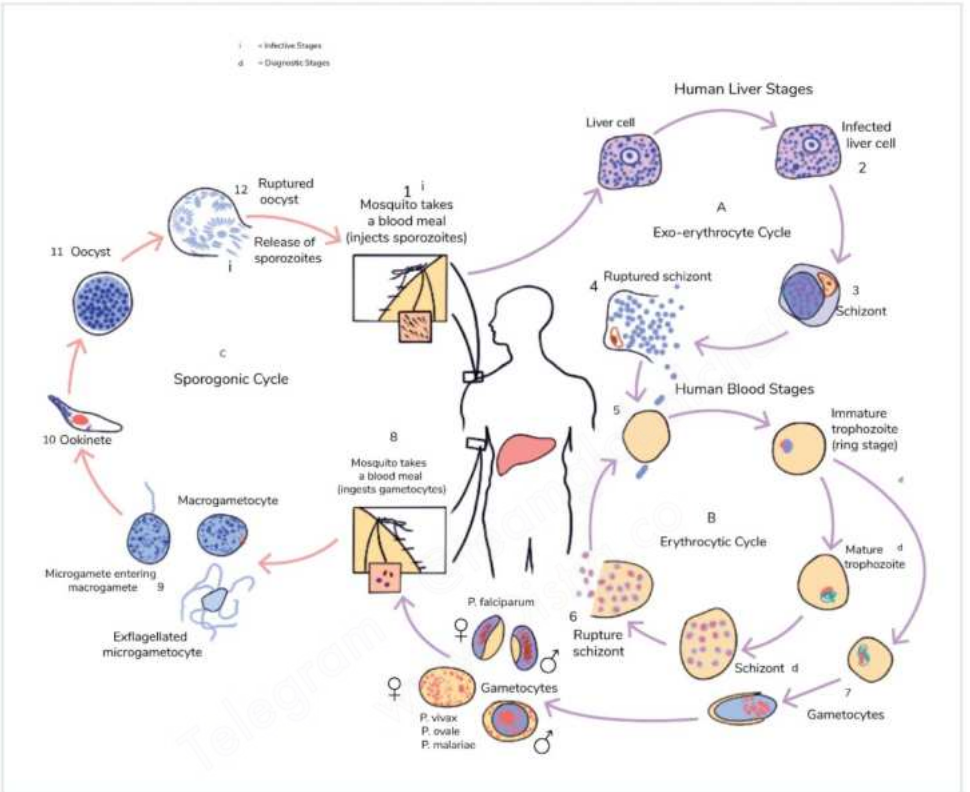

i = Infective Stages
d = Diagnostic Stages
Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocyte Cycle
3 Schizont
4 Ruptured schizont
Human Blood Stages
5
Immature trophozoite (ring stage)
B
Erythrocytic Cycle
Mature trophozoite
Schizont
7 Gametocytes
6 Rupture schizont
P. falciparum
Gametocytes
P. vivax
P. ovale
P. malariae
1 Mosquito takes a blood meal (injects sporozoites)
12 Ruptured oocyst
Release of sporozoites
11 Oocyst
C
Sporogonic Cycle
10 Ookinete
8 Mosquito takes a blood meal (ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete 9
Exflagellated microgametocyte

Image 39.2

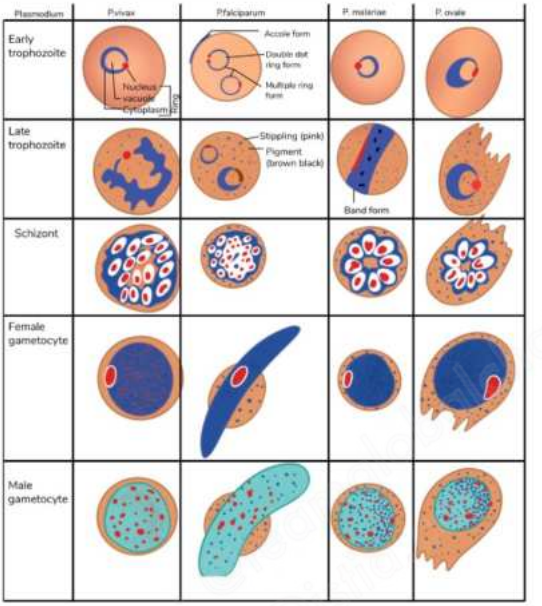
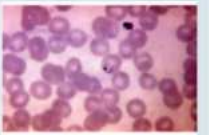
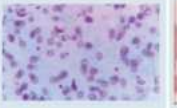
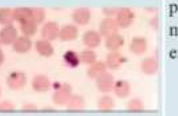
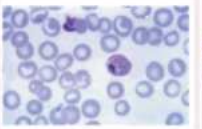
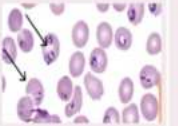


Image 39.3

		P. vivax	P. falciparum	P. malariae	P. ovale
Trophozoites (Ring form)	Ring form (early trophozoites) 	• One Ring • One Dot- Chromatin 	Multiple rings Multiple dots 	Band form 	All similar to earlier plasmodium microscopic examination
	Developing Trophozoites 	Amoeboid Shape 	Accole/applique form- Margination of ring 		

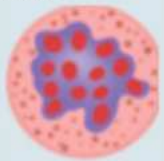
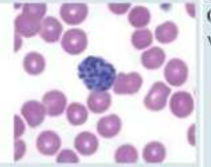
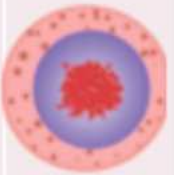
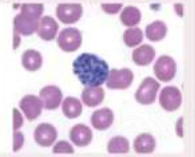
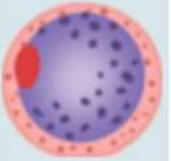
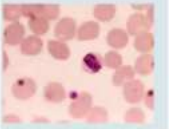
Schizonts	Immature  Mature 	Merozoites 	Not seen in the blood sample Only seen in brain vasculature		
Gametocytes	Micro-male gametocytes 	Oval Gametocyte Central Chromatin 	Banana shaped gametocyte for micro(males) Broad ends Chromatin spread out 		
	Macro - female Gametocytes 	Oval Gametocyte Eccentric Chromatin  	Banana shaped gametocyte for macro (females) Pointed ends Chromatin orgnaised  		

Image 39.4

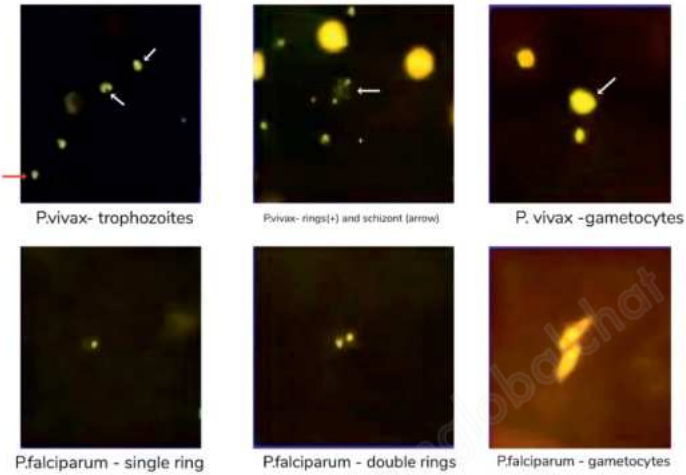
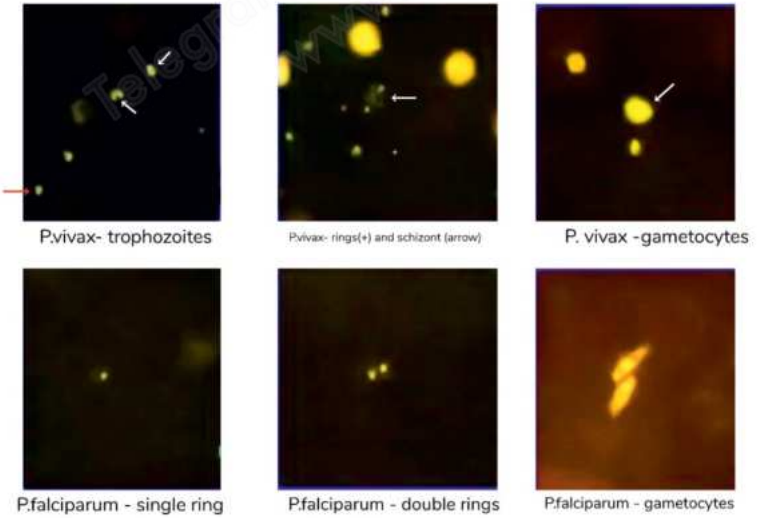
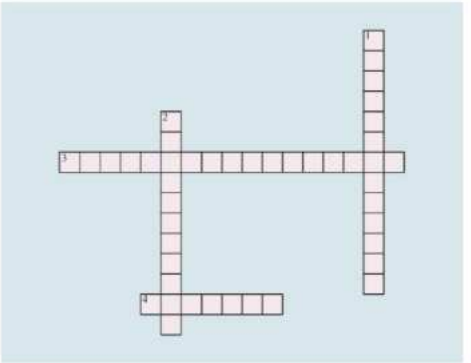


Image 39.5



CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Renal manifestation
4. Absent in blood transfusion

Down

1. Febrile paroxysms
2. MALARIA

40 CESTODES

- Cestodes
  - Also called tapeworms
  - Flat like shape
- Trematodes - flukes
  - leaf like
- Nematodes - worms

Features	Cestodes	Trematodes	Nematodes
Body cavity	-	-	+
Alimentary canal	-	-	+
Segmentation	+	-	-
Sexes	Hermaphrodites	Hermaphrodites	Dioecious - male and female

Definitions

00.01.25

- **Definitive host**:
  - In which sexual reproduction happens
  - Eggs are laid
- **Intermediate host**:
  - In which Asexual reproduction happens
  - Human beings are intermediate host in
    - → Babesia
    - → Echinococcus
    - → Sarcocystis
    - → Tenia solium
    - → Toxoplasma
    - → Plasmodium
- **Paratenic host**:
  - Larval stage is able to survive but shows no further development.
  - Only transmission of larva
- **Accidental or dead-end host**
  - Example
    - → In Echinococcus - humans are accidental or dead-end host
- **Biological vectors**
  - **Propagative** - Replication will occur in this vector
    - → Replication +
    - → Development -
    - → Example - Yersinia in rat flea
  - **Cyclopropagative** - Replication and development occur in this vector
    - → Replication +
    - → Development +
    - → Plasmodium - malarial parasite - female anopheles
    - → Babesia
  - **Cyclodevelopmental** - Only development occur in this vector
    - → Replication -
    - → Development +
    - → Wuchereria - filaria
    - → Leishmania
    - → Trypanosoma

- **Stool sample processing**

1. **Microscopy**
   - Saline mount
     - → Stool mixed with saline, seen under microscope
     - → Motility can be assessed in saline mount only
   - Iodine mount - trophozoites are immobilized.

2. **Concentration techniques**
   - If parasites are less in number
   - Either they can be made into sediments
   - Or make the eggs float
     - → Saturated salt - Floatation technique
     - → Formol ether - Sedimentation technique

Cestodes

00.08.46

- Tapeworms
- Segmented

Different Types

00.09.05

- **Head** helps in differentiating between different cestodes.

	Taenia solium	Taenia saginata	Hymenolepis nana	Hymenoiepis diminuta	Diphytiobothrium latum	Echinococcus granulosus
Heads						
	4 suckers 2 rows of hooks	4 suckers No hooks	4 suckers Single row of 20-30 hooks	4 suckers No hooks	2 suctorial grooves or bothria, no suckers, No hooks	4 suckers 2 rows of hooks

- All of them have suckers except DIPHYLLOBOTHRIUM LATUM
  - D. latum is Leaf like
  - Two grooves are present and no suckers.
- Hooklets
  - Tenia solium - hooklets +
  - Tenia saginata - no hooklets
  - Hymenolepis nana - hooklets +
  - Hymenolepis diminuta - no hooklets
  - Diphyllobothrium latum - no hooklets
  - Echinococcus granulosus - hooklets +
- Single row of hooklets - in H.nana, rest have two rows

Refer Table 40.1

- Intestinal
  - Tenia saginata
  - Tenia solium
  - H.nana - smallest tapeworm
  - D. latum - longest tapeworm
- Somatic
  - Echinococcus
  - T. solium
  - T. multiceps
  - Spirometra

Definitive host 00.13.43

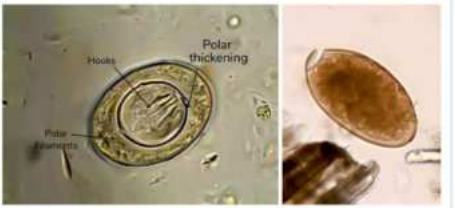
- In all of them is man
  - Except Echinococcus:
    - → Dog is the definitive host
    - → It is called dog tapeworm
- All of them requires 2 hosts
  - Except
    - → H.nana - single host - man
    - → D. Latum - 3 hosts

Important Information

H. nana - nano
- Smallest tapeworm
- Single arrow of hooklets
- Single host
- Most common cestode infection

Diphyllobothrium latum
- Longest tapeworm
- 3 hosts

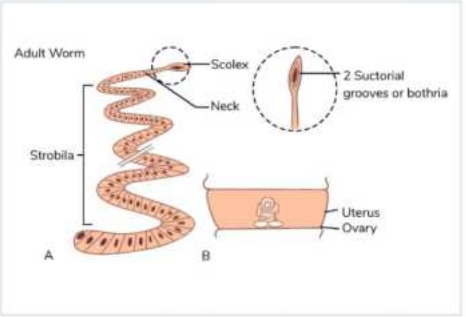
Eggs 00.15.50



- Egg with a lid on top of it - operculated egg
  - Seen in
    - → Spirometra
    - → Trematodes except schistosoma
    - → D. latum
- Hexacanth embryo –
  - 6 hooklets present
- Outer membrane
- Inner membrane
- Yolk granules between the membranes
- Polar thickening from which Polar filaments comes out.

Diphyllobothrium latum

00.18.43



- Longest
- Looks like a leaf.
- No suckers
- No hooklets
- 2 grooves for attachment.
- **3 hosts**
  - Definitive host - man
  - First intermediate host - Cyclops
  - Second intermediate host - Fish
- **3 larvae**
  - L1 - Coracidium
  - L2 - Procercoid
  - L3 - Plerocercoid - infective form

Life cycle

00.21.05

Refer Image 40.1

- Eating fish → Raw or undercooked containing L3 or plerocercoid form → Gets into human → D. latum goes to the small intestine and terminal ileum → Lays down egg → comes out of feces → L1 form is eaten by cyclops → L1 turns into L2 in cyclops → Cyclops are eaten by fish → Inside fish L2 transforms into L3 form.
- Terminal ileum is where vitamin B12 gets absorbed.
  - So, patient has megaloblastic anemia.

Clinical features

00.23.53

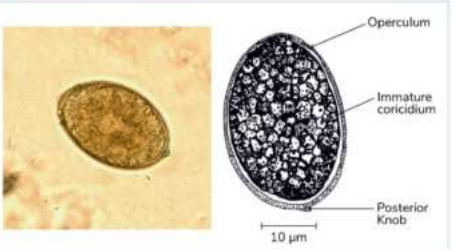
- Abdominal pain
- Anemia
- Nausea
- Bothriocephalus anemia - B12 deficiency

Important Information

- Parasite associated with
  - Iron deficiency anemia - Hookworm
  - Megaloblastic anemia - D latum

Egg

00.24.44



- Lid and on the opposite end has knob

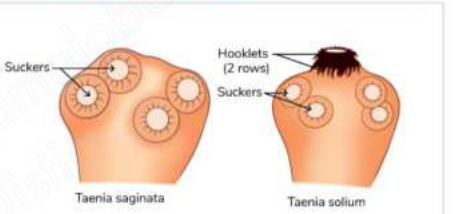
Treatment

00.25.22

- Praziquantel

Tenia saginata and Tenia Solium

00.25.30



- 4 suckers in both
- No hooklets in T.saginata

	Taenia saginata	Taenia solium
Length	5-10 meter	2-3 meter
Scolex	Large quadrate	Small and globular
	Rostellum and hooks are absent	Rostellum and hooks are present
	Suckers may be pigmented	Suckers not pigmented
Neck	Long	Short
Proglottids	1000-2000	Below 1000
Measurement (gravid segment)	20 mm × 5 mm	12 mm × 6 mm

Expulsion	Expelled singly	Expelled passively in chains of 5 or 6
Uterus	Lateral branches 15-30 on each side; thin and dichotomous	Lateral branches 5-10 on each side; thick and dendritic
Vagina	Present	Absent
Testes	300-400 follicles	15-200 follicles

- Most important features to differentiate between T.saginata and T.solium
  1. Proglottids
  2. Uterine branches

	T.saginata	T.solium
Definitive host	Man	Man
Intermediate host	Cattle	Pigs
Larva	Cysticercus bovis Only in cow, not in man	Cysticercus cellulosae Present in pig and man
Egg	Not infective to man	Infective to man
Disease	Intestinal infection	Intestinal infection and cysticercosis

T.solium
(4 suckers on head end and hooklets are present)

Life cycle 00.32.16

- Man with cattle - T.saginata
- Man with pig - T.solium

Refer Image 40.2

- Man consumed undercooked meat (cattle/pig) → Larva develop into adults → Eggs are formed → Hexacanth embryo → Eggs come out to the environment → Eaten by cattle/pig → They deposit in the muscles of the animal → They develop into cysticerci (larva)

Taeniasis 00.34.00

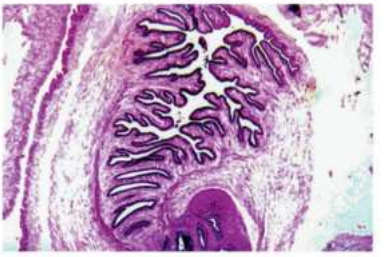
- **Intestinal**
  - Due to larval form
  - Both T.solium and T.saginata can cause intestinal infection
  - Man - definitive host
  - Intermediate host
    → Cattle in T.solium
    → Pig in T.saginata
- **Cysticercosis**
  - Organ involvement
  - Due to egg of T.solium
  - Man is the definitive and intermediate host

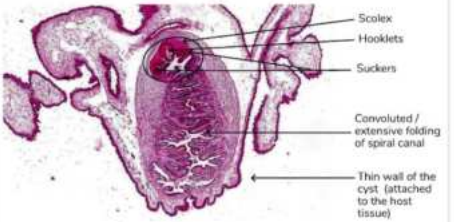
Cysticercosis 00.35.18

- Adults are in the intestine → They lay eggs → It penetrates the intestine and go to organs → causes cysticercosis
  - CNS > muscle > eye
- In CNS it causes neurocysticercosis
  - Most common site is brain parenchyma.
  - Most common presentation - seizures
- If it goes into ventricles - it causes hydrocephalus
- Subarachnoid space – meningitis
- Spinal cord

Lab diagnosis 00.36.39

- **Intestinal disease**
  - Stool examination
    → Eggs are hexacanth.
  - Concentration technique - Formol ether technique
- **Neurocysticercosis**
  - Biopsy





- Suckers with extremely convoluted spiral canal with a thin wall around it
- Head with spiral tail
  - Other investigations
    - → X- ray - If in soft tissue
    - → CT - for calcified cyst
    - → ELISA

Treatment

- Praziquantel

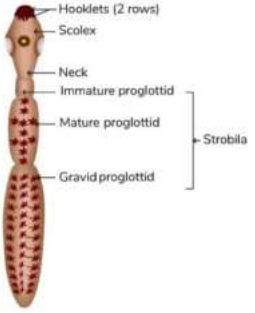
Taenia saginata asiatica 00.40.54

- Definitive host - Human
- Intermediate host - Pig
- Life cycle similar to T.solium

Tenia multiceps 00.41.31

- Definitive host - dog
- Intermediate host - sheep
- Man becomes accidental host
- Similar to Echinococcus (dog tapeworm)

Echinococcus granulosus / Dog Tapeworm 00.42.21



- 4 suckers
- 2 rows of hooklets
- Definite host - Dog
- Intermediate host - sheep and other animals
- Man is the accidental host.

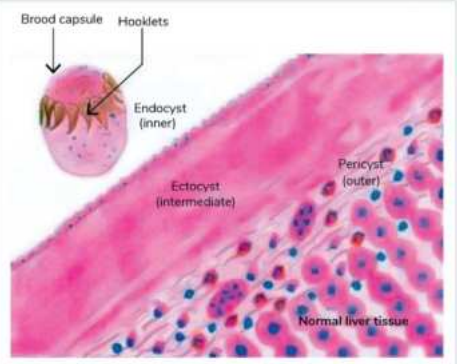
Life cycle 00.43.06

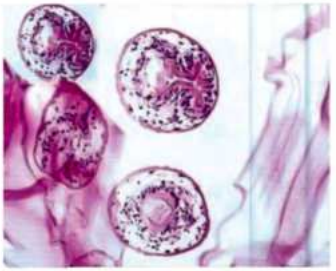
Refer Image 40.3

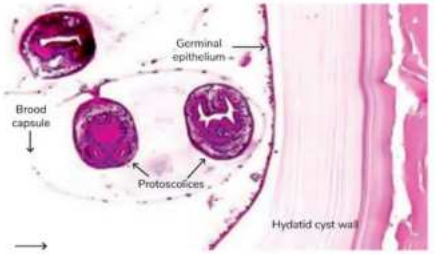
Hydatid cyst 00.43.44

- Multiple pearly white cysts
- Contain
  - hydatid cyst fluid
  - pH- 6.7
  - Salts
- Cyst fluid is Antigenic - cause anaphylaxis
  - So FNAC is contraindicated.

Layers of Hydatid Cyst 00.45.01







- Shows 3 Layers

1. **Pericyst** -
   - Host derived
   - Layer of inflammatory cells
2. **Ectocyst**-
   - Paint brush appearance
3. **Endocyst** –
   - Brood capsules (Organisms or Germinal layer)

- Fluid contains Hooklets
  - ZN stain +
- Common site - Right side of liver
  - Presents with Hepatomegaly
  - May complicate as portal hypertension
- 2nd most common site - Lungs
  - Presents with Cough with Sputum /Chest pain.

Lab diagnosis 00.48.13

- **Imaging** - Water Lily sign
- FNAC contraindicated.
- Cyst fluid - hooklets present which are **ZN stain** + (Acid fast)
- Skin test - Casoni test
  - It has become obsolete
  - As the cyst fluid is antigenic and can cause anaphylaxis
  - It is a Type 1 hypersensitivity test.
- Newer test - Arc -5 immunoelectrophoresis

Treatment 00.49.38

- **P**uncture the cyst
- **A**spirate
  - Don't let the fluid spill into the peritoneum
- **I**njection with 95%ethanol
  - It is a Scolicidal agent
- **R**easpirate once the scolices are killed

Echinococcus Multilocularis 00.51.04

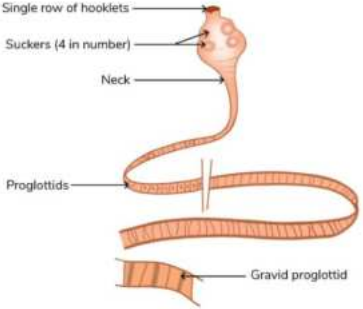
- Inside every cyst - multiple locules are present
- Called alveolar hydatid cyst (structure looks like alveoli)
- Most common site - Liver
- 2nd most common - Lungs

Life cycle 00.52.12

- Intermediate host - Rat (rodent)
- Man is accidental host.

Refer Image 40.4

Hymenolepis Nana / Dwarf tapeworm 00.53.11



- Smallest
- Single host
- Single row of hooklets
- Most common cestode infection

H.Diminuta / Rat tapeworm

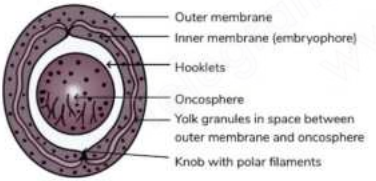
00.53.49

Difference between H.Nana and H.Diminuta

00.54.19

	H. nana	H. diminuta
	Dwarf tapeworm	Rat tapeworm
Host	Man	Man + rat
Egg	6 hooklets - hexacanth Yolk granules Polar knobs	6 hooklets No yolk granules No polar knobs

Egg of H.nana



Life cycle

00.55.57

- Man only - H.nana
- Man + Rat - H.diminuta
- In H.nana also - sometimes rat can be present

Refer Image 40.5

Clinical features

00.56.23

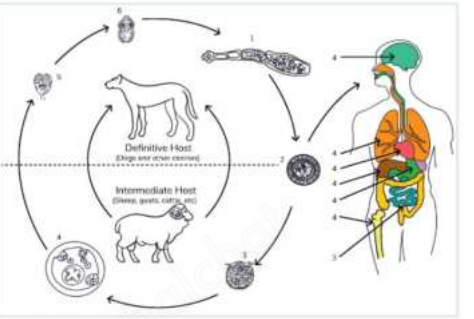
- Abdominal pain
- Nausea
- Vomiting
- Diarrhea

Treatment

- Praziquantel

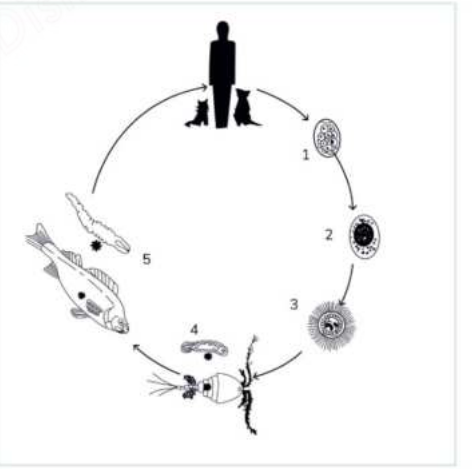
MCQs

Q. Identify the life cycle as shown below



A. Tenia solium
B. Echinococcus
C. Trichuris trichura
D. Diphyllobothrium latum

Q. Identify the life cycle as shown below



A. Tenia solium
B. Entamoeba Histolytica
C. H.nana
D. Diphyllobothrium latum

A. Diphyllobothrium latum
B. Tenia saginata
C. Schistosoma mansoni
D. Echinococcus granulosus

Q. Which of the following has grooves on its scolex for attachment in the definitive host
A. Diphyllobothrium latum
B. Tenia saginata
C. Schistosoma mansoni
D. Echinococcus granulosus

Q. Infective form of diphyllobothrium is
A. Proceroid larva
B. Plerocercoid larva
C. Metacercaria
D. Cysticercus

Q. Are ... countercurrent electrophoresis test on serum is diagnosis of
A. Cysticercus bovis
B. Cysticercus cellulosae
C. Hydatid cyst
D. Cysticercoid

Q. Larva form of which of the following cestode is not seen in man.
A. Tenia multiceps
B. Echinococcus granulosus
C. Tenia saginata
D. Tenia solium

Table 40.1

Head		T.solium	T.saginata	H. nana	H.diminuta	D. latum	E. granulosus
	Suckers	4	4	4	4	No suckers 2 grooves	4
	Hooklets	+	-	+	-	-	+
	Rows of hooklets	2 rows	2	Single row	2	-	2

Image 40.1

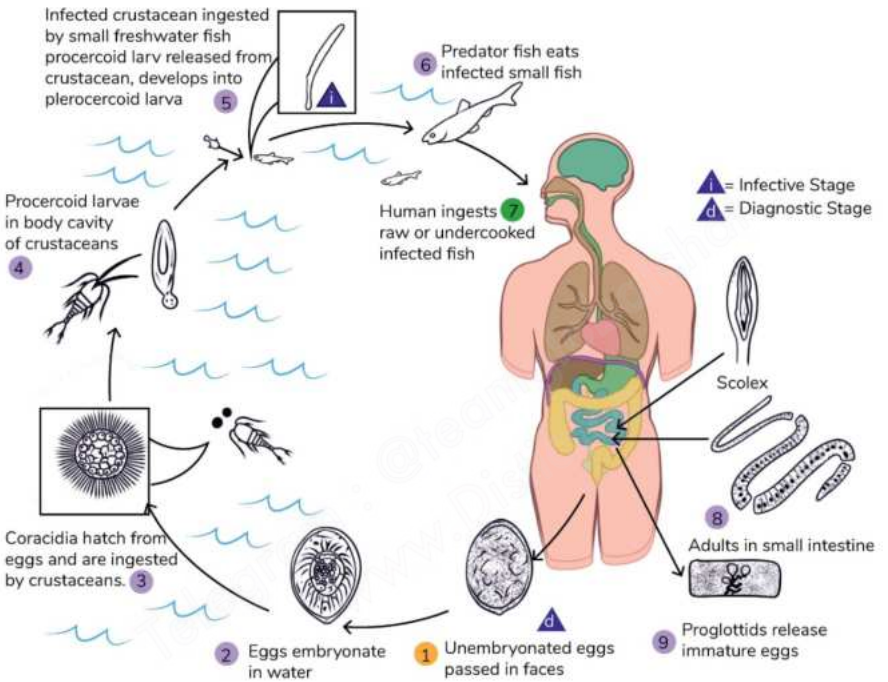

Infected crustacean ingested by small freshwater fish procercoid larv released from crustacean, develops into plerocercoid larva
5
i
6 Predator fish eats infected small fish
i = Infective Stage
d = Diagnostic Stage
Procercoid larvae in body cavity of crustaceans
4
Human ingests 7 raw or undercooked infected fish
Scolex
8
Adults in small intestine
Coracidia hatch from eggs and are ingested by crustaceans. 3
d
9 Proglottids release immature eggs
2 Eggs embryonate in water
1 Unembryonated eggs passed in faces

Image 40.2

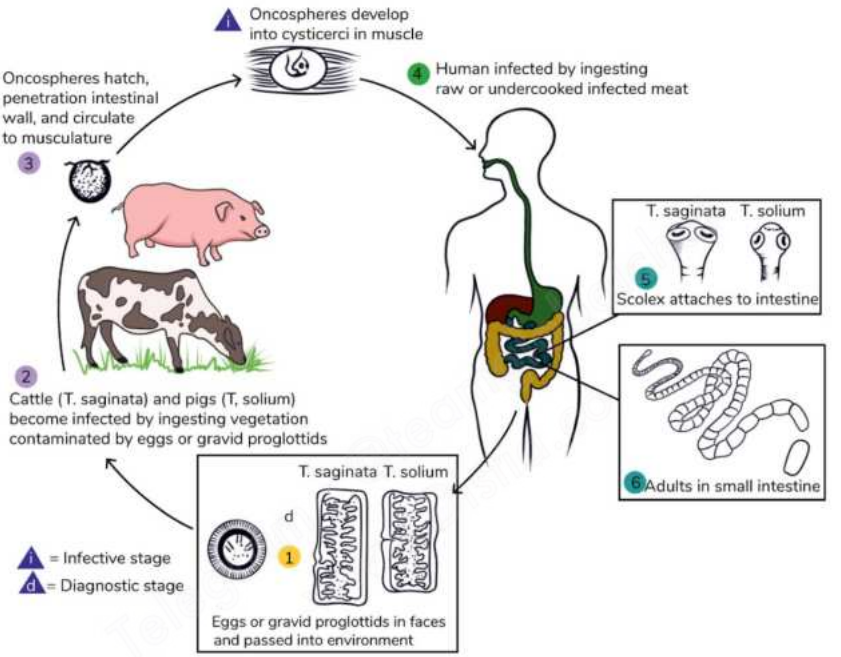
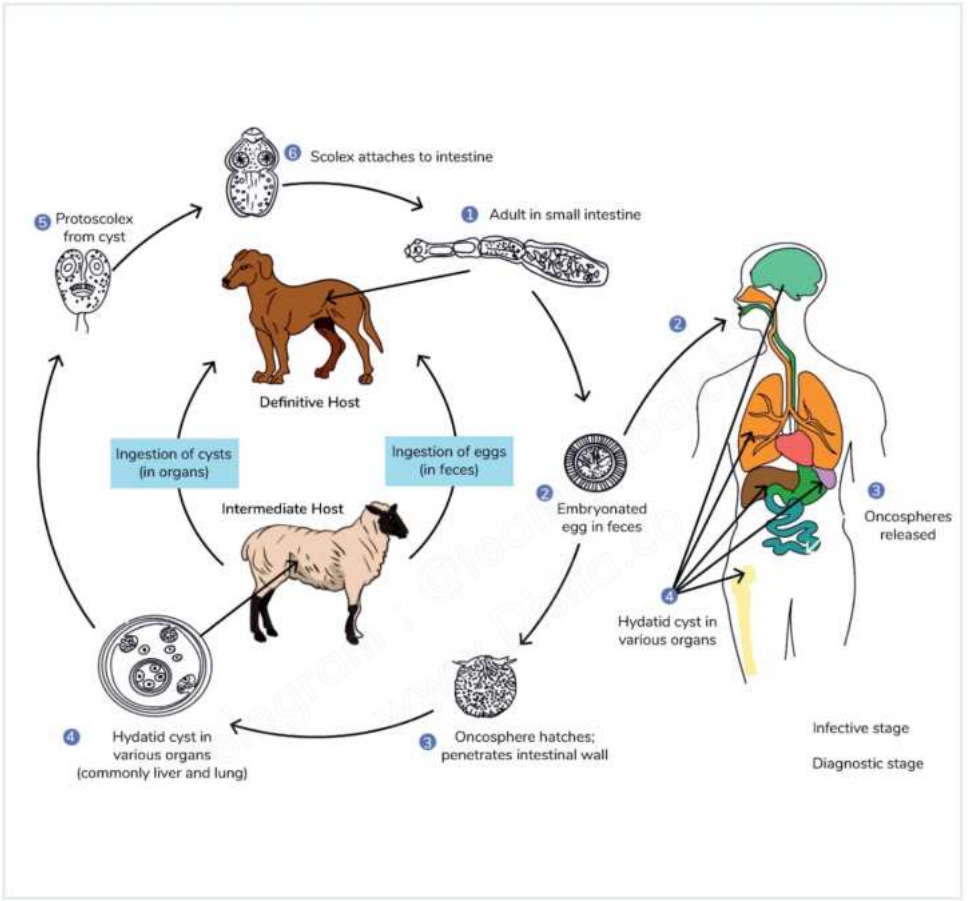

i Oncospheres develop into cysticerci in muscle
Oncospheres hatch, penetration intestinal wall, and circulate to musculature
3
4 Human infected by ingesting raw or undercooked infected meat
T. saginata T. solium
5 Scolex attaches to intestine
2
Cattle (T. saginata) and pigs (T, solium) become infected by ingesting vegetation contaminated by eggs or gravid proglottids
6 Adults in small intestine
T. saginata T. solium
d
1
i = Infective stage
d = Diagnostic stage
Eggs or gravid proglottids in faces and passed into environment

Image 40.3


6 Scolex attaches to intestine
1 Adult in small intestine
5 Protoscolex from cyst
Definitive Host
Ingestion of cysts (in organs)
Ingestion of eggs (in feces)
Intermediate Host
2 Embryonated egg in feces
2
3 Oncospheres released
4 Hydatid cyst in various organs
4 Hydatid cyst in various organs (commonly liver and lung)
3 Oncosphere hatches; penetrates intestinal wall
Infective stage
Diagnostic stage

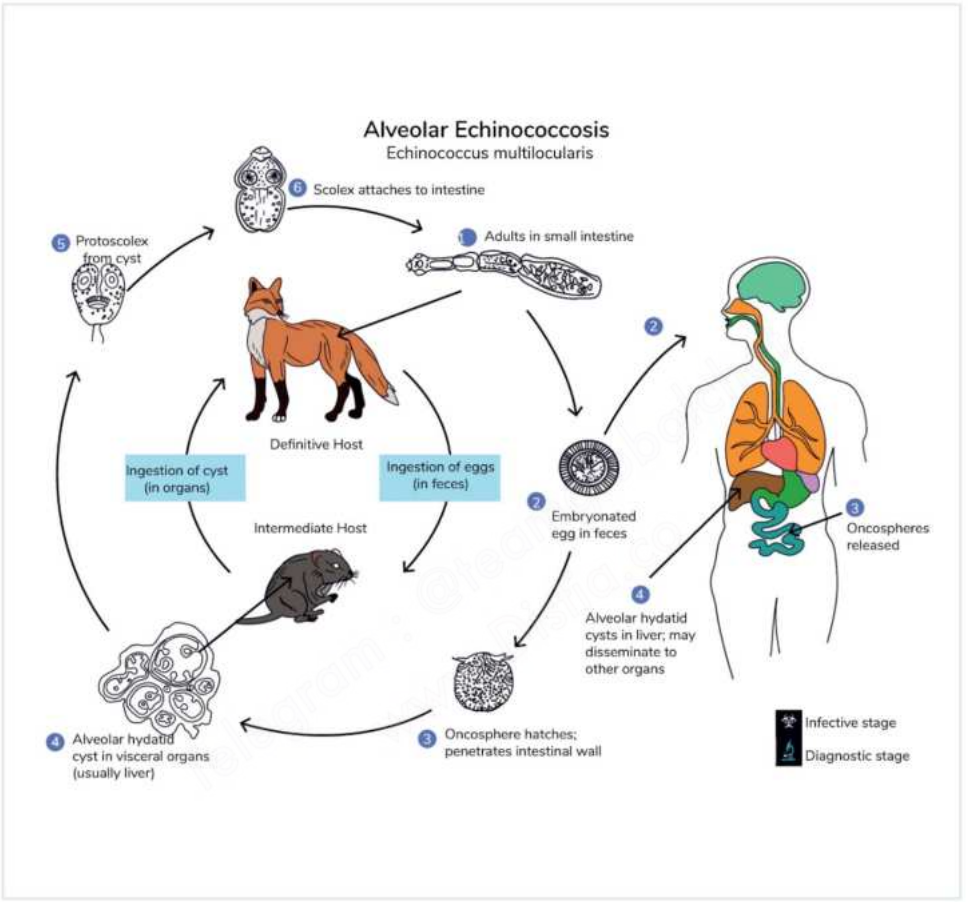
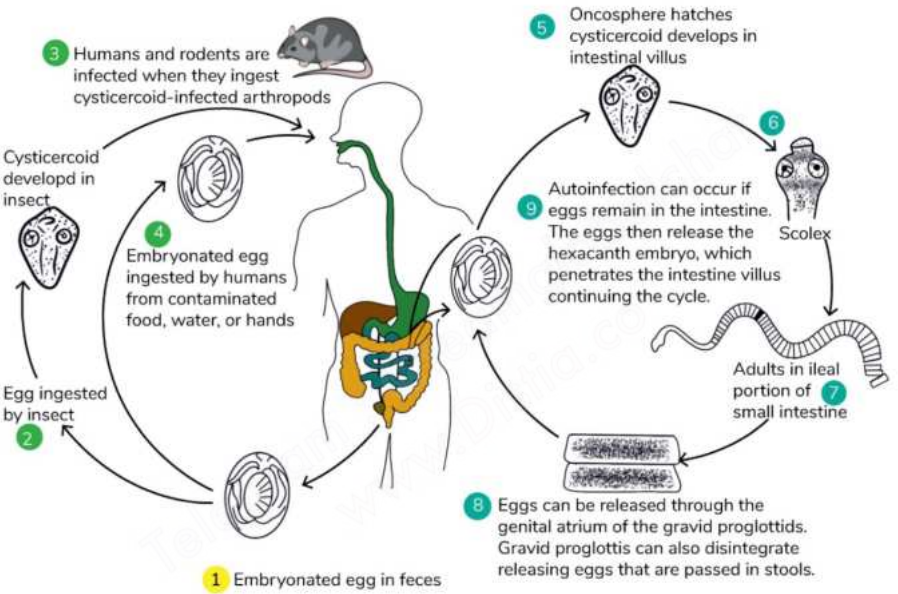

Alveolar Echinococcosis
Echinococcus multilocularis
6 Scolex attaches to intestine
1 Adults in small intestine
5 Protoscolex from cyst
Definitive Host
Ingestion of cyst (in organs)
Ingestion of eggs (in feces)
Intermediate Host
2 Embryonated egg in feces
2
3 Oncospheres released
4 Alveolar hydatid cysts in liver; may disseminate to other organs
4 Alveolar hydatid cyst in visceral organs (usually liver)
3 Oncosphere hatches; penetrates intestinal wall
Infective stage
Diagnostic stage

Image 40.5

i Infective stage

d Diagnostic stage



41 TREMATODES

- Trematodes - aka flukes.
- Extremely flat

Types of Trematodes

00:00:13

- Group 1
  - Schistosoma: Blood fluke
- Group 2
  - Paragonimus westermani: Lung fluke
  - Clonorchis sinensis: Chinese liver fluke
  - Opisthorchis: Cat liver fluke
  - Fasciola hepatica: Sheep liver fluke
  - Fasciolopsis buski: Intestinal fluke

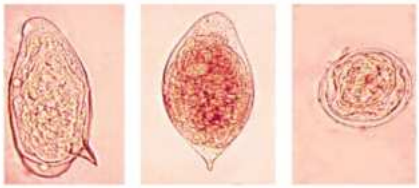
Rules

00:00:37

- Infective Form: Metacercaria Larva

	Group 2	Group 1
Infective Form	Metacercaria Larva	Cercaria larva
Mode of infection	Ingestion	Skin penetration
Eggs	Operculated eggs 	Spinous eggs 
Hermaphrodites	All	Separate sexes
Drug of choice	Praziquantel • Fasciola hepatica is an exception. ○ It is treated with triclabendazole	

- Operculated eggs:
  - Spirometra
  - Trematodes
    → Except: Schistosoma
  - D.latum
- Spinous eggs:



- Image A - Schistosoma Mansoni: Lateral spine
- Image B - Schistosoma Haematobium: Terminal spine
- Image C -Schistosoma Japonicum: Rudimentary spine:

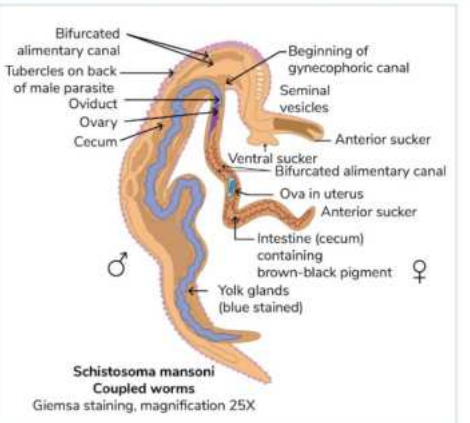
Trematode	Definitive host	First intermediate host	Second intermediate host
Schistosoma	Human	Snail	None
Paragonimus westermani • Pulmonary fluke			Crab Crayfish
Clonorchis sinensis			Fish
Opisthorchis			
Fasciola hepatica • Sheep liver fluke	Human and sheep		Fresh Aquatic plants
Fasciolopsis buski			

Schistosoma (Blood Fluke)

00:07:21

- Schistosoma haematobium
- Schistosoma mansoni
- Schistosoma japonicum

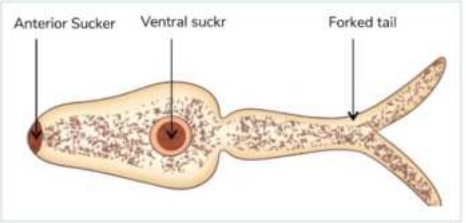
Adult worm



Schistosoma mansoni
Coupled worms
Giemsa staining, magnification 25X

- There are 2 suckers.
- Just behind the gynaecophoric canal where the female is held

Cercaria Larvae



- Infective form of Schistosoma
- Forked tail.

Life cycle

Refer Image 41.1

- Only humans and snail are involved.
- Infection- skin penetration.
- Sexes are separate.
- Eggs have spine.
- Larvae formed:
  - Miracidia
  - Cysts
  - Cercaria
- In Schistosoma - Redia and metacercaria larva are not seen.

Clinical features: 00:12:40

Schistosoma Haematobium	Schistosoma Mansoni	Schistosoma Japonicum
Resides in pelvic and vesical venous plexus.	Resides in Inferior mesenteric venous plexus draining sigmoid rectal region	Resides in Superior mesenteric venous plexus draining ileocecal region.
• Hematuria • Squamous Cell Carcinoma>> Transitional Cell Carcinoma of urinary bladder.	Swimmer's Itch	• Severe swimmer's itch • Genital schistosomiasis • CNS schistosomiasis

- Acute schistosomiasis:
  - **Katayama like fever:** Serum sickness like illness
- Chronic schistosomiasis
  - Granuloma
  - **Pipestem fibrosis (Periportal)**
    - → Occurrs in the liver
    - → Brown part is liver
    - → White bands are pipestem fibrosis

Schistosoma Intercalatum 00:16:56

- Has terminal spine
- Can be acid fast at times
- Seen in africa
  - Not present in India

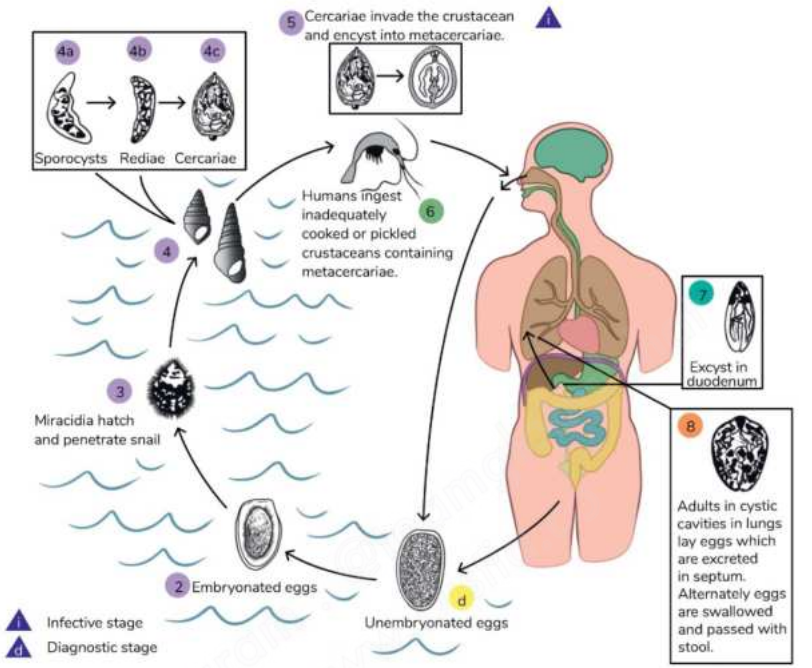
Paragonimus Westermani (Lung Fluke) 00:18:26

- Pulmonary
  - **Endemic focus-** Manipur
  - Pulmonary symptoms
    - → Cough
    - → Sputum- contains eggs.
- Operculated
- Golden brown

- Extrapulmonary symptoms
  - Abdominal involvement

Life Cycle

00:19:50



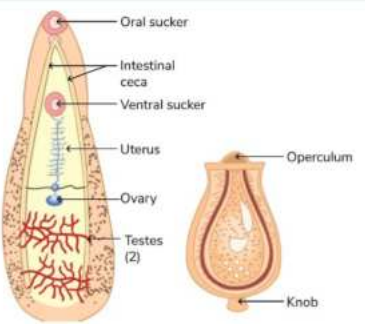
- Involves human, snail and crayfish.
- Paragonimus is present.
- Lung is involved.
- Mode of transmission- Ingestion.
- Crayfish→ Metacercaria larva→ ingestion→Metacercaria larva enters body →becomes Hermaphrodite adults →Exist in duodenum→eggs→comes out through cough or feces →larvae

Liver Flukes

00:22:28

- **Clonorchis sinensis:** Chinese liver fluke
- **Opisthorchis viverrini:** Southeast asian liver fluke
- **Opisthorchis felineus:** Cat Liver fluke
- **Fasciola hepatica:** Sheep liver fluke
- **Fasciola gigantica:** Giant liver fluke

Clonorchis Sinensis



- Looks like a spatula
- Narrow end

Lifecycle
Refer Image 41.2

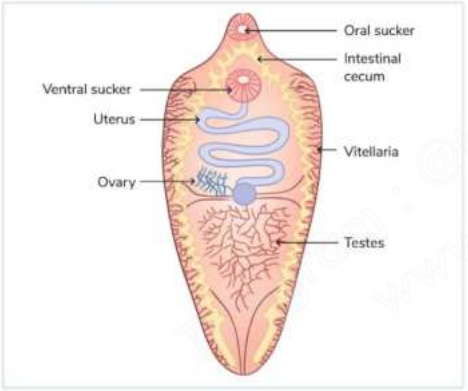
- Causes
  - Colangitis
  - Cholangiocarcinoma

Important Information

- Schistosoma Haematobium
  - SqCC>>>TCC
- Clonorchis
  - Colangiocarcinoma

- **Mode of infection:** Ingestion
- **Larva:** Metacercaria
- Hermaphrodite
- Operculated eggs

Fasciola Hepatica



- Flat
- Bile duct obstruction
- Right upper quadrant pain, jaundice, pain
- Halzoun: Suffocation due to laryngeal edema

Eggs

- Operculated
- 2nd intermediate host will be fresh aquatic plants

Intestinal Trematodes

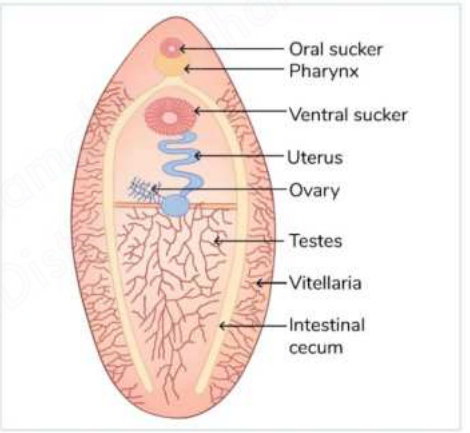
00:28:25

- Fasciolopsis Buski (giant intestinal fluke)
- Heterophyes (small intestinal fluke)
- Metagonimus yokogawai (small intestinal fluke)
- Echinostoma ilocanum
- Gastrodiscoides hominis (colonic fluke)

Important Information

- **Gastrodiscoides hominis (colonic fluke):** Only one in large intestine

Fasciolopsis Buski



- Intestinal
- Clinical features
  - Diarrhea
  - Abdominal pain
  - Malabsorption
- 2nd intermediate host is fresh aquatic plants

To remember

- If liver involved: **Fasciola hepatica**
- If Intestine involved: **Fasciolopsis buski**

Q. Which of the following life cycles is incorrectly matched?
1. Clonorchis: man-snail-crab-man
2. Paragonimus westermani: man-snail-fish-man
3. Fasciola hepatica: man-snail-aquatic plants-sheep
4. Fasciolopsis buski: man-snail-aquatic plants-man
5. Schistosoma: man-snail-crayfish -man

B. 1,2,5
C. C. 1,2,4,5
D. 2,3,5
Answer: b

Q. Which of the following have separate sexes?
A. Fasciola
B. Taenia
C. Schistosoma
D. Paragonimus
Answer: c. Schistosoma

Q. Operculated eggs are not produced in?
A. Paragonimus westermani
B. Clonorchis sinensis
C. Schistosoma haematobium
D. Diphyllobothrium latum
Answer: c. Schistosoma haematobium

Q. The infective form of man in case of Schistosoma infection is?
A. Metacercaria
B. Cercaria
C. Coracidium
D. Operculated egg
Answer: b. Cercaria

Q. Radiae are not present in the life cycle of?
A. Paragonimus
B. Clonorchis sinensis
C. Schistosoma mansoni
D. Opisthorchis viverrini
Answer: c. Schistosoma mansoni

Q. Which of the following localizes in veins draining the ileocaecal region?
A. Schistosoma japonicum
B. Schistosoma mansoni
C. Schistosoma haematobium
D. Fasciola hepatica
Answer: a. Schistosoma japonicum

A. Schistosoma haematobium
B. Schistosoma mansoni
C. Schistosoma japonicum
D. Schistosoma intercalatum
Answer: c. Schistosoma japonicum

Q. Terminal spine eggs are seen in?
A. Schistosoma haematobium
B. Schistosoma japonicum
C. Schistosoma mansoni
D. Clonorchis sinensis

Answer: a. Schistosoma haematobium

Q. A man a few months after returning from a trip to Malaysia complains of pain abdomen, jaundice with raised enzymes and hyperbilirubinemia. USG shows biliary tree blockade. What is the likely cause?
A. Fasciolopsis buski
B. Strongyloides
C. Clonorchis
D. Gnathostoma

Answer: c. Clonorchis

Q. A 35 year old man presents to the OPD with productive sputum since 5 days. Sputum for AFB is negative. He gives a history of recent visits to China and ingestion of crab pickle over there. What is the likely diagnosis?
A. Clonorchiasis
B. Diphyllobothriasis
C. Paragonimiasis
D. Fascioliasis

Answer: c. Paragonimiasis

Image 41.1

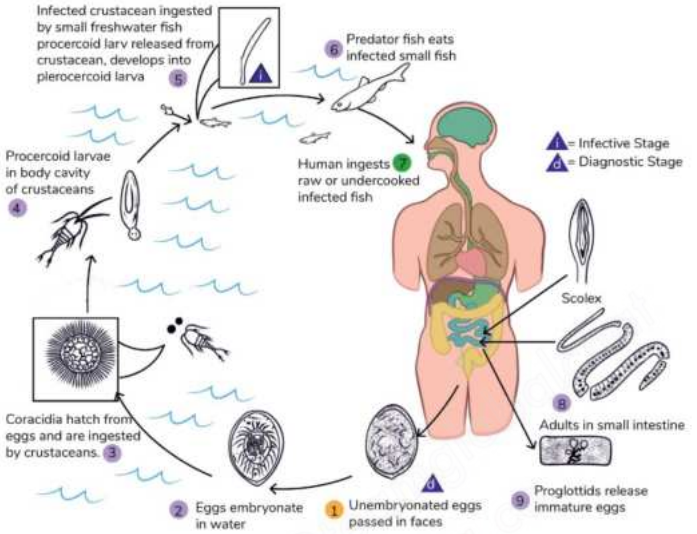
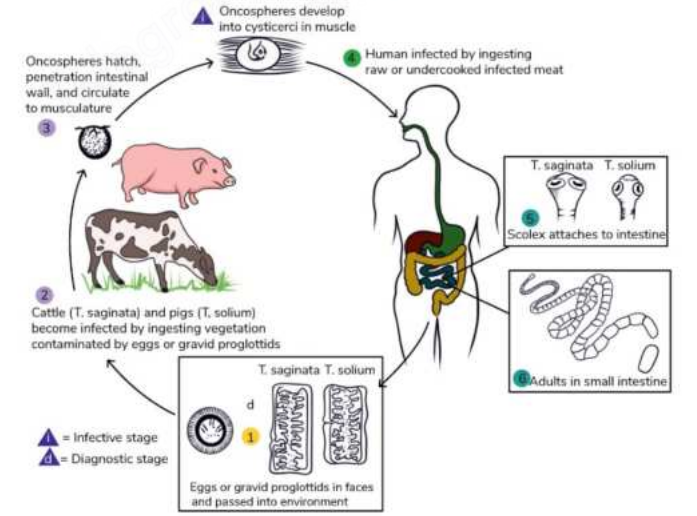


Image 41.2



42 NEMATODES

Classification

00:00:08

As Per Habitat

Intestinal		Somatic
Small (Also goes into lungs)	**Large**	
• Roundworm • Hookworm • Strongyloides	Enterobius Trichuris	• Filaria • Trichinella • Dracunculus

As per Eggs/Larva

00:02:37

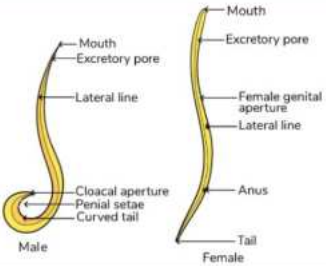
Viviparous	Oviparous	Ovoviviparous
Directly lays the larva	Lay eggs later on larva comes out	Larva Immediately hatch out the egg
Filaria Trichinella Dracunculus	Roundworm Hookworm Enterobius Trichuris	Strongyloides

Roundworm/Ascaris Lumbricoides

00:05:07

- Most common helminthic infection in the world.
- Sexes:
  - **Male:** Has curved posterior end
  - **Female:** Longer

Adults worms of Acaris Lumbricoides

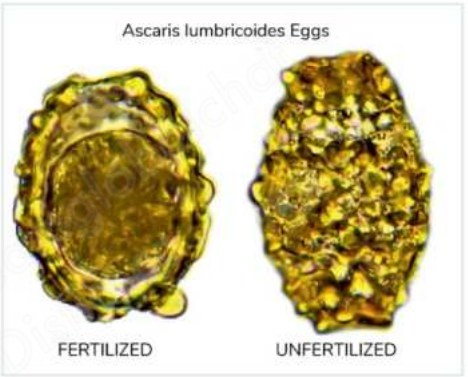


- **Infective stage:** Embryonated Egg
- **Route:** Oral route
- **Location:** Small intestine
- **Lung stage:** Involved
- **Incubation period:** 60-75 days
- **Treatment:** Albendazole

Eggs

00:06:52

- Fertilized eggs and unfertilized egg



	Fertilized	Unfertilized
Albumin coat	Yes	No
Crescentic space	Yes	No
Float in saturated salt solution	Yes	No

Life Cycle

00:08:14

Refer Image 42.1

- Adults form in small Intestine.
- Lung involvement

Clinical Features

00:09:31

- Malabsorption:
  - Fat
  - Immunoglobulins
- Loeffler's syndrome / Eosinophilic pneumonitis: Involvement of lung

Visceral Larva Migrans/Ocular Larva Migrans

00:10:08

- Ingestion of non-human roundworms

Life Cycle

Refer Image 42.2

- Causes
  - Toxocara cati canis (dog)
  - Toxocara(cat)
  - Ascaris suum (Pig)

Rhabditiform Larva

↓

Filariform Larva

↓

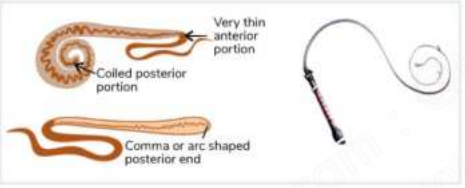
Pass through lymph and blood and reach the right side of the heart through the pulmonary circulation.

↓

Invades visceral organs

Trichuris Trichiura/Whipworm

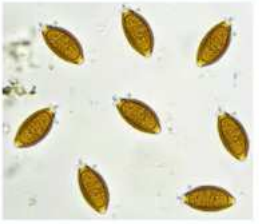
00:12:30



- Looks like a whip of a hunter.
- Broad posterior end
  - **Males:** coiled
  - **Females:** not coiled
- **Infective stage**: Embryonated egg
- **Route**: ingestion
- **Location**: Large Intestine
- **IP**: 70 to 90 days
- **Treatment:** Albendazole

Eggs

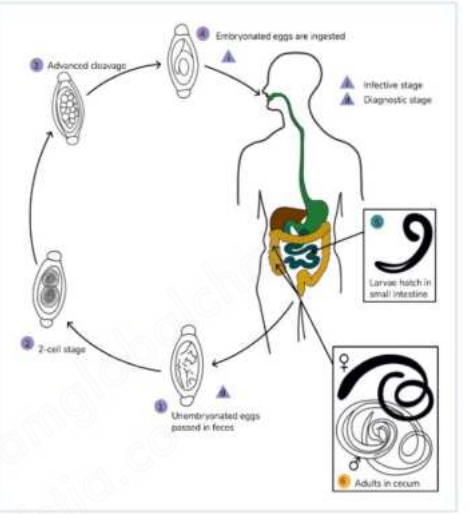
00:13:47



- Bomb barrel shaped.
- Brown colored spots: Bile stained.
- Mucous plugs at both ends are non-bile stained.

Life Cycle

00:15:10



Clinical Features

00:15:47

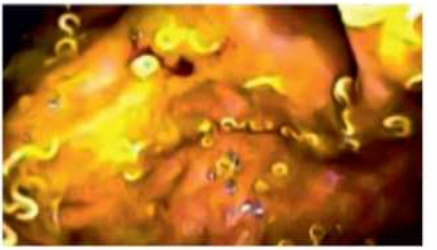
- Dysentery
  - Blood in stools
- IDA
- **Complication:** Recurrent rectal prolapse.

Diagnosis

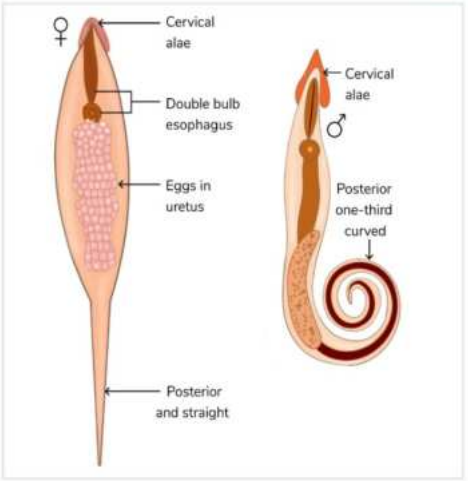
00:16:18

- Endoscopy
  - Coconut cake rectum

Endoscopy often shows adult worms attached to the bowel mucosa (coconut cake rectum)



00:16:47



- Vermiform Appendix (Tip)
- **Identification of adult worm:**
  - **Male:** coiled posterior
  - **Female:** Long
  - **Cervical alae (3):** Anterior projections
- **Infective stage:** Embryonated egg
- **Route:** Oral
- **Location:** Large intestine
- **Lung stage:** No
- **Ib:** 35-45 days
- **Treatment:** Albendazole
- **Autoinfection:**
  - Perianal pruritus → Scratched by hand →Then eggs get into nails → Ingestion
- **Retroinfection:** Larva hatches and goes back in the intestine

Life Cycle

00:19:08

Refer Image 42.3

Worms mate in cecum
↓
Male dies after mating
↓
Female worm in night moves towards the anal canal (Perianal) and lays eggs and move back
↓
Perianal pruritus

- Perianal pruritus
  - Can spread to genital area and causes genital infections.

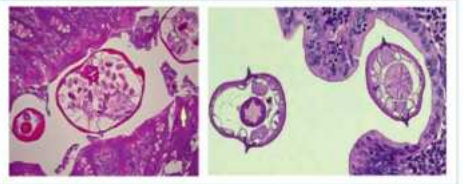
Diagnosis

00:23:15

- Collection of the eggs
  - Can done by NIH swap
  - In Children- Scotch tape method is used

- Microscopy:
  - D shape
  - Plano convex shaped
  - Eggs have tadpole inside it.

- Biopsy of appendix:
  - Projection
  - Cervical alae



	Infective form	Route
Trichuris Enterobius Ascaris Lumbricoides	Embryonated egg	Oral

	Infective form	Route
Strongyloides Hookworm	Larva	Skin

Ancylostoma/Hookworm 00:27:57

- **New world:**
  - Necator Americanus
- **Old world:**
  - Ancylostoma Duodenale

Life Cycle 00:28:36

Refer Image 42.4

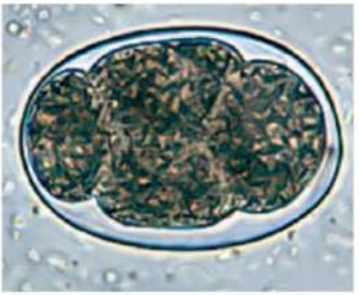
- Filariform larva comes via the skin→ Small intestine →Lung involvement is there→Rhabditiform larva →Filariform larva
- **Infective stage:** Filariform larva
- **Route:** Skin
- **Location:** Small intestine
- **IP:** 40-100 days
- **Treatment:** Albendazole

Clinical Features 00:30:27

- Skin:
  - Ground itch
  - Creeping eruption
- Small intestine
  - IDA
- Pulmonary symptoms

Diagnosis 00:31:21

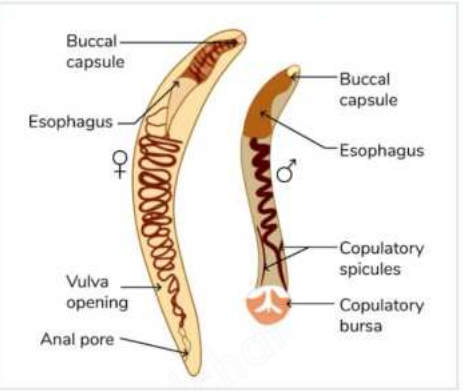
- Un-embryonated egg - fresh stool
- Larva- old stool
- Egg with blastomeres



Chandler Index 00:32:24

- It is the average number of hookworm eggs per gram of feces for the entire community.
- If above 300 it is problem

Difference between sexes 00:33:11



	Male	Female
Size	Short	Longer
Copulatory bursa	Present	Absent
Posterior end	Umbrella like expansion	

Difference between new and old world 00:33:57

Features	Ancylostoma Duodenale (Old world)	Necator Americanus (New world)
Eggs	Eggs with blastomere	Same
Rhabditiform larva	Same	Same
Adult worms	Larger (Joint family)	Smaller (nuclear family)
Filariform worms	Curvature at anterior end same as the body	Opposite
Pathogenicity	More pathogenicity	Less pathogenicity More ground itch

Refer Image 42.5

Ancylostoma Duodenale	Necator Americanus
4 ventral teeth and 2 dorsal teeth	4 chitin plates
Free spicules	Fused spicules

Cutaneous Larva Migrans

- Due to Ancylostoma braziliense
- Skin penetration by larva
- Treatment - Ivermectin

	Visceral Larva migrans	Cutaneous Larva migrans
Causative parasite	Roundworm (toxocara)	Hookworms
Infective stage	Embryonated egg	Filariform larva
Route infection	Ingestion	Skin penetration
Diagnosis	Toxocara specific Antibody tests PCR is more specific	Physical examination of the advancing serpiginous track on the skin.

Strongyloides Stercoralis

00:40:19

- Smallest nematode known to cause human infection

Life Cycle

00:40:42

- Ovoviviparous, larva comes out immediately from egg

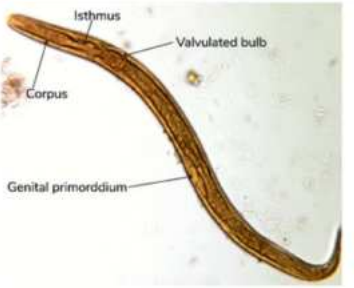
Refer Image 42.6

- Infective Filariform larva penetrate the intact skin of definitive host
- Enters small intestine, shows lung phase
- Males are present.
- Females are capable of asexual reproduction (Parthenogenesis)
- Ovoviviparous eggs release larva
- Rhabditiform larva comes first, has 3 ways
  1. **Autoinfection**
     - Penetrate intestine, produce Filariform larva
  2. **Direct cycle**
     - Rhabditiform larvae exits body, transforms into Filariform larva re-enters body
  3. **Indirect cycle**
     - Rhabditiform larva forms adult, lays eggs
     - Eggs develop into Rhabditiform larva
     - Rhabditiform larva transforms into Filariform larva
- Infective stage: Filariform larva
- Route: Skin / autoinfection
- Location: Small intestine
- Lung stage: Present
- IP: 17-28 days
- Treatment: Ivermectin

Important Information

- Ivermectin is also given to Cutaneous larva migrans
- Egg is not seen in Strongyloides Stercoralis

Features	Filariform larva	Rhabditiform larva
Form	Infective	Diagnostic
Identification	Notched end	Double bulb esophagus Tapering end
Seen in	Hyperinfection and disseminated infection may show in sputum, stool and body fluids	Stool

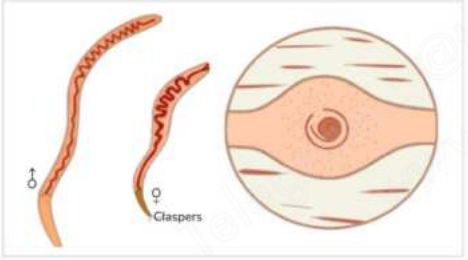


- Cutaneous Larva currens
- Pulmonary lung infection
- Small intestinal infection
- Filariform larva is associated with
  - Hyperinfection syndrome (severe form)
    → GIT
    → Lung
  - Disseminated Infection- all over the body

	Infective form	Route
Ascaris (Roundworm)	Embryonated eggs	Oral
Enterobius (Pinworm)	Embryonated eggs	Oral
Trichuris trichiura	Embryonated eggs	Oral
Ancylostoma (Hookworm)	Filariform larva	Skin
Strongyloides Stercoralis	Filariform larva	Skin
Trichinella spiralis	Encysted larva	Oral
Dracunculus medinensis	Cyclops	Oral

Trichinella Spiralis

00:49:02



- Spiral form
- Females are 2 times longer than males.
- Claspers in male hold female during mating in posterior end
- **Host:** Pig
- **Alternate/ dead end:** Human
- **Infective form:** Encysted larva

Life Cycle

00:50:20

Refer Image 42.7

- Primarily in pigs
- Accidentally in humans, by ingestion of undercooked meat (pork)
- Encysted larva in striated muscle of pork
- Digestive enzymes (acidic) in stomach breaks the cyst.
- In pork we eat muscles→ Insisted larva →Due to digestive enzymes and acidic conditions, the larva comes out →Male and female start the mating process →settle in Human muscle (dead end)

Laboratory Diagnosis

00:53:02

- **Biopsy:** Tendon insertion of Deltoid
- Bachman intradermal test
- Xenodiagnosis: Animal studies

Treatment: Albendazole

Dracunculus Medinensis/Guinea Worm

00:53:40

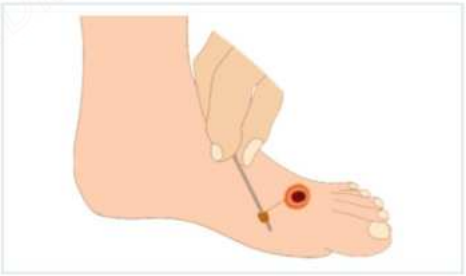
- **Eradicated:** Under national guinea worm eradication programme.
  - Steps taken:
    → Drink clean water.
    → Abate: Larvicide
- Caused by drinking dirty water.

Life Cycle

00:54:31

Refer Image 42.9

- Cyclops are consumed Goes to subcutaneous tissue Pregnant female makes subcutaneous tunnels (Blister formation) Discharge larvae through skin into the water.



Filarial Nematodes

00:57:35

Refer Table 42.1

Life Cycle

01:03:37

Refer Image 42.10

- Mosquito bite→Larva will be deposited→Lymph nodes →Adult worm formed →Mating →Microfilaria →Blood →Consumed by mosquito →Different larval stages

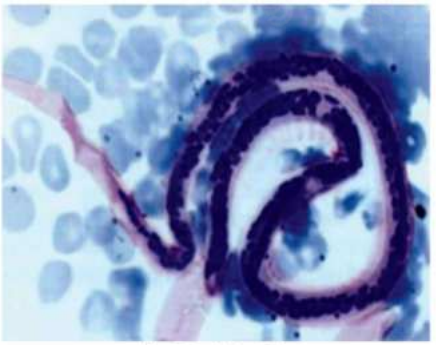
- Lymphatic filariasis

1. **Acute filariasis** –
   - Fever
   - Lymphadenitis
   - Lymphangitis

2. **Chronic filariasis:**
   - 10-20 years later due to lymphatic obstruction: Elephantiasis
   - Hydrocele: Genital involvement
   - Chyluria: Lymphatic fluid coming out into the urine.

- Urine sample
  - Has milkish white look
  - Sample of chyluria

- Microfilaria in blood



Microfilaria in blood film

  - It has the sheath
  - Vanished nuclei
  - Adult worm is in lymph node

	Classical Filariasis	Occult Filariasis
Cause	Due to adult and developing worms	Hypersensitivity to microfilarial antigen
Basic lesion	Lymphangitis Lymphadenitis	Eosinophilic granuloma formation
Organ involved	Lymphatic vessels and lymph node	Lymphatic system, lung, liver, spleen, joints
Microfilaria	Present in blood	Present in tissues but not in blood
Serological test	Complement fixation test. Not so sensitive	Prompt response to diethylcarbamazine (DEC) DEC provocation test

Lab diagnosis 01:10:15

- Peripheral blood
- DEC provocation test:
  - Diethylcarbamazine is given.
  - Force the microfilaria to come to blood.
- **If low parasitemia**
  - Quantitative Buffy Coat
  - Concentration methods
    → Membrane filtration
    → Knott's centrifugation

- **USG scrotum -** Filarial dance sign
- **Antibody-** Nonspecific
- **Antigen-** specific
  - ELISA (AD12Ag)
  - ELISA (Og4C310)

Treatment 01:12:04

- **Drug of choice**
  - DEC
- DEC + albendazole
- DEC + ivermectin

Brugia Malayi 01:12:19

- **Zoonotic -** from monkey via mansonia
- Difference from Wuchereria Brancrofti:
  - Leg below knee involved
  - No genital involvement
  - No hydrocele

Onchocerca Volvulus/ Convoluted Worms 01:13:28

- Seen in Africa
- Caused by black flies.
- Black population more affected.

Clinical Features 01:13:56

- **Skin-**
  - Dermatitis
  - Leopard skin- formation of thick dark skin
  - Onchocercoma – nodule formation
- **Eye -** River blindness
- **Lymph Node -** Hanging groin

Diagnosis 01:14:53

- Skin biopsy
- Mazotti skin test (DEC patch test)

Treatment 01:15:28

- DOC
  - Ivermectin

Loa Loa 01:15:39

- African eyeworm
- **Definitive host** – humans
- **Intermediate host-** chrysops/ deer flies
- Diurnal
- Asymptomatic infection
- Calabar / fugitive swelling - hypersensitivity reaction to adults, subcutaneous tissues, pruritus, fever
- **Treatment -** DEC

Mnemonic To Remember

Bile Non-Stained

- **N** – Necator
- **E** – Enterobius
- **H- H**. Nana
- **A -** Ancylostoma

Autoinfection

- **C-** Capillaria philippinensis
- **C** – Cryptosporidium
- **H- H**.Nana
- **E-** Enterobius
- **S-** Strongyloides
- **T- T**. solium

Does Not Float on Saturated salt solution

- **U -** Unfertilised eggs of Ascaris
- **L-** Larva strongyloides
- **T-**Taenia Egg
- **O**perculated egg (Trematodes)

Fish And Intermediate Host

Trematodes

- Clonorchis
- Opisthorchis
- Paragonimus(Crayfish)

Cestode

- D.Latum
- Spirometra

Nematodes

Capillaria philippinensis (Fish)

Q. The 2 pairs of ventral teeth are found in the buccal capsule of?

A. Ancylostoma duodenale
B. Necator americanus
C. Capillaria philippinensis
D. Ascaris lumbricoides

Answer: a. Ancylostoma duodenale

Q. Hookworm and Strongyloides infection are usually acquired through?

A. Bite of the adult
B. Ingestion of contaminated food
C. Contact of skin with infected soil
D. None of the above

Answer: c. Contact of skin with infected soil

Q. Tail tip contains nuclei in case of microfilariae of?

Answer: Brugia Malayi

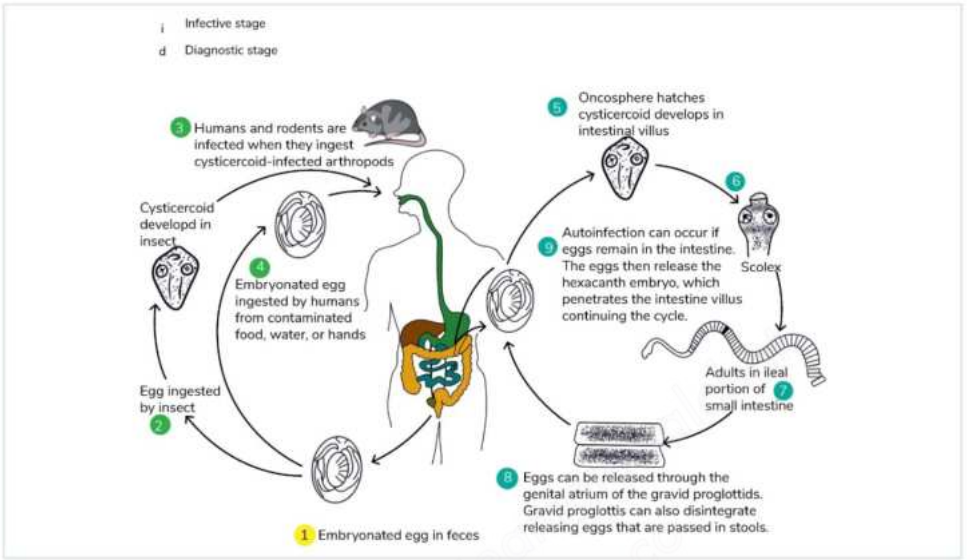

i Infective stage
d Diagnostic stage
3 Humans and rodents are infected when they ingest cysticercoid-infected arthropods
Cysticercoid developd in insect
4 Embryonated egg ingested by humans from contaminated food, water, or hands
Egg ingested by insect
2
1 Embryonated egg in feces
5 Oncosphere hatches cysticercoid develops in intestinal villus
6
Scolex
9 Autoinfection can occur if eggs remain in the intestine. The eggs then release the hexacanth embryo, which penetrates the intestine villus continuing the cycle.
Adults in ileal portion of small intestine 7
8 Eggs can be released through the genital atrium of the gravid proglottids. Gravid proglottis can also disintegrate releasing eggs that are passed in stools.

Image 42.2

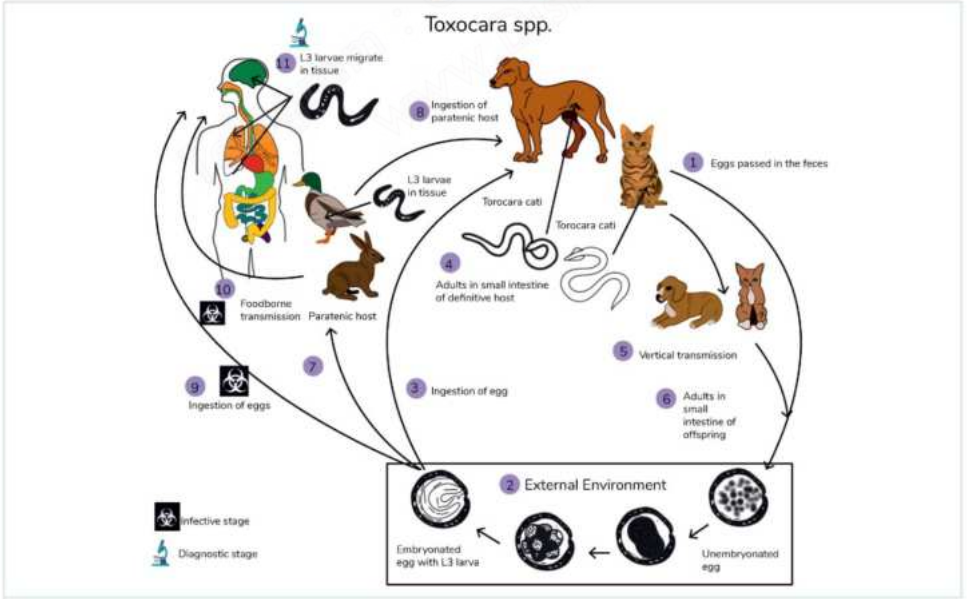

Toxocara spp.
11 L3 larvae migrate in tissue
8 Ingestion of paratenic host
1 Eggs passed in the feces
L3 larvae in tissue
Torocara cati
Torocara cati
4 Adults in small intestine of definitive host
10 Foodborne transmission
Paratenic host
5 Vertical transmission
9 Ingestion of eggs
7
3 Ingestion of egg
6 Adults in small intestine of offspring
2 External Environment
Embryonated egg with L3 larva
Unembryonated egg
Infective stage
Diagnostic stage

Image 42.3

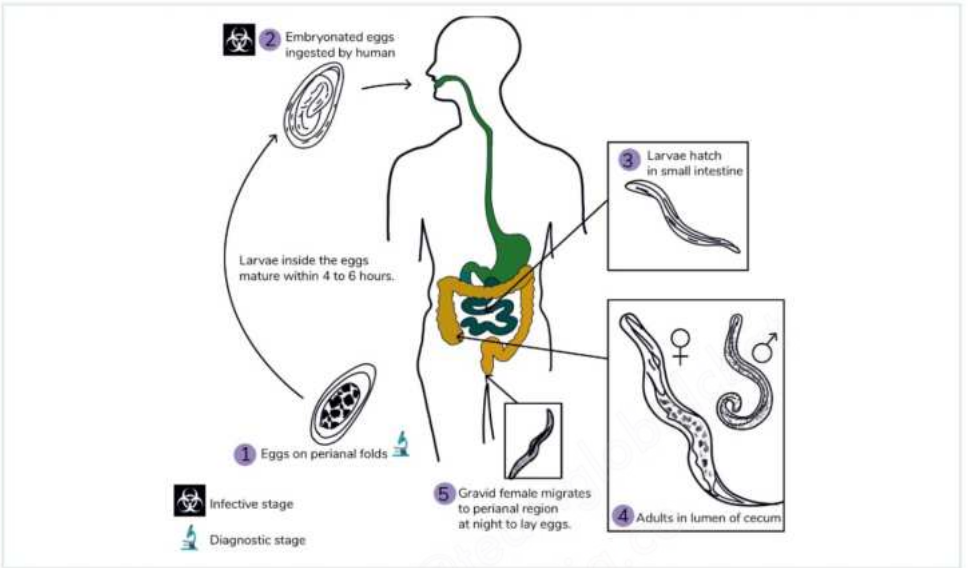


Image 42.4

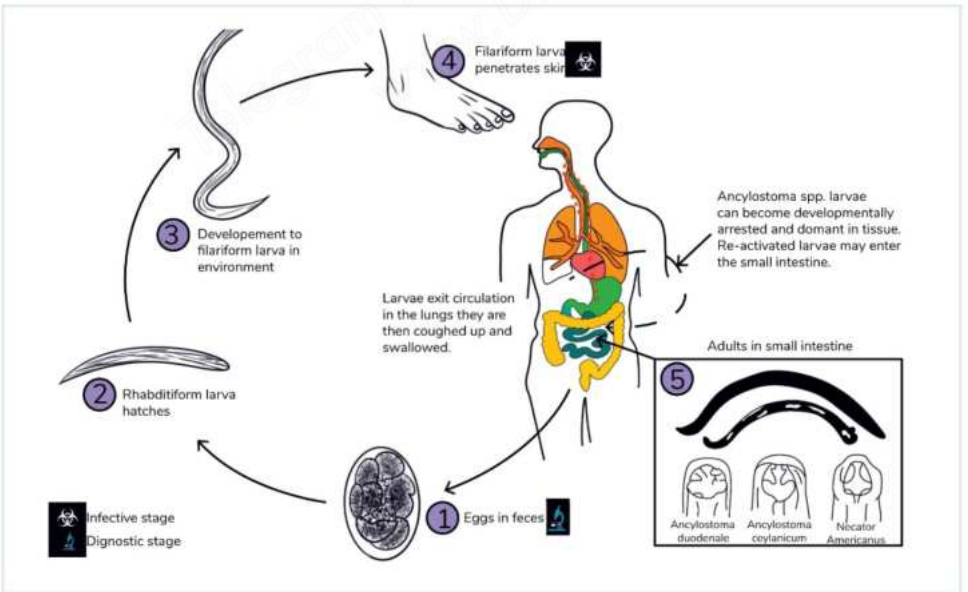


Image 42.5

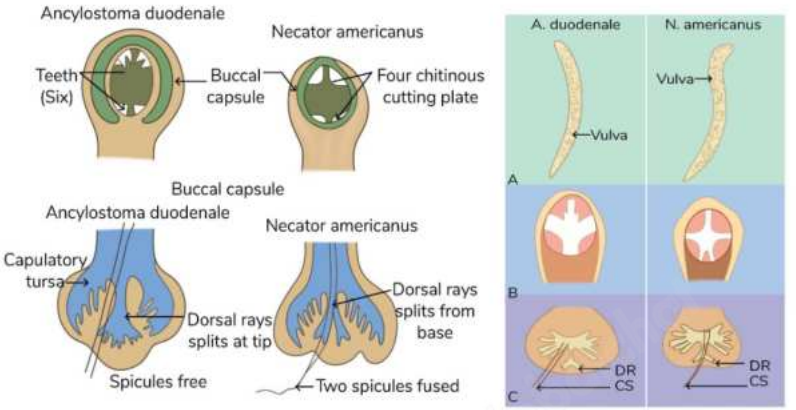


Image 42.6

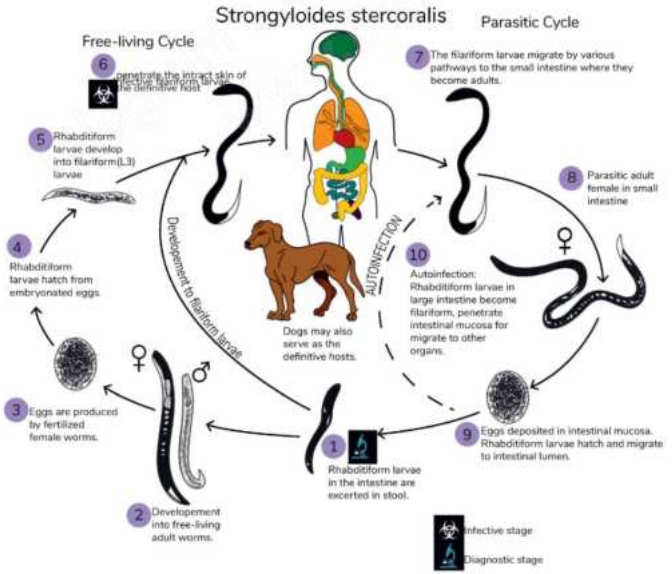


Image 42.7

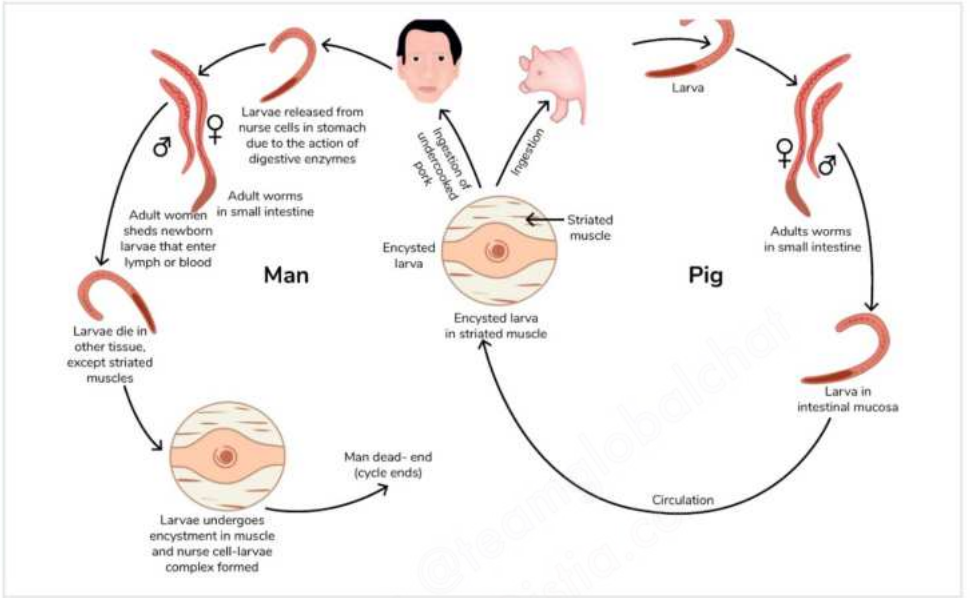


Image 42.8

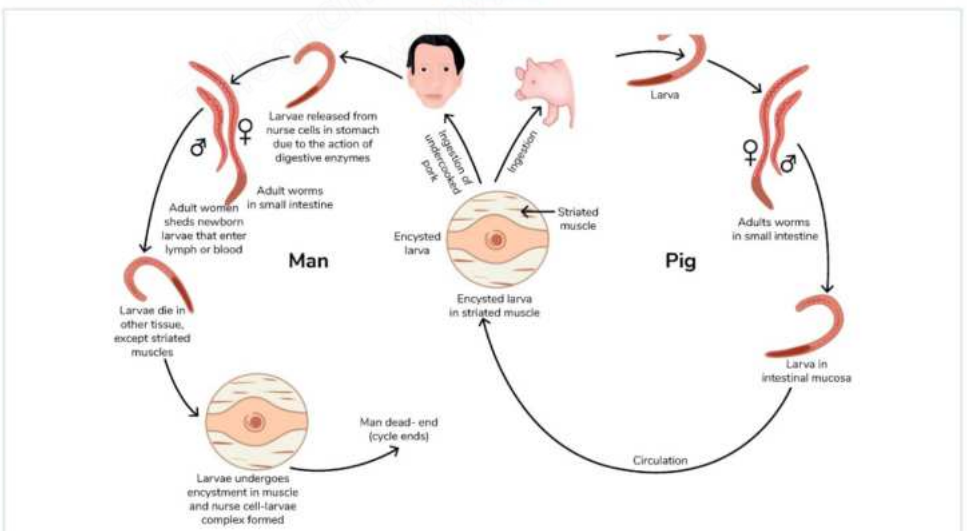


Image 42.9

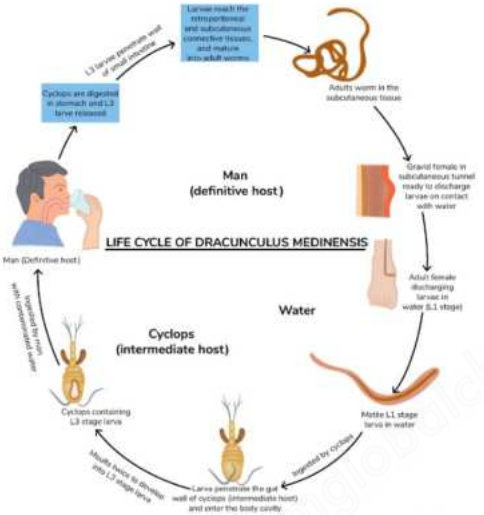
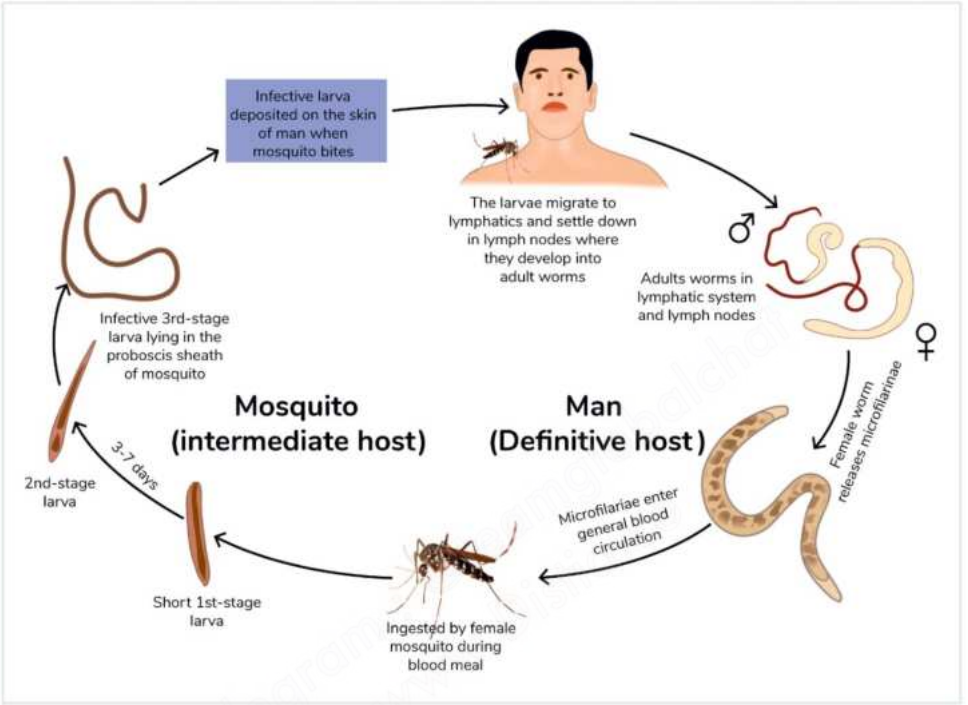


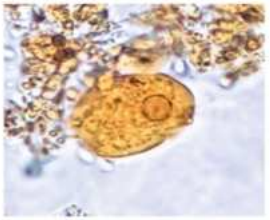
Table 42.1

Species	Wuchereria bancrofti	Brugia malayi	Loa loa	Mansonelia prestans	Mansonella ozzardi	Onchocerca volvulus
Shape						
Posterior end						
Tail nuclei	Nuclei do not extent to the tip of tail	2 nuclei at the tip of the tail	Nuclei from continuous row in the tip of the tail	Nuclei extend to the tip of the tail	Nuclei do not extend to the tip of the tail	Nuclei do not extent to the tip of the tail
Sheath	Sheathed	Sheathed	Sheathed	Unsheathed	Unsheathed	Unsheathed"
Periodicity	Nocturnal	Nocturnal	Diurnal	Non periodic		
Vector	Culex, anopehelesaedees	Mansonia	Deer fly	Midges	Black fly	

Image 42.10

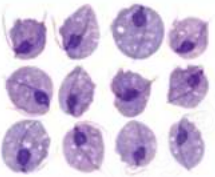

Infective larva deposited on the skin of man when mosquito bites
The larvae migrate to lymphatics and settle down in lymph nodes where they develop into adult worms
Adults worms in lymphatic system and lymph nodes
♂
♀
Female worm releases microfilarinae
Microfilariae enter general blood circulation
Ingested by female mosquito during blood meal
Short 1st-stage larva
3-7 days
2nd-stage larva
Infective 3rd-stage larva lying in the proboscis sheath of mosquito
Mosquito (intermediate host)
Man (Definitive host)

Q. Patient presented with colicky pain &diarrhoea. No history of fever & Stool sample is given for investigation & showed in the image diagnosis? (FMGE Dec 2020)



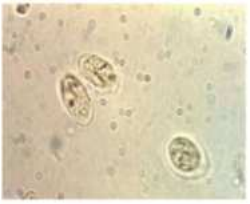
A. E. Coli
B. E. Histolytica
C. Giardia Lamblia
D. Shigella

Q. Identify image below? (FMGE Aug 2020)



A. Trichomonas
B. Plasmodium falciparum
C. Entamoeba histolytica
D. Giardia lamblia

Q. Cyst are parasite seen in stool microscopy. What is the organism? (AIIMS Nov 2017)

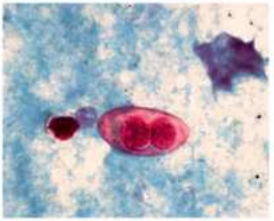


A. Entamoeba Dispar
B. Balantidium Coli
C. Giardia Lamblia
D. Taenia Solium

Q. Post renal transplant patient on Immunosuppressants complains of chronic diarrhoea. Stool examination reveals cyst of 4-6 micron in size. Positive on Kinyoun staining which of the following statements is true regarding the causative agent of this clinical condition? (AIIMS Jun 2020)

A. MC cause is Cryptosporidium
B. Oocyst becomes ineffective immediately after coming out in stools
C. These are obligate intracellular organisms
D. Autoinfection is seen with them

Q. A known HIV patient on anti-retroviral therapy presented with diarrhoea of six months duration. Stool microscopy was done in which 10-30 micrometre cyst were seen. Kinyoun Stain was positive. What is most likely. Diagnosis? (AIIMS May 2018)



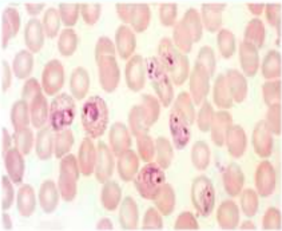
A. Cystoisospora
B. Cryptosporidium
C. Balantidium Coli
D. Strongyloides

Q. Intracellular parasites of hepatocytes? (AIIMS Nov 2019)
A. Malaria
B. Leishmania
C. Babesia
D. Toxoplasmosis

Q. A female from a tribal area of Jharkhand reports with fever for last 3 days peripheral blood is collected & stained with Giemsa. A diagnosis of malaria is made. The smear is shown in the figure. What is the likely cause? (NEET Jan 2020)

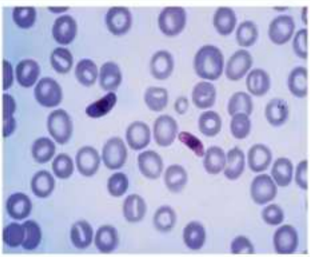
A. P. Falciparum
B. P. Vivax
C. P. Malariae
D. P. Ovale

Q. Identify organism based on peripheral smear finding given below? (FMGE Dec 2020)



A. Plasmodium vivax
B. Plasmodium falciparum
C. Babesia
D. Plasmodium ovale

Q. Banana shaped gametocytes are typically formed by: (FMGE Aug 2020)



A. Plasmodium vivax
B. Plasmodium ovale
C. Plasmodium malariae
D. Plasmodium falciparum

Q. Malaria relapse common with which type plasmodium species? (AIIMS Nov 2017)
A. Plasmodium malariae & vivax
B. Plasmodium falciparum & vivax
C. Plasmodium vivax & ovale
D. Plasmodium ovale & malariae

43 TYPES OF IMMUNITY

Types of Immunity

Acquired Immunity/ Adaptive immunity	Native/ Naive/ Natural/ Innate Immunity
• It is acquired after exposure to particular bacteria, virus or any infection. • It has memory • It has specificity • Macrophages present	• Since birth • No memory • It has no specificity • Macrophages present • Alternate complement system

Components of Innate Immunity

- **Epithelial barriers**: Skin, Mucosa
- **Cells**: Neutrophils, Macrophages (They help in phagocytosis)

- **Complement system**
  - **Other Name:** Alternate complement system (God has given)

- Innate lymphoid cells/ NK cells
- Pattern recognition receptors

Natural Killer Cells (NK Cells)

00:04:15

Other Names

- Innate lymphoid cells (ILC)
- Large granular lymphocytes
- MHC unrestricted cells
- Non B non T cells
- Null cells

NK Cells: Markers

- CD 16 (Receptor for Fc IgG)
- CD 56
- CD 94 (New marker)

NK Cells Towards Self Cells

- One arm is called activating arm(NKG2d)
- The other arm is inhibitory arm(CD94)
- Each self cell of our body contains MHC I
- When MHC1 cells are found, NK cells recognise and put the inhibitory arm forward and it doesn't kill it.

NK Cells towards Foreign Cells

- Viruses & cancer decrease the expression of MHC1.
- The activating arm kills the cell.

NK Cells

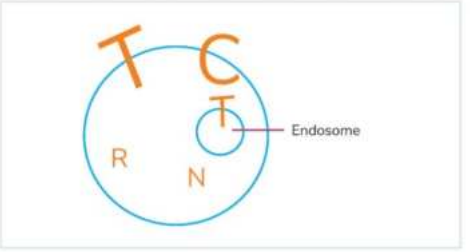
- Stimulation of NK cells:
  - Il2
  - Il15
- Activates killing of target cells
  - IL 12

Antibody Dependent Cellular Cytotoxicity (ADCC)

- Neutrophils
- Macrophages
- Eosinophils
- NK cells
- If any cell gets infected with virus/cancer, it gets coated by opsonin, IgG.
- To kill this virus IgG is picked up by these cells.
- For eg. NK cells are supposed to be attached with the FC portion of IgG.
- CD16 is the receptor for the FC portion of IgG.
- This is known as Antibody Dependent Cellular Cytotoxicity.

Pattern Recognition Receptors

- Toll like receptors
- C type lectin receptors
- RIG receptors
- NOD receptors
- These are the receptors which will recognise the pattern of bacteria, virus or any parasite.



PRR

- There are two receptors which are present on the membrane of the cell. These are membranous;
  - Toll like receptors
  - C type lectin receptors
- These are also present on membranes of organelles like endosomes.
- RIG and NOD receptors are cytoplasmic

Functions:

- Toll-like receptors are going to identify a bacteria.
- C type lectin receptors will identify fungal organisms.
- RIG receptors will identify viral organisms.
- NOD receptors will identify;
  - **N:** Necrotic or dead cells
  - **O:** organisms
  - **D:** associated with Diseases like Diabetes mellitus

  NOD2 mutation in inflammatory bowel disease.
- Cell death associated with NOD2 receptors - Pyroptosis

TLR (Toll like receptors) (activate the NF Kappa B pathway).	Binds To
2	Gram positive(teichoic acid), TB
3	dsRNA
4	Gram negative
5	Flagellin
6	Mycoplasma (fried egg colonies)
7, 8	Ssrna (M/C)
9	CpG DNA

Update – Interferonopathies

- RIG Receptor is Retinoic Acid Inducible Gene
- It activates STING - Stimulator of Interferon Gene
- It increases IFN Alpha that results in a group of diseases known as Interferonopathies.

Acquired Immunity

- Acquired Immunity is two types
  - Cellular immunity: B and T lymphocytes
  - Humoral immunity: Immunoglobulins

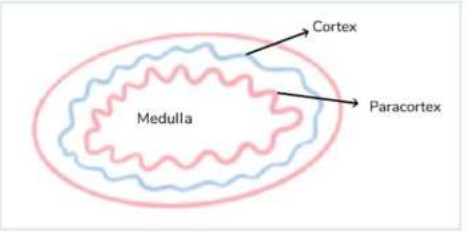
Cellular Immunity- B and T Cells

00:27:20

Characteristic	T cells	B cells
Percentage	60-70%	20-30%
Formation	Bone marrow	Bone marrow
Maturation	Thymus	Bone marrow
Sites		
Lymph node	Paracortex	Cortex
Spleen	White pulp (PALS)	White pulp
GIT	IEL	MALT/PEYER'S patches

Lymph Node

00:30:10



- Cortex have B cells
- Paracortex have T cells
- Medulla and it has Macrophages (M for M).

T Cells

- Every T cell has T cell receptor (TCR)
- 95% cases are alpha beta receptors.
- In 5% cases it is the gamma, delta receptors

Gamma Delta T cells

(Recent PYQ)

- These are present only 5-10%.
- These are present in GIT, genitourinary tract.
- They don't require any MHC.
- They process lipids, peptides.

CD Markers of T Cells

- T cells have: CD 1, 2, 3, 4, 5, 6, 7, 8, 28, 40L.
- The Pan T cell marker is Cd3
- CD3 is going to take signal transduction molecules.
- Helper T cell is CD4 (TH)
- Cytotoxic T cell is CD8. They participate in killing the antigen
- CD4:CD8 ratio is 2:1

To Remember

In entire immunology, there is a Rule of 8 to study.

- CD4:CD8 = 2:1
- Rule of 8
- CD4:CD8 ratio reduces → CD4 reduces → HIV
- CD4:CD8 ratio increases → CD4 increases → sarcoidosis

Q1. Who is the master regulator of the immune system?
Answer: CD4 cells or TH cells are considered as the master regulators of the immune system.

Relation of the T-cells in Cancer

- T- cells has a PD receptor
- The cancer cell will code itself with PDL1
- When this receptor and ligand bind together, suppression of immunity (Immunosuppressive response)

Treatment:

- Drugs like anti-PD-1 (Pembrolizumab) will block the PD receptors to prevent immunosuppression.

B Cells

- These cells have the B cell receptor with IgM and IgD antibodies.
- CD markers of B-cells
  - CD 10 (CALLA)
    - → C: Common
    - → ALLA: ALL Antigen
  - CD 19
  - CD 20, 21, 22, 23
  - CD 40
  - CD 79a, 79b
- **Pan B cell marker:** CD 19
- **Signal transduction molecule:** CD 79ab

Q2. How does the Epstein Barr virus enter the body?
Answer: Through B cells

Q3. How does it enter through B cells?
Answer: CD 21/CR2 marker

Interaction between B and T Cells

- B cells have IgM and IgD antibodies
- IgM and IgD are converted into IgG, IgA, IgM, IgD, and IgE by a process called Isotype switching (Change)
- Mnemonic: **Ch**ange
  - **CH**aalis: 40
  - **Ch**aar: 4
- B cell will have CD 40 receptor
- T cell will have CD 40 ligand
- When a foreign body (Antigen) enters into the body then it will get attached to IgM antibody
- Then this B cell give the antigen to T Cell
- T Cell further converts them into the Il4
- The IL4 will convert the IgM and IgD into IgG, IgA, IgM, IgD, and IgE

Q4. Which interleukin is needed for Isotype switching?
Answer: Il4

Q5. Which CD marker is needed for Isotype switching?
Answer: CD 40

Humoral Immunity 00:51:41

- Formation of antibodies is generally considered as humoral immunity
- IgG (maximum concentration)
- IgA
- IgM
- IgD
- IgE (minimum concentration)

Types of APCs	Description
Professional APCs	• These are further divided into ○ Dendritic cells ○ B cells ○ Macrophages • These dendritic cells are present in two regions ○ Skin: They are called as Langerhans cells ○ Lymph node: Follicular dendritic cells → Reservoirs for HIV (Langhans cells are not antigen presenting cells, these are the giant cells generally found in the TB condition)
Non - professional APCs	It includes • Thymic epithelial cells • Endothelial cells • Fibroblasts

- First this antigen will be presented on the MHC and then it will be given to T cells

Major Histocompatibility Complex 00:57:40

- It was originated from the HLA gene that is presented on the chromosome 6p (Short arm)

Chromosome 6

The genes present in the chromosome 6 includes

- HLA gene
- HFE gene (Gene for hemochromatosis)
- Autosomal recessive polycystic kidney disease

Structure of HLA Gene

- It has many parts
  - HLA gene I
  - HLA gene III
  - HLA gene II
- HLA gene I has HLA IA, HLA IB, HLA IC, HLA IE, and HLA IG
- HLA gene II has DP, DQ, and DR
- HLA gene III has no subdivisions
- HLA E will be the self recognition target for Natural Killer cells
- HLA G (Gestationalis) responsible for the feto-maternal tolerance
- HLA I gene → MHC I
- HLA II gene → MHC II
- HLA III:
  - Complementary proteins like C2, C4
  - Properdin
  - Tumor necrosis factor alpha
  - Heat shock proteins (HSP)

Characteristic	MHC I	MHC II
Structure	• It has arms o Alpha 1 o Alpha 2 o Alpha 3 o Beta2 microglobulin	• It has o Alpha 1 o Alpha 2 o Beta 1 o Beta 2
Antigen binding cleft	At the junction of alpha 1 and alpha 2	At the junction of Alpha 1 and the Beta 1
Present on cells	• All nucleated cells in the body • Platelets	Present in all the antigen presenting cells
Not present	• RBC • Sperms	
Present to cells	CD 8 cells	CD 4 cells
Diagnosis	Allo antisera testing	Mixed lymphocyte reaction testing

Important Information

- As RBC doesn't have the MHC I, to survive they will adsorb the MHC I from the surroundings

Rule of 8	Rule of 8 is also studied in
MHC I: CD 8 (1 ×8 = 8) MHC II: CD 4 (4 ×2 = 8)	CD4 /CD8 = **2** /1 MHC I = CD8 MHC II = CD4

Types of T Cells

It is of two types

- CD4 cells
- CD8 cells
- It is further divided into
  - TH 1
  - TH 2
  - TH 17

TH 1	TH 2	TH 17
• It releases IL 2 • IL 2 will activate TH1	• It releases IL4, IL5, IL13 (Responsible for asthma)	• It releases IL17 and Il22
• It has an association with IL12 and TNF gamma (Controlled) • INF gamma will help in the formation of granuloma	• IL4: Helps in isotype switching • IL5: It results in eosinophilic activation • IL13: Helps in mucous production	• They are responsible for neutrophils and monocytes

To Remember: All the asthma allergies are hypersensitivity type 1

- These are cytotoxic T cells

Mechanism of Cytotoxic cells

- It has two mechanisms

Mechanism of Cytotoxic Cells	
Mechanism I	**Mechanism II**
CD95 and CD95L will help in Apoptosis ↓ An Apoptic cell will have CD95 ↓ Now a T cell will reach there with CD95L ↓ Hence they help in apoptosis	**Name: Perforin Granzyme Pathway** CD8 T cells have perforin and granzyme ↓ When the foreign body enters then perforin will enter the foreign body and then it will make some perforation ↓ Then granzymes will enter through the perforations ↓ Hence it kills the foreign body (Bacteria)

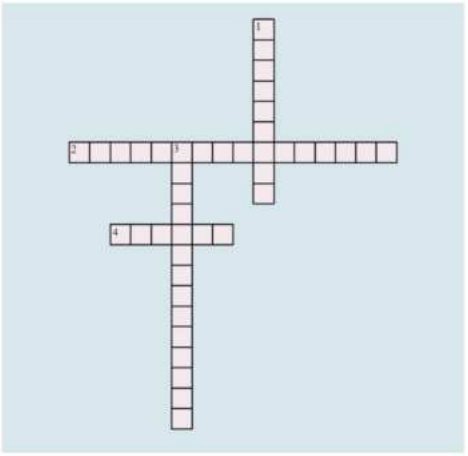
Q6. True statements about MHC are all except?

A. Chromosome 6 harbors gene for MHC
B. Gene encoding complements are adjacent to MHC
C. Monocytes have MHC class II molecules on their surfaces
D. Class III MHC does not encode complement

Answer: Class III MHC does not encode complement

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. Immunity acquired after exposure to particular bacteria, virus or any infection
4. The outermost area of the Lymph Node is called

Down

1. These are also present on membranes of organelles
3. God gifted immunity

44 TYPES OF HYPERSENSITIVITY REACTIONS

Immunity - Hypersensitivity Reactions

Gell & coomb classification

- Type I- Anaphylaxis
- Type II- Cytotoxic
- Type III- Immune Complex

} Antibody mediated

- Type IV- Delayed Type → Cell mediated

Type I Hypersensitivity Mechanism 00:02:18

- The first exposure: allergen.
- Antigen-presenting cells pick up the allergen & going to keep the allergen on MHC and present it to the T cell.
- T cells differentiates into TH2 cells.
- TH2 cells produce interleukin 4, interleukin 5, and interleukin 13.
- Interleukin 4: isotype switching → IgE production
- Interleukin 5: eosinophils activation happens.
- Interleukin 13: mucus production will occur.
- IgE coats the mast cells leading to sensitization.
- When the second exposure happens, it will bridge the gap in the mast cell & undergoes polymerization. This will cause degranulation of the mast cell.
- As a result, the mast cell bursts, will release histamine as the mast cell is a rich source of histamine.

Q. What is the special stain for mast cells and basophils?
Ans - Toluidine blue

Type I Hypersensitivity Early Phase

- Occurs within 2 hours
- Preformed mediators - release of histamine & Tryptase.
  - Patients with allergies have a rise in serum tryptase.
- Newly formed mediators
  - PG
  - Leukotrine
  - Platelet Activating Factor
- All of these above mediators cause bronchoconstriction.
- Histamine causes vasodilation in the blood, and it causes bronchoconstriction in the lungs.

Type I Hypersensitivity Delayed Phase

- Occurs after 2 to 24 hours.
- Due to eosinophils
- Interleukin 5 is associated with it.
- Eosinophils release MBP (Major Basic Protein)

Difference between Anaphylaxis and Anaphylactoid

- Anaphylaxis: IgE is involved here
- Anaphylactoid reaction: IgE is not involved

Type I Hypersensitivity Examples

a. Allergies (allergic dermatitis, rhinitis, conjunctivitis.)
- Other names of allergic conjunctivitis - VKC (Vernal KeratoConjunctivitis)
- Drug of choice for VKC - Olapatadine
- Olapatadine is a mast cell stabilizer. It also has antihistamine properties.
- ATOPY - Allergy with a genetic determinant.
  - Associated with chromosome 5.

b. Bronchial Asthma

c. Casoni Test
- The test is done for Echinococcus Granulosus.

d. Drugs (Penicillin - Test dose)
- Some other hypersensitivity reaction examples include
  - PK reaction
  - Theobald smith phenomenon

Type II Hypersensitivity Mechanisms 00:17:33

Mechanisms	
Opsonization and Phagocytosis	Antibody and antigen reactions IgG will opsonize it, and phagocytosis will occur.
ADCC	Once the antigen is covered with IgG, it will be identified by NK cells and macrophage and all resulting in ADCC (Antibody dependent cellular cytotoxicity.)
Complement activation and inflammation	Antigen and antibody reactions activate the complement system.

Type II Hypersensitivity Examples

- My blood group is Rh+ve (mnemonic)
  - My: Myasthenia Gravis
  - Blood: blood transfusion reaction
  - Group: Good pasture syndrome and Graves disease
  - Is: Immune hemolytic anemia (IHA)
    → Immune thrombocytopenic purpura (ITP)
  - Rh: Rh incompatibility,RHD
  - Positive: Pemphigus (Bullous) and Pernicious Anemia
- Myasthenia Gravis and Graves disease are now classified as Type V hypersensitivity.
  - In Myasthenia Gravis, Antibody is against acetylcholin receptor
  - In Graves disease, the antibody is against the TSH receptor
  - Antibodies against cellular receptor → type V hypersensitivity

Type III Hypersensitivity - Phases 00:27:10

- Immune complex
- Occurs in 3 phases:
  - **Immune complex formation** - Antigen and antibody both are mobile and combine to make an immune complex
  - **Immune complex Deposition** - These deposit in tissues (joints, skin & serosa)
  - **Complement Activation** - leading to signs and symptoms
  - Duration - 10 to 14 days
- Difference with Type II
  - In Type II, the antigen is fixed.
  - In Type III, both antibodies and antigens are mobile

Type III Hypersensitivity – Examples

- **S** - SLE
- Serum sickness (anti-tetanus / anti-rabies serum)
- Schick test C. Diphtheriae)
- **H** - Henoch Schonlein Purpura (IgA Vasculitis in children)
- **A** - Arthus reaction (localized immune complex disease)
- **R** - Raji Assay, Reactive Arthritis
- **P** - PSGN (Poststreptococcal Glomerulonephritis) cola color urine
- PAN,
- Parasitic diseases (eg, malaria → nephrotic syndrome → affecting kidneys)

Type IV Hypersensitivity 00:36:20

- Delayed type hypersensitivity
- Only cell-mediated
- It has CD4 T cells and CD8 T cells
- T cell to form TH1 cells.
- TH1 releases interferon-gamma, forms granuloma.
- CD8 T cells work as well in apoptosis and perform granzyme pathways.

Type IV Hypersensitivity – Examples

- Granuloma
- Skin test
  - Mantoux test
  - Lepromin test
- IBD (Inflammatory Bowel Disease)
- Multiple sclerosis
- Contact dermatitis
  - Most common cause worldwide- poison ivy
  - Most common cause in india- detergent usage and artifical jewellery

Type V Hypersensitivity – Examples 00:41:45

- Myasthenia Gravis and Graves disease.
- There are antibodies against cellular receptors.

Controversial Hypersensitivity

- Rheumatoid arthritis. - Type 3 and Type 4
- Hypersensitivity pneumonitis - Type 3 and Type 4
- Transplant rejection
  - Hyperacute - Type 2
  - Acute - Type 2 and Type 4
  - Chronic - Type 4
- SLE: combination of type 2 <<< type 3 hypersensitivity.

Q. A 30-year-old HBsAg positive female complaints of feeling generally ill and fatigued, having fever, and loss of appetite and weight loss. Occasional episodes of muscle and or joint pains are reported. On GPE, the skin sores are noted. There is no cardiopulmonary discomfort noted. Her BP is raised. On arteriography, irregular narrowing and dilation of the blood vessels is noted. Biopsy of the vessels show fibrinoid necrosis. Which of the following hypersensitivity reactions play a role in this disease?

a. Type I
b. Type II
c. Type III
d. Type IV

Ans - Type III

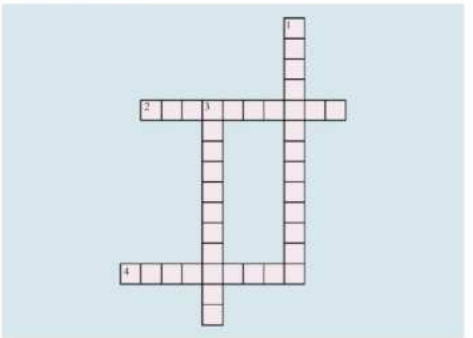
Q. A 42-year-old patient presents with cough and sputum. He also complains of weight loss, fever, and night sweats. On sputum examination, there are multinucleated giant cells along with lymphocytes , and macrophages. Numerous scattered cells with slipped shaped nuclei are noted. Which of the following hypersensitivity reactions play a role in this disease?

a. Type I
b. Type II
c. Type III
d. Type IV

Ans- Type IV

CROSS WORD PUZZLES

Crossword Puzzle



Across

2. The test is done for Echinococcus Granulosus
4. The special stain you have for mast cells and basophils

Down

1. This is a classical hyperthyroidism
3. Drug of choice for VKC

- Tolerance to self-antigen
- Two types of tolerance:
  - Central tolerance (Bone marrow, Thymus)
  - Peripheral tolerance

Central Tolerance

00:01:12

Two mechanisms:

1. Deletion/negative selection

- All the self-reactive T Cells are going to be deleted.
- The regulator of this entire process: Autoimmune regulator (AIRE) gene.
- Defect in the AIRE gene: Autoimmune Polyendocrinopathy (AIPE) (diabetes Mellitus, Addison's disease, etc.)

2. Receptor editing

- B cells (self-reactive)

Peripheral Tolerance

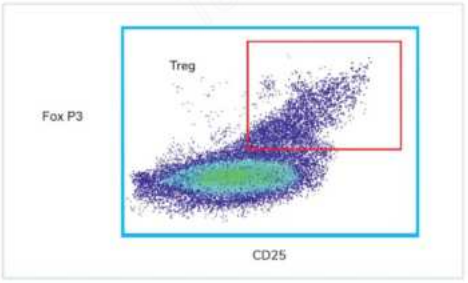
00:06:06

- If central tolerance fails, peripheral tolerance will work.

1. T regulatory cells

- In peripheral tolerance, T regulatory cells are present.
- This is a Type of T cell that decreases immunity. It is called immunosuppression.
- They are a type of CD4 T cells that are CD25+/IL2Rc.
- FOXP3 genes regulate them.

Flow Cytometry



- CD25+ & FOXP3+ = T regulatory cells
- T regulatory cells are regulated by an increase in CTLA4 and PD1 and this causes immunosuppression
- Interleukin 2 receptor mutation:
  - Inflammatory bowel disease
  - Multiple sclerosis.
- FOXP3 gene mutation: there will be IPEX.
- Two genes: FOXP3 gene and FOXL2 gene.
- FOXP3 - causes IPEX Syndrome.
  - I- Immune dysregulation
  - P- Polyendocrinopathy
  - E- Enteropathy
  - X- X-linked disorder
- FOXL2
  - Granulosa cell tumours of the ovary.

2. Killing of self reactive cells

- These cells will be killed.
- Killing happens by Apoptosis.
- Apoptosis is mediated by Cd95
- If there is CD95 or Fas mutation: Autoimmune lymphoproliferative syndrome (ALPS)

3. Anergy

00:13:44

- It is applicable to T and B cells.
- **For T cells:**
  - When an antigen comes into a cell, the antigen-presenting cell is going to present it via an MHC to T cells.
  - T Cell receptor is alpha and beta.
  - Signal 1: When APC and MHC is going to give antigen to T cell (T Cell receptor)
  - But T cells need one more signal.
  - APC increases molecules on it's surface, and these are known as B7.1 (CD80) and B7.2 (CD86)
  - They will go and bind to Cd28.
  - This will be Signal 2, known as the costimulatory signal.
  - Now, the T cell will get activated.
  - CD28 is a costimulatory signal and will activate the T cell.
  - So, when there is a foreign antigen, signal 1 and 2 signal are needed. If there is self-antigen, then no signal 2.
  - Not having signal 2 is known as Anergy.

- **For B cells**
  - The B cells have IgM and IgD.
  - They will pick & give antigens to the T cell this is Signal 1.
  - Signal 2: B cell has CD40 receptor, and T cell has CD40 ligand.
  - Binding between these two occur & are known as costimulatory signals.
  - It activates T cells and releases Interleukin 4 and this will change IgM and IgD into IgGAMDE.
  - When there is self-antigen, signal two will not be there and this will be known as Anergy.

Cryptic/Hidden Antigen

Sites where antigens are not shown:

- B - Brain antigens except for area postrema,
  - Hidden by a blood-brain barrier.
- E - Eyes except for optic Nerve
  - Hidden by a blood-ocular barrier.
  - Eg. Trauma eye → B-O-B breaks → Traumatic uveitis
- T- Tests except for epididymis,
  - hidden by a blood testicular barrier.

Autoimmune Disorders 00:25:10

Systemic Lupus Erythematosus

- It is a combination of type 2 and 3 hypersensitivity reactions.
- It is associated with early complement deficiency = C1, C2, C4.
- More common in females.
- Also associated with Klinefelter syndrome.
- There is also a cell death associated with NETs.

SLE- Criteria

There are a total of 11 Criteria; 4 are needed for the diagnosis.
Mnemonic: **MD SOAP BRAIN**

- **M** - **M**alar Rash
  - Butterfly-shaped rash on face
  - Spares nasolabial fold.

- **D**- **D**iscoid Rash (Round like a discoid)

- **S**- **S**erositis
- **O**- **O**ral Ulcer (Aphthous; painless)
- **A**- **A**rthritis (non-erosive arthritis)
- **P**- **P**hotosensitivity
- **B**- **B**lood
  - Pancytopenia (low Haemoglobin, low WBC, low platelets)
  - LE cells

- **R**- **R**enal
  - Lupus Nephritis
    - → Has total of 6 classes, from Class I & VI
    - → Microscopically: Most characteristic lesion - Wire loop lesion - Class III, IV&V
    - → Maximum wire loop lesion - Class IV

- **A**- **A**NA (antinuclear antibody)
- **I**- **I**mmunological (antibody)
- **N**- **N**eurological (Psychosis)

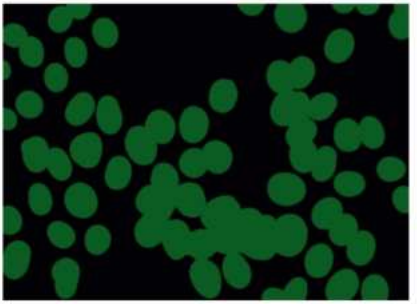
Antibodies

- Most sensitive: ANA (Antinuclear antibody)
- Most specific: ASA (anti-Smith antibody)
- An antibody that is both sensitive and specific: Anti-double-stranded DNA antibody (anti-dsDNA antibody)
- SLE resulting in psychosis - Anti ribosomal P antibody
- SLE has happened in children - Neonatal Lupus - anti-Ro antibody

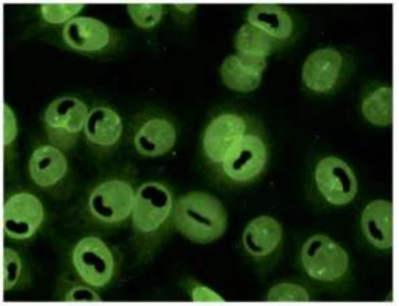
Q. How do you detect antinuclear antibodies?
Ans: Immunofluorescence

Patterns of ANA
Mnemonic - **HAPPENS (HPNSC)**

H - **H**omogenous technique

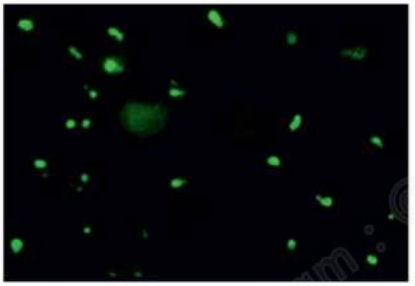


P - **P**eripheral or rim pattern



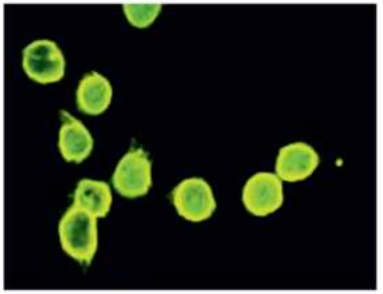
- It happens when antibodies are only against anti dsDNA

N - Nucleolar



- It happens when antibodies are against RNA.

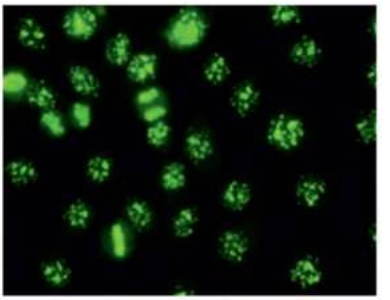
S - Speckled pattern



- Most common patterns in autoimmune disorder
- Ab Against
  - Anti-Smith: most common for SLE
  - Anti RO and Anti LA: related to Sjogren syndrome

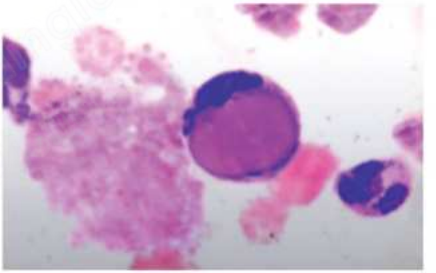
- Least specific pattern

C - Centromeric pattern



- Related to CREST syndrome

LE cell



- Lupus erythematosus cell
- It is a neutrophil or a monocyte that has engulfed damaged nuclear material (Hematoxylin body).
- The damaged nuclear material left is called the Hematoxylin body (blue-colored body)
- These dead cells will then be engulfed with either a neutrophil or a monocyte.

LE Cell	Tart Cell
Seen in SLE Related to autoimmune disorder	Normal cell (macrophages, monocytes engulfed other cells)

Other Organs affected by SLE

- Heart- Libman Sacks Endocarditis
- Lungs- Patient shows shrinking lung
- Spleen- Onion skinning pattern around the arterioles

Drug-induced Lupus (DIL)

- Variant of SLE
  - SHIP Drugs
    - → S - Sulfonamides
    - → H - Hydralazine
    - → I - Isoniazid
    - → P - Procainamide
    - → D-Dapsone

SLE	DIL
• Antibody's: ANA, ASM, dsDNA • Organs - CNS, Renal • Treatment - steroids	• Antibody: Anti-histone antibody • Brain and kidney does not get affected • Treatment - Withdraw the drug

Sjogren syndrome

- Known as dry eyes and dry mouth
- Antibodies against salivary glands:
  - Dry mouth
  - Halitosis (Bad breath)
- Antibodies against lacrimal glands:
  - Dry eyes
  - Gritty sensation in the eyes
- Antibodies are:
  - Anti-SS-A (Referred to as Ro) - Most sensitive
  - Anti SS-B (Referred to as La) - Most specific
- **Diagnosis**
  - Serology to find the antibody
  - Lip biopsy: Lip has minor salivary glands
  - M/E: Lymphocytes
- Sjogren syndrome is also associated with Primary biliary cholangitis in the liver, marginal zone lymphomas.

Scleroderma

00:55:05

- Sclero: stiff or hard/thick
- Derma: Skin

Sclerodactyly

- Sclerosis of digits
  - Claw-shaped digits due to stiffness
- This can happen anywhere in the body
- Two forms of scleroderma

Limited

- Caused by anti-centromere antibody
- Limited involvement of skin
- A limited form of scleroderma is associated with CREST syndrome.

Diffused

- There is diffuse involvement of skin
- Caused by anti-Topoisomerase antibody / Anti SCL 70 (most specific)

CREST syndrome

- Associated with anti-centromere antibody
- **C** - Calcinosis cutis
- **R** - Raynaud's phenomenon
- **E** - Esophageal dysmotility
- **S** - Sclerodactyly
- **T** - Telangiectasis (problems in blood vessels)

Mixed Connective Tissue Disorder

- Mixed features of SLE, Sclerosis, Polymyositis
- Antibody: Anti U1 RNP (RIbonucleoprotein) antibody
- Treatment - Steroid

Myositis

- Three types of myositis

1. Dermatomyositis
2. Polymyositis
3. Inclusion body myositis

Dermatomyositis

- Myo - Proximal muscles will be involved
  - Causing weakness in patients
- Derma - Involvement of skin
  - Causing rash around the eyes
    - → Heliotrope rash

  - Papules present on the knuckles and phalanges
    - → Gottron papules

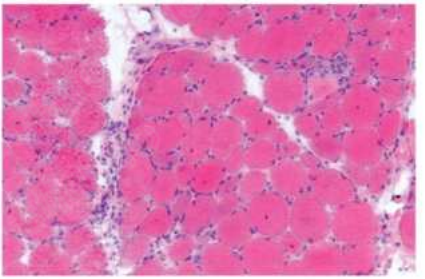
- Rash on the skin of the neck and back
  → Shawl sign

- V-shaped rash on neck
  → Necklace sign

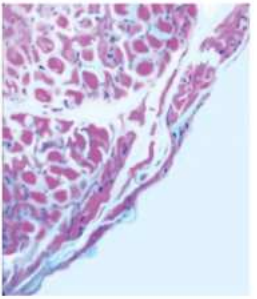
- Presence of cracked hands
  → Mechanic hands

Muscle biopsy:

- All the inflammation is perifascicular (around the fascicle)



- Perifascicular atrophy has also occurred



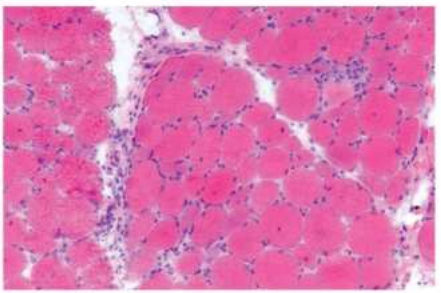
- The muscle fibres in the centre are bigger, and at the periphery, the muscle fibres have become very small, called peri fascicular atrophy

Antibodies:

- ANA+ve
- Anti Jo 1 Ab+ve: Mechanic hand
- Anti Mi2 Ab+ve: skin features
- Anti P 155 Ab+ve: Paraneoplastic syndromes Anti P
- 140 Ab+ve: Juvenile Dermatomyositis

Polymyositis

- Proximal muscle weakness.
- There is no derma problem.
- The inflammation here is Endomysial inflammation.



- In Dermatomyositis, there was perimysial atrophy.
- But in Polymyositis, there is Endomysial inflammation and no atrophy.

Inclusion Body Myositis

- IBM happens more in adults.
- Muscle biopsy shows rimmed vacuoles

- Stain: Gomori Trichrome stain also shows rimmed vacuoles that contain Amyloid like inclusions.
- Stain for Amyloid will be Congo red.

Q. Which of the following hypersensitivities can be noted in SLE?

A. I, III
B. II, III
C. III, IV
D. I, IV

Q. Which of the following inflammatory cells is more commonly seen in a lip biopsy of a patient of Sjogren syndrome?

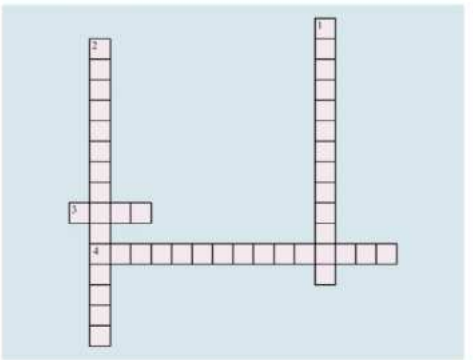
A. Neutrophil
B. Lymphocyte
C. Monocyte
D. Basophil

Q. LE cell is a_____ that has engulfed a_____?

A. Neutrophil, lymphocyte
B. Neutrophil, hematoxylin body
C. Macrophage, lymphocyte
D. Macrophage, neutrophil

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. (CD25+) - (FOXP3+)
4. Known as dry eyes and dry mouth

Down

1. Dot dot appearance inside the nucleus
2. There is a Type of T cell that decreases immunity

46 IMMUNODEFICIENCY DISORDERS

Types of immunodeficiency

- Primary immunodeficiency
- Secondary immunodeficiency

1. Secondary immunodeficiency
- Causes
  - Malnutrition (most common)
  - HIV/Aids
  - Cancer
  - Chemotherapy

Primary immunodeficiency 00:01:50

It is divided into 3 groups

1. Defect in Lymphocytes maturation
2. Defect in lymphocytic activation and function
3. Systemic disease

Defect in Lymphocytes maturation

1. SCID
2. Bruton's hypogammaglobulinemia
3. Bare lymphocyte syndrome
4. Digeorge syndrome

1. SCID (Severe Combined Immunodeficiency)

- B, T, NK cells are affected.
- It is of two types

XLR (X linked recessive variety)

- Most common problem in the gamma subunit of cytokine receptors: IL2, IL4, IL7, Il15).
- T and NK cell are affected. B cells are not affected

AR (Autosomal recessive variety)

- ADA (Adenosine deaminase) deficiency(most common) Involves all cells (B, T, NK)
- JAK3 defect:(T and NK cells are affected. B cells are normal)
- RAG1 and RAG2 (Recombinant activation gene) defect: B and T cell affected. NK cells normal
- If all the B, T, and NK cells are defective, then it will be ADA deficiency that is autosomal recessive.
- If B and T cells are defective and NK cells escape, it will be RAG 1,2.

Clinical features

- Morbilliform rash
  - Red rash on face of child
  - Maternal T cells attack featal skin
- Pneumonia
- Pneumocystis
- CMV

Treatment

First disease to be treated with gene therapy

- For Gamma subunit (XLR)
- T cell proliferation started (Leukemia)

Enzyme replacement used now

- ADA deficiency

2. Bruton's hypogammaglobulinemia

- Occurs most commonly in boys, XLR
- BTK gene defect
- B cell defect

$$\text{Immature B cells} \xrightarrow{\text{Bruton tyrosine kinase}} \text{mature B cells} \longrightarrow \text{plasma cells and antibodies}$$

- BTK is not there, so B cells won't mature, then plasma cells and antibodies will not be formed.
- Less antibodies will cause hypogammaglobulinemia.

Clinical features

- Enterovirus infections
- Polio
- ECHO
- Coxsackie virus infection.
- Live polio vaccine is always contraindicated.
- Reduced IgG → defective optimisation

3. Bare lymphocyte syndrome

- MHC II is missing

4. Digeorge syndrome- mnemonic- CATCH22 00:18:22

- Also known as Velo Cardio Facial syndrome.
- There is deletion 22q11 (TBX 1 gene is going to be deleted) → 3rd and 4th pharyngeal pouches are not developed. Thymus and parathyroid hormones will not form.
- Reduced parathyroid hormones, reduced calcium levels → hypocalcaemia.
- T cells mature in Thymus, if thymus won't mature and cause thymic aplasia and T cell defect will be there.
- also known as velo cardio facial syndrome.
- Tetralogy of fallot.
- Abnormal facies are there: Cleft in lip and cleft palate.

Diagnosis

- FISH: diagnosis of choice
- TBXgene: seen in digeorge syndrome and schizophrenia.

Defect in lymphocytic activation and function

1. **Common variable Immunodeficiency (CVID)**
- It is a B and T cell problem
- B cell problem is because of the BAFF gene defect and the T cell problem is because of ICOS gene defect.

Differences between Bruton's hypogammaglobulinemia and CVID. 00:24:24

Bruton's hypogammaglobulinemia	CVID
• BTK gene defect • Occurs in boys • B cell defect • Hypoplastic germinal centers	• BAFF gene defect • Occurs in boys & girls • B & T Cell defect • Hypergerminal centers

2. **Absence of CD ligand or CD receptor leading to hyper IgM**
- The patients of hyper IgM syndrome are at the risk of pneumocystis carinii.

3. **Hyper IgE Syndrome**
- Mutation: STAT 3 defect
- It is also known as job's disease.

4. **Isolated IgA Syndrome**
- IgA is a mucosal antibody present in mucosa (GIT, respiratory mucosa)
- when IgA goes down, mucosal immunity goes down that causes mucosal infection.
- GIT affected diarrhoea
- Respiratory mucosa affected respiratory infections
- In the patients of Isolated IgA deficiency, washed RBCs are given to patients.

5. **XLP disorder (X linked lymphoproliferative syndrome)**
- SAP defect (SLAM activating protein)
- SLAM: Signal Lymphocyte Activation Molecule
- Needed by B/T/NK cells
- Increased risk of EBV infections

Systemic disease

1. **Wiskott Aldrich Syndrome (WAS)**
- X linked recessive disorder and WASP gene defect.
- Patients have bleeding manifestations (because of platelet defects, small size platelets)
- Infections
- eczema.
- Ig profile: IgM decreased, IgA and IgE increased

2. **Ataxia Telangiectasia**
- It is associated with ATM gene
- Immunodeficiency is also there.

MCQ's

Q. A new born child presents with a morbilliform rash. Mother also gives a history of recurrent diaper rash. Examination reveals oral candidiasis and failure to thrive?

A. LAD 1
B. LAD 3
C. Bruton's agammaglobulinemia
D. SCID

Q. BAFF genetic defect associated with germinal centre hyperplasia leading to hyperplasia of peyer's patches and tonsils?

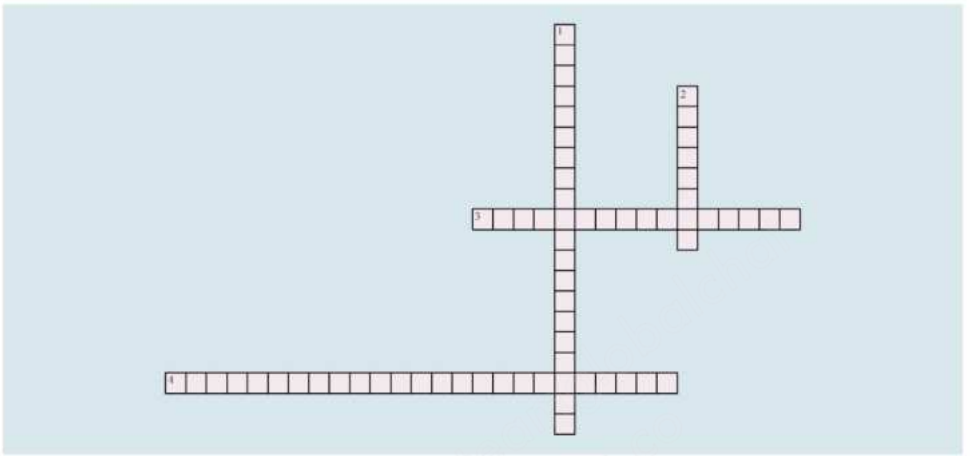
A. Bruton's agammaglobulinemia
B. SCID
C. CVID
D. CGD

Q. Select the incorrect match?

A. Del 22p - DiGeorge syndrome
B. Baff - CVID
C. ADA deficiency - SCID
D. BTK - Bruton's hypogammaglobulinemia

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. It is also known as velo cardio facial syndrome
4. Full form of AR

Down

1. This converts immature B cells into mature B cells
2. It makes plasma cells and antibodies from mature b cells

47 TRANSPLANT IMMUNOLOGY

Pre-Transplant

Types of Grafts

00:00:52

- Autograft - SELF
- Isograft - Identical twins
- Allograft - Grafting between the same species.
  - Kidney transplant or bone marrow transplant
  - Human to human
- Xenograft - Grafting done between different species
  - Example: Prosthetic heart valves, that are taken from different animals
  - Porcine, bovine different types of heart valve

Prior to Transplant

ABO Matching	Matching of blood group between Donor and Recipient
HLA Matching	Between Donor and Recipient

Criteria for HLA matching

Bone Marrow	100%
Kidney	50%
Cornea	0% - because it is a avascular structure
Heart/ liver / Lung	Alongwith HLA matching- viability test first priority • Vitality will be gone if waited

HLA matching

00:03:15

Adults	
Ideal Matching • Genes that are targeted: HLA, A, B, C, DQ and DR • **Criteria:** Minimum 8 on 10 should be matched	Practical Matching • Genes that are targeted: HLA, A, B, C & DR • **Criteria**: minimum 6 out 8 should be matched
Children	
• HLA A, B, DR genes to be considered • **Criteria**: minimum 4 out of 6 should be matched Identical Twins get a perfect 6/6 alleles matched.	

Q. Which is the important HLA gene that has to be matched?
Ans: The most important matching is DR matching

Post-Transplant

Transplant Rejection & Graft v/s Host Disease (GVHD)

00:10:52

- Recipient reject donor graft
- Donor attacks recipient

Types of Transplant Rejection

- **Hyperacute Rejection**
  - Within first 48 hours
  - Type 2 Hypersensitivity reaction
- **Acute rejection**
  - Few days to weeks and rejection done after that
  - Type 2+4 Hypersensitivity reaction
- **Chronic Rejection** (most common)
  - Type 4 Hypersensitivity reaction

Hyperacute Transplant Rejection

- Donor graft has been rejected within 48 hours.
- Most of the time it happens on the operation table itself
- Reason: PREFORMED ANTIBODIES

For example: A kidney (donor graft) is placed and anastomoses the blood supply.
But the recipient rejected it.

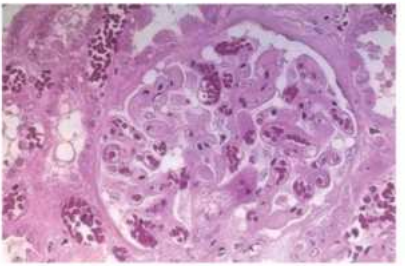
Q. Why does this happen?

- Due to Preformed antibodies

Q. Which types of patient will have pre-formed antibodies?

- Previous multiple blood transfusion
- Multiparous women
- **Blood vessels-** fibrinoid necrosis
- **Kidney-** coagulative necrosis

Microscopically:



1. Fibrinoid necrosis in the blood vessels
2. Intraluminal thrombi
3. Coagulative necrosis in the solid organs
4. Necrosis always show inflammation - neutrophilic infiltration

Q. How will the surgeon know about the rejection?
- Kidney becomes floppy and turns blue due to no supply of blood
- Few drops of blood in urine

Acute Transplant Rejection

Refer Table 47.1

Summary of Pathways
- **Direct Pathway:** Acute Cellular Rejection
  - Donor's antigens cells are creating the problem
- **Indirect Pathway:** Chronic Rejection
  - My own APC were having problems

Chronic Transplant Rejection
- Type 4 HS and is related to cells Rejection
- Self antigen presenting cells are going to activate CD 4 T cells
- CD4 cells differentiate into: T helper 1 cell
- IFN gamma- granuloma
- Epithelioid cells
- Type 4 hypersensitivity

Question: What is going to happen to the Kidney?

G- GBM duplication - Glomerular basement membrane duplicate
O- Obliteration/sclerosis of blood vessels
T- Tubular atrophy - Atrophied
I- Interstitial fibrosis

Hyperacute Rejection	Acute Rejection	Chronic Rejection
Within 48 hours	Within few days to weeks	Few weeks to months
HS 2	HS 2+4	HS 4
PREFORMED ANTIBODIES (Blood Transfusion or multiparous pregnancy)	• Humoral antibodies ○ CD 4 ○ Rejection vasculitis • Cellular Rejection ○ Donor APC - DIRECT PATHWAY	Indirect Pathway Self APCs that create problems **G-**GBM duplication - glomerular basement membrane duplicate **O-** Obliteration /sclerosis of blood vessels **T-** Tubular atrophy - atrophied
	○ Tubulitis and Endotheliitis	**I-** Interstitial fibrosis
Blue, floppy kidney Blood in urine		
M/E • Coagulative necrosis • Fibrinoid necrosis		

Graft Versus Host Disease 00:39:03
- Donor graft attacks recipient
- Eg. Kidney attacks the receiver
- Donor is immunocompetent
- Recipient is immunocompromised

Acute GVHD	Chronic GVHD
Within 100 days	After 100 days
Skin- Rash Intestinal - Diarrhea Liver- Jaundice	Skin - Fibrosis - nodular & sclerosis. Intestine - stricture Liver - Fibrosis

Should we suppress DONOR T CELL?
- NO
- Why?
- Engraftment (graft has to make a place for itself) is done by DONOR T CELL

In case of bone marrow transplant
- Leukemia: these T cells from outside are normal and helps in killing the leukemic blasts
- GVL - Graft vs Leukemia

Post-Transplant Complications
- Cytomegalo Virus infection
- BK virus: belongs to POLYOMAVIRUS DNA family
- Urine microscopy examination shows decoy cells
- Immunosuppressive state of patient:
  - Human papilloma virus - cervical cancer
  - EBV
  - HHV8

MCQs

Q. What is the graft between different members of the same species termed as?

A. Autograft
B. Isograft
C. Xenograft
D. Allograft

Q. Which of the following histological changes is suggestive of chronic transplant rejection

A. C4d positivity along peritubular capillaries

B. Presence of glomerular basement membrane double contours

C. Presence of inflammatory cells within glomerular capillaries

D. Presence of inflammatory cells within peritubular capillaries

Q. Which of the following organs is least likely to be affected in GVHD?

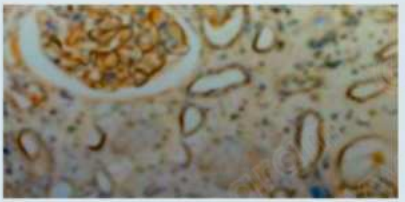
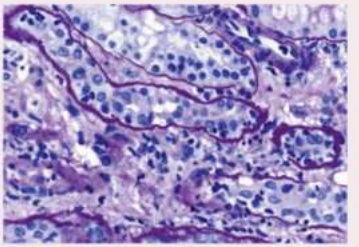
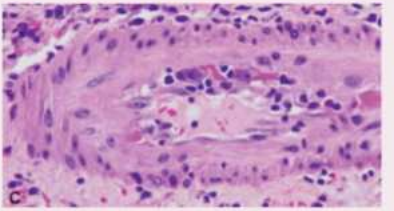
A. Liver

B. Skin

C. Intestine

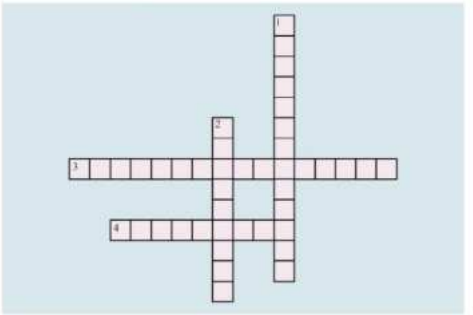
D. Lung

Table 47.1

Acute Humoral Rejection	Acute Cellular Rejection
• Hypersensitivity 2 reaction • Donor kidney carries its own MHC1 & 2 • These act as antigens for recipients to make antibodies • Immune complex is formed • Complement activation	• Hypersensitivity 4 reaction • Donor's antigens presenting cells to recipient • CD 4 & CD 8 T Cells • So much inflammation which results in:
IHC:  C4D around Vessels (Rejection Vasculitis)	Tubulitis  Endotheliitis 

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Big big cells
4. Grafting done between different species

Down

1. Dark blue color cells are called?
2. Grafting between the same species

48 ANTIGEN AND ANTIBODY

Antigen

00:01:08

- Immunogenicity
  - To induce an immune response
- Antigenicity
  - To bind to the antibody
    → Ability to Bind
- All antigens with antigenicity might not show immunogenicity.
- All antigens with imagenicity will also show antigenicity.

Hapten/Pro-Antigen/Incomplete Antigen

- **Immunogenicity:** Absent
- **Antigenicity:** Shown binding
- Converted to complete antigen by binding to a protein carrier.
  - Combining Hapten with a protein barrier
    → Incomplete antigen is converted to a complete antigen.

Factors Affecting Immunogenicity

- **Size of antigen:** More the size the more immunogenic potential (Midsize antigens have maximum immunogenicity).
- **Chemical nature:** Maximum is protein > Polysaccharides, lipids, etc.
- **Susceptibility of antigen to tissue enzymes:** If the antigen is ready to break into mid-size, then it can be more immunogenic.
- **Structural complexity**: More complex, more immunogenicity
- **Foreignness to host:** More the foreign nature, the more the immunogenicity.

Optimal Dose of Antigen and Route

- Too little dose → Fails.
- Too large dose → Will not create any reaction.
- Mid or required dose is mandatory.
- Adjuvant → enhances the immunogenicity of an antigen.
  - Alum.
  - Freund's adjuvant.
  - Non-bacterial products (thiomersal).
  - Other bacteria (Tt, Dt).

Q. When an antigen comes to the body, will it be dependent on the T cell or not?

T- independent antigen	T- dependent antigen
Simple antigens LPS, capsule, flagella	Complex
No memory	Memory present
No macrophage processing	Macrophage processing seen
IgM and IgG3 produced	All classes produced
Directly acts on B cells and causes polyclonal B cell proliferation	Involves CMI and humoral immunity

- **T-dependent cells:** Whenever an antigen enters the body, the antigen presenting cell picks it up and gives it to the T cell, Then T cell informs the B cell, and the B cell will make antibodies.
- **T independent:** If antigen directly acts with B cell.
  - For simple antibodies.
  - **Ex:** Epstein–Barr virus.

Heterophile Antigens

00:14:24

Antigen → shares epitopes

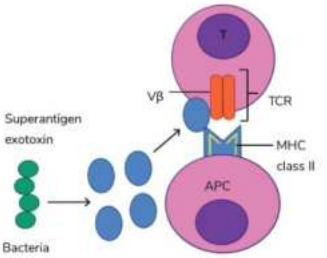
- For diagnosis of rickettsia, we use the Weil felix test.
- We use antigens of proteus in this test.
- Different family antigens (hetero) and share (Phile)

Antibody → Cross react.

- **Examples**
  - Cold agglutination test (mycoplasma)
  - Weil felix test (rickettsia)
  - Paul Bunnell test (EBV)
  - Forssman antigen→ lipid - carbohydrate complex present in many animals, plants, bacterias, but not in rabbits.

Superantigen

00:17:21

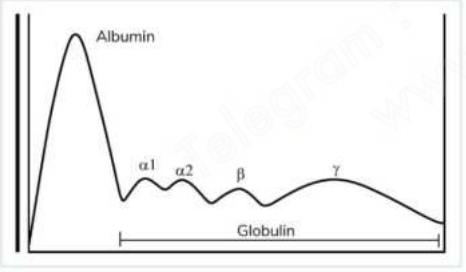


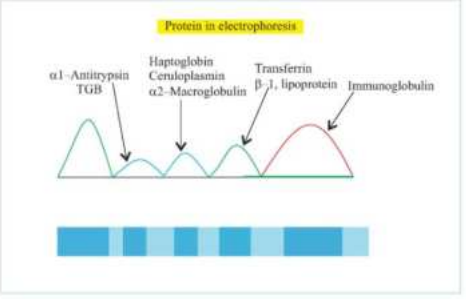
- Antigen in any form, ideally binding with antigen presenting cell then gives to T cell.
- Ideally the antigen will have a center of attachment.
- Superantigen attaches laterally (vβ part of TCR receptor).
- More than 20% of T cells get activated.
- Massive cytokine and lymphokine release will occur.
- It will result in toxic shock syndrome.
  - **Example:** staphylococcus bacteria
- Staphylococcal antigen (TSST)
  - Toxic Shock Syndrome Toxin Aka Enterotoxin F
- Streptococcal toxin (SPE-A&C).
- Yersinia enterocolitica.
- Yersinia pseudotuberculosis.
- M.tuberculosis
- EBV
- CMV
- Rabies
- Malassezia furfur

Antibodies

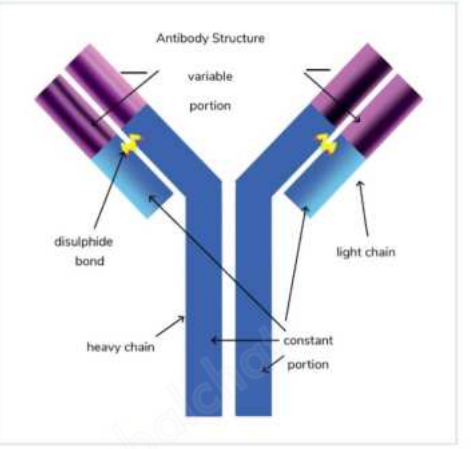
00:21:52

- Structurally they are glycoproteins (proteins>>>> sugars).
- A little component of sugar included.
- Aka γ globulins (proteins)
- Technique to find out the proteins in the body: Serum protein electrophoresis.

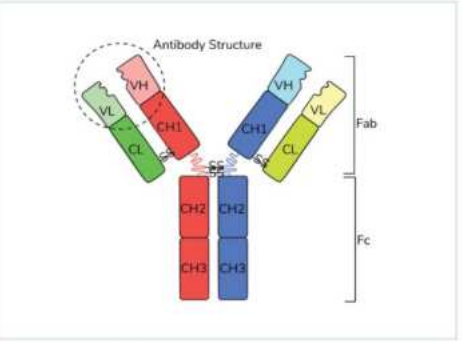




Structure of an Antibody

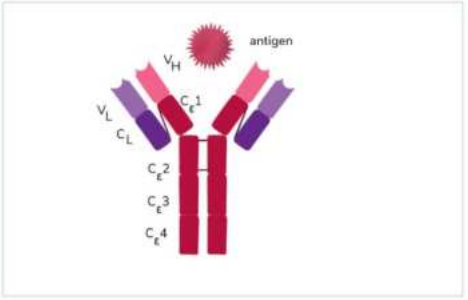


- **2 heavy chains:** 50000 Dalton
- **2 light chains:** 25000 Dalton (Kappa - K, Lambda - λ)
- **Ig:** Basal of heavy chains
  - G: γ
  - A: α
  - M: μ
  - D: δ
  - E: ε
- Polyclonal and monoclonal depend on light chains.
- **If the same type of light chain is produced:** Monoclonal.
- **If different types of light chains:** Polyclonal.
- Chains are connected by disulphide bonds



- C: Constant region
- V: Variable region
- Heavy and light chains have a constant and a variable region.
- Heavy chain has 1 Variable and 3 constant regions.

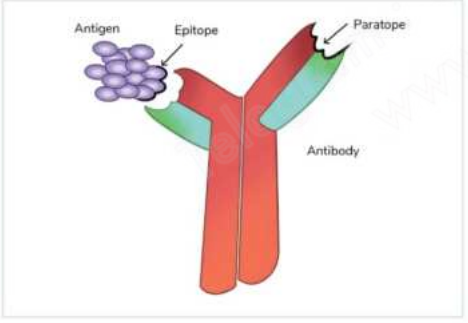
- IgG, IgA, IgD have 3 constant regions
- ME: 4 constant regions
- Have longer heavy chains CHI-4



- In main structure it has
  - Fab end: Where does the antigen bind.
- Fc end: Antigen bind in the variable region.

Refer Image 48.1

- It goes to the hypervariable region (Exact location).
- Complement determining region.



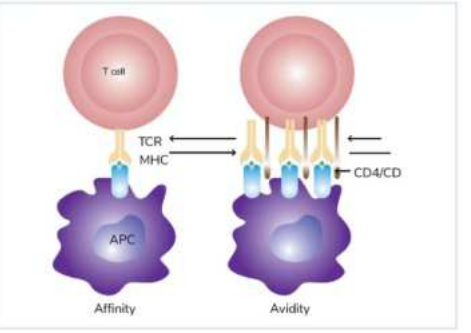
- Epitope of the antigen binds with the paratope with the antibody.

Definitions

00:33:33

Affinity

- Strength of interaction of Ab with Ag at single point
- IgG better affinity than IgM.



Avidity

- Strength of interaction of Ab with Ag at multiple points
- IgM has better avidity than IgG

Isotype/Iso specificity

- Depends on constant regions of the heavy chain.
- Isotypes are.
  - IgG = gamma
  - IgA = alpha
  - IgM = mu
  - IgD = delta
  - IgE = epsilon

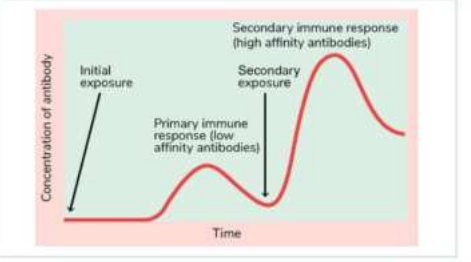
Allotype

- Amino acid differences in the Ch regions.

Idiotype

- Variations in the variable region.

Types of Active Immunity



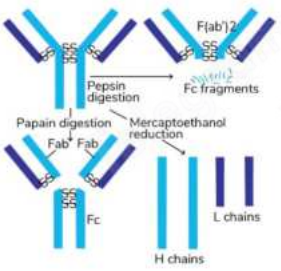
Primary immune response	Secondary immune response
Slow Sluggish Short lived	Prompt Powerful Prolonged
IgM	IgG
LONGER LAG	SHORTER LAG
Less titre of antibodies	Higher titre

- Kappa - lambda = 1.5: 1
- H gene- Ch 14
- L chain kappa- Ch 2
- L chain lambda- Ch 22

Enzymatic Cleavage of Antibodies

00:40:00

- **Pepsin:**
  - Breaks below the disulphide bond.
  - Fab portion has gone as 1 single unit.
- **Papain:**
  - Breaks above disulphide bond.
  - 2 fabs and 1 Fc.
- **Mercaptoethanol:**
  - All heavy chains and light chains are separated

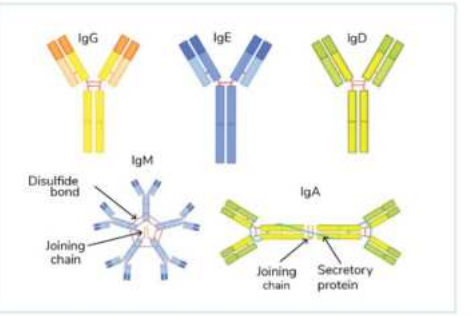


Immunoglobulins

00:41:50

- IgG
- IgA
- IgM
- IgD
- IgE

Max concentration to least concentration

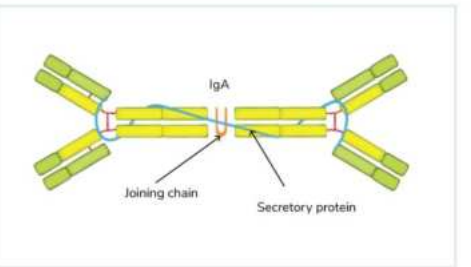


IgG

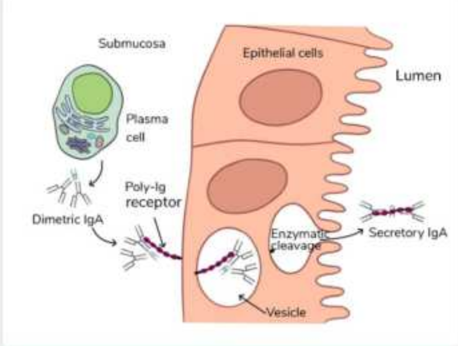
- **Placenta:** Transplacental spread.
  - Ig G_1, Ig G_2, Ig G_3, Ig G_4
  - Least chance to cross the placenta is Ig G_2.
  - Ig G_4
    → Fibrosis
    → Reidle's Thyroiditis
- Concentration: Maximum.
- **Valency:** 2 antigens.
- Part of chronic inflammation.
- IgM and IgG activate the classical complement system.
- **Best opsonin:** G1 and G3.

IgA

- Subclasses: Ig A_1 and Ig A_2
- Activates the Alternative complement system.
- **Monomeric:** Serum.
- **Dimeric:** mucosa secretions.

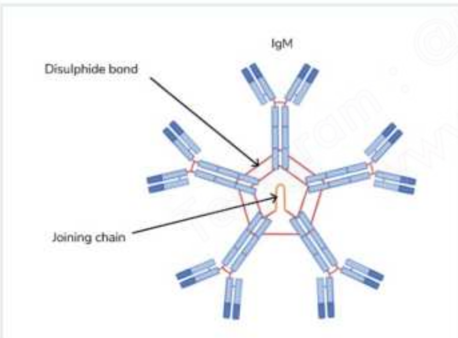


- Known as mucosal antibody.
- Known as a secretory antibody.



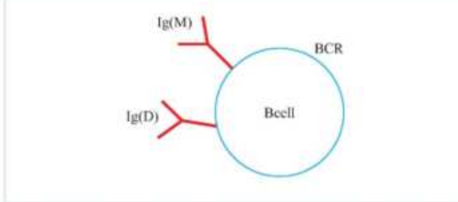
- Intestinal cells give secretory protein.
- Adding a secretory piece, it is made resistant to enzyme attack.

IgM
- **Millionaire antibody:** Maximum molecular weight
- **Pentameric:** 5 molecules come together.
  - There is a joining chain.



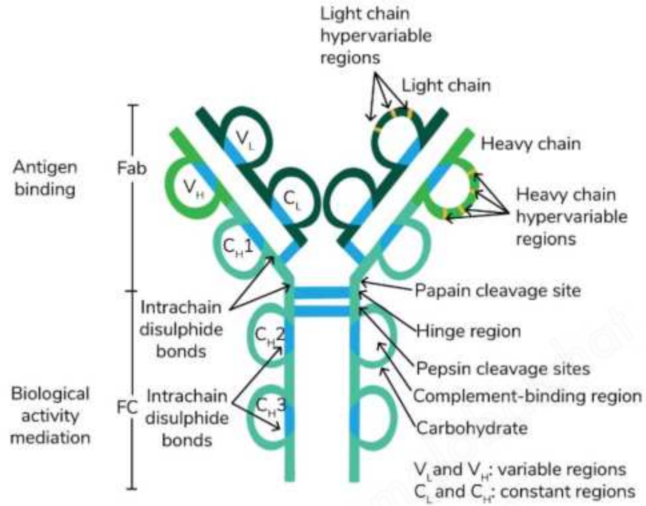
- **Valency:** 10 antigens.
  - Practically 5-6 antigens.
  - **Example:** Public transport.
- 80% of IgM is in blood.
- **Fetus:** 20th week.
- **Inflammation:** Acute
- IgM and IgG activate the classical complement system.

IgD
- **BCR:** B cell receptor
  - IgM and IgD are B Cell receptors.
- Alternate complementing system.



IgE
- Least concentration
- **Heat:** Labile
- Aka homocytotropic antibody and reaginic antibody.
  - Act on mast cells
- **Allergy:** Any kind of allergy.
- Parasitic infection
  - IgE blood test.
  - Eosinophils test.

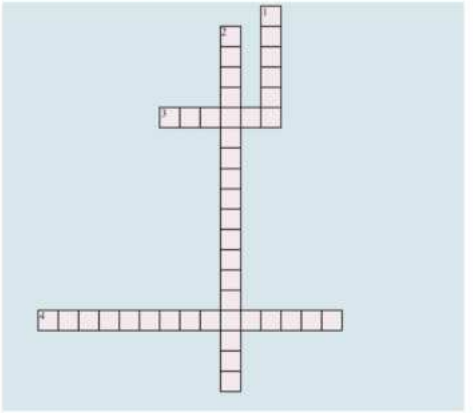
Image 48.1



Light chain hypervariable regions
Light chain
Heavy chain
Heavy chain hypervariable regions
Antigen binding
Fab
V_L
V_H
C_L
C_H1
C_H2
C_H3
Papain cleavage site
Intrachain disulphide bonds
Hinge region
Pepsin cleavage sites
Biological activity mediation
FC
Intrachain disulphide bonds
Complement-binding region
Carbohydrate
V_L and V_H: variable regions
C_L and C_H: constant regions

CROSS WORD PUZZLES

Crossword Puzzle



Across

3. Fab portion has gone as 1 single unit
4. All heavy chains and light chains are separated

Down

1. 2 fabs and 1 Fc
2. IgE blood test

49 ANTIGEN ANTIBODY REACTIONS

Types of Reactions

00:00:07

- **Precipitation:** Soluble antigen (Ag) and antibody (Ab).
- **Agglutination:** Insoluble (Particulate) antigen and antibody.

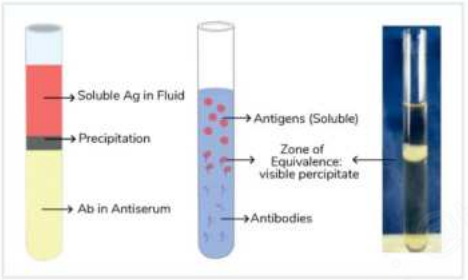
Precipitation Reactions

00:00:40

- Liquid medium
  - Ring test
  - Flocculation test
- Gel medium

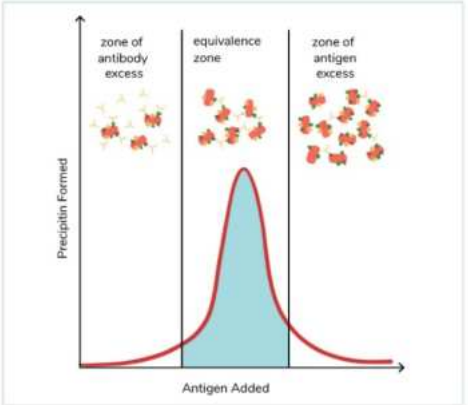
A. Precipitation Reactions in Liquid Medium

- **Ring test**

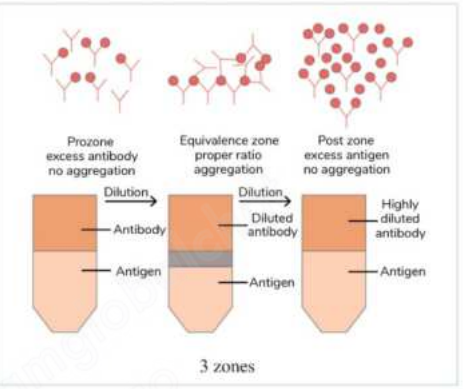


- **Example**
  - Streptococcal lancefield grouping - Beta haemolytic streptococcus
  - Ascoli thermo ring precipitation test - Anthrax

Marrack Lattice Hypothesis



- **Zone of equivalence:** Antibody = Antigen (ring forms)
- **Pro zone:** Antibody > Antigen
- **Post zone:** Antibody < Antigen



Flocculation test

- Ag-Ab complexes floats/ suspended as floccules
  - Slide flocculation test - VDRL (most common)
  - Tube flocculation test - Kahn test
  - (both are done for T.pallidum)

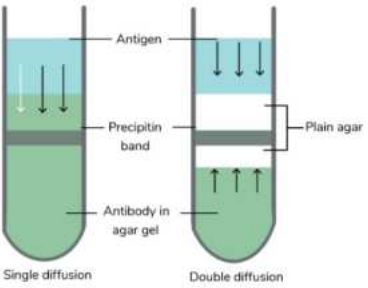
B. Precipitation Reactions in Gel Medium

- 1% Agarose gel is used
- **Diffusion**
  - **Single diffusion -** Only antigen moves
  - **Double diffusion -** Both antigen and antibody moves

- **Dimension**
  - **If reaction occurs in** Test tube **-One dimension**
  - **If reaction occurs in** Slide-**Two dimension**

One Dimension

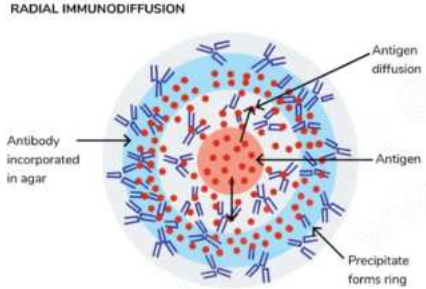
- **Mnemonic:** OOTD
  - O - Single diffusion (Oudin test)
  - O - Double diffusion (Oakley-Fulthorpe test)
  - T - Tube
  - D - Dimension (1D)

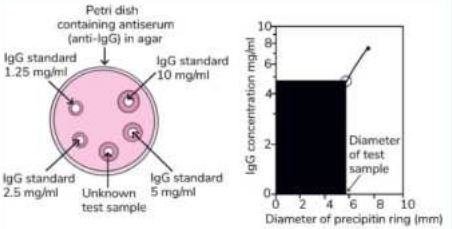


Oudin test and Oakley-Fulthorpe test

Two Dimension

- **Single diffusion**
  - Radial immunodiffusion
  - Agar contains antibody
  - Wells contain antigen
  - Only antigen moves

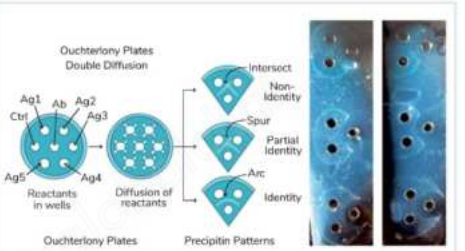




- Diameter is measured
- Concentration and sub classes of different Ag and Ab are given
- Other name: **Mancini method**

- **Double diffusion**
  - **O**uchterlony **D**ouble **D**iffusion test - **2D**
  - Agar contain antibody
  - Wells contain antigen
  - **Ex:** Elek's gel precipitation test - C.diphtheria

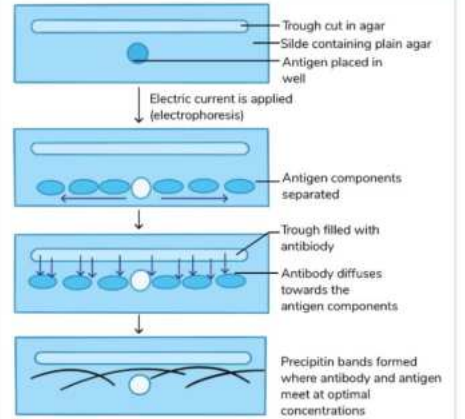
- Mnemonic: **D**ee **D**ee **D**ee **D**E**e** - **O**uch
- **D**-DOUBLE
- **D**-DIFFUSION
- **D**-2-D
- **EE**-ELEK'S GEL PRECIPITATION /OUCHTERLONY



Ouchterlony Double Diffusion test

- Different Ag are added
- Ag will forms Arcs
  - → **Total hug (Arc) -** Identical Ag
  - → **Formal hug (Spur) -** Partial identical Ag
  - → **Intersect -** Non-identical Ag

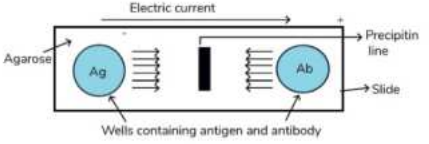
- **Electroimmunodiffusion**



Electroimmunodiffusion

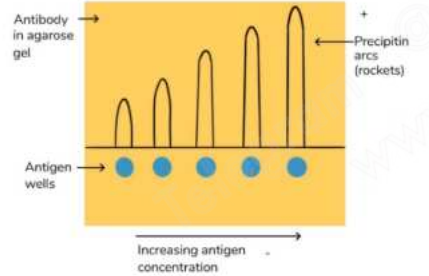
- Ag is added to wells
- Electric current is passed
- Ag will separate to different parts
- Form proper lines
- Faster method
- Current is passed only from one side and only the antibody moves in one direction

- **Counterimmunoelectrophoresis**



- More faster
- Both Ab and Ag diffuse at same time

- **Rocket electrophoresis**



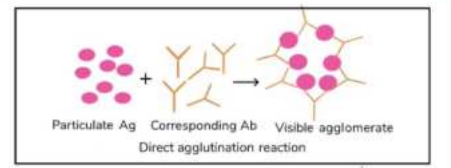
- For quantitative assessment of antigen
- Ag placed in increasing concentration
- Ab placed in agar
- Ag concentration ∝ Rockets

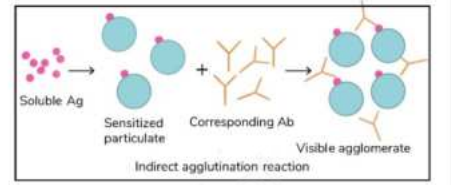
Agglutination Reactions

00:22:25

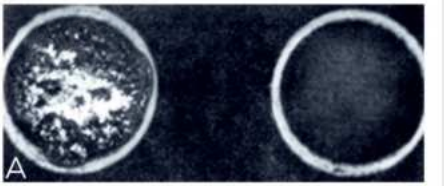
- Better than precipitation reactions
- **2 tests**
  - Direct
    - → Slide
    - → Tube
    - → Microscopic
  - Indirect/passive agglutination reaction

A. Direct Agglutination

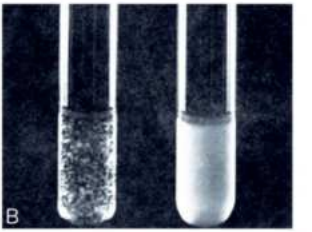




- Particulate Ab
- Ag is added
- Agglutination is done directly
- **Slide agglutination**
  - Blood grouping



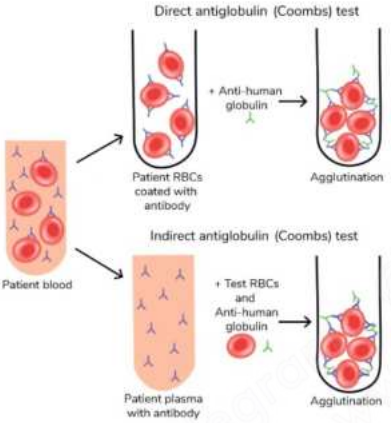
- **Tube agglutination**
  - WIDAL test (typhoid)
  - SAT
  - CAT
  - Weil felix (Rickettsia)
  - Paul Bunnell (Episteinbar Virus)
  - COOMB's test



- **Microscopic agglutination test**
- Eg: MAT

Mnemonic: CAT SAT on a MAT (My Bi Lii - Hindi)

- **CAT - My**
  - **C**old **A**gglutination **T**est
  - **My**coplasma
- **SAT - Bi**
  - **S**tandard **A**gglutination **T**est
  - **Bi**rucella
- **MAT - Lii**
  - **M**icroscopic **A**gglutination **T**est
  - **Lii**ptospira



Direct and Indirect COOMB's test

- **Direct-for fetal ab,in vivo sensitization-AIHA**
  - Fetal cells are coated with Ab (erythroblastosis fetalis)
  - Add COOMB's reagent (Anti Human Globulin)
  - Gap is filled with AHG
  - Agglutination is seen (+ve)
- **Indirect-for maternal ab,in vitro sensitization**
  - Ab serum (mother)
  - Add AHG and RBCs
  - Agglutination is seen (+ve)

B. Indirect Agglutination

- Soluble Ab are converted to Particulate Ab (adsorbed on RBC/ latex particulate)
- **Latex agglutination reaction (IMP)**
  - Polystrene latex particles used
  - Can absorb many antigens
  - **Ex:** Antistreptolysin O (ASO) antibody

Complement Fixation Test 00:31:35

- Wasserman test (No longer done)
- Treponema Pallidum Immobilization test (TPI) (most specific test)
- **Principle**

Complement Fixation Test Principle

  - Ag and Ab combine
  - Forms complex with complement (fixed)
  - So, RBCs are not killed (as no complement for lysis)

Important Information

- **RBC Intact -** Has Ab
- **RBC lysis -** No Ab

Neutralisation Test 00:33:46

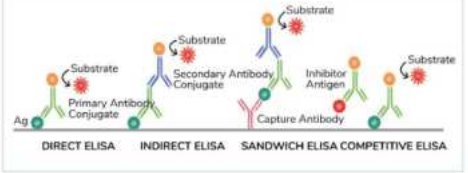
- Toxin + Antitoxin = Neutralised
- **Schick test -** Corynebacterium Diphtheriae
- **Nagler's reaction -** Clostridium Perfringens

*Refer the respective chapters

ELISA - Enzyme Linked ImmunoSorbent Assay 00:34:43

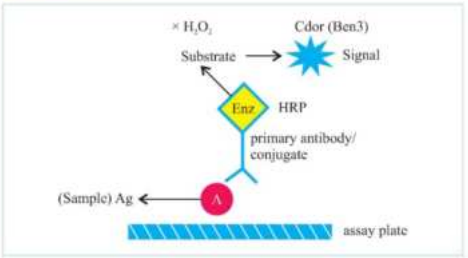
ELISA plate

- 12 x 8 = 96 wells, thus 96 testings can be done at a time
- **Enzyme:** HRP - Horse raddish Peroxidase
- **Substrate:** H_2O_2
- **Chromogen:** Tetramethyl benzidine
- **4 types of ELISA**



- **Direct** - Ag
- **Indirect** - Ab
- **Sandwich** - Ag
- **Competitive** - Ag

1. Direct ELISA



- For Ag detection
- Add pt sample (Ag)
- Add Ab + Enzyme (HRP)
- Add Substrate (H2O2)
- Add Tetramethyl benzidine
- +ve if color comes out
- Intensity of color ∝ Ag conc.

2. Indirect ELISA

- For Ab detection
- Add Ag
- Add Ab from pt sample
- Add Ab + Enzyme
- Add Substrate
- Add Tetramethyl benzidine
- +ve if color comes out
- Intensity of color ∝ Ab conc

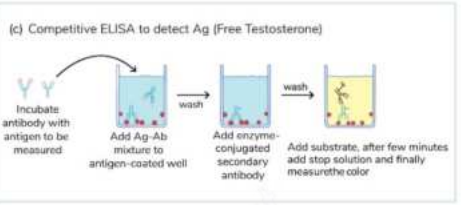
3. Sandwich ELISA

Type of direct ELISA

- To measure antibody in patients
- Add Ab
- Add detection Ag (pt sample)
- Add Ab + Enzyme
- Add Substrate
- Add Tetramethyl benzidine
- +ve if color comes out
- Intensity of color ∝ Ag conc.

4. Competitive ELISA

- For Ag detection



- Incubate Ab with Ag from patient
- Add Ag to well
- Add the Ab+Ag mixture to antigen coated well and wash
- Add Ab + Enzyme
- Add Substrate
- Add Tetramethyl benzidine
- -ve outside well if color comes out
- +ve outside well if color doesn't come out
- Intensity of color ∝ 1/Ag conc.

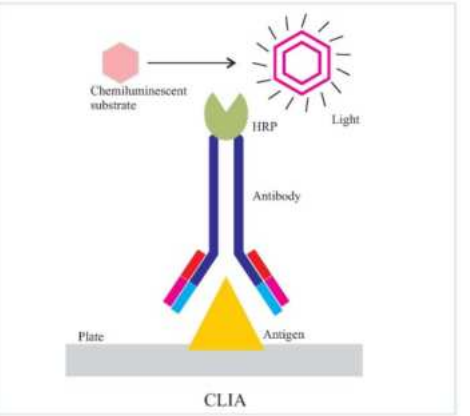
Important Information

- **Direct, Sandwich:** More color - More Ag
- **Indirect:** More color - More Ab
- **Competitive:** More color - Less Ag

CLIA - Chemi Luminescence ImmunoAssay

00:45:23

- Shinny chemical light is the end point

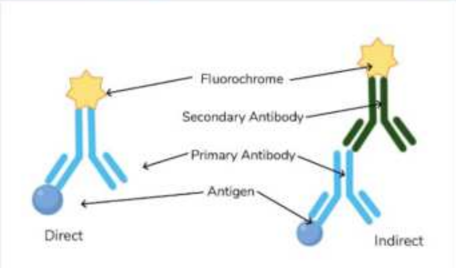


Important Information

- Luminol/ Acridinium Esters are used in place of Tetramethyl benzidine

Immunofluorescence

00:46:28



3 types of Immunofluorescence

Direct

- Ag detection
- Add pt Ag sample
- Add Ab
- Add signal (FITC - Fluoro-Iso-Thio-Cyanate)
- +ve if Ag present

Indirect

- Both Ag and Ab detection
- **Ab detection**
  - Add pt Ab sample
  - Add Ag
  - Add signal (FITC - Fluoro-Iso-Thio-Cyanate)
  - +ve if Ag present
- **Ag detection**
  - Add pt Ag sample
  - Add Ab
  - Add signal (FITC - Fluoro-Iso-Thio-Cyanate)
  - +ve if Ag present
- **Eg for indirect:** Fluro Treponemal Antibody (FTA-Abs)

CARD Test

00:48:49

- Many in number
- **Examples**
  - HBV
  - HCV
  - Malaria
  - COVID
  - Urine pregnancy test
  - Typhi dot
- **Principle**
  - Based on ICT - Immuno Chromatography
  - **Control line** - All normal and always present
  - **Test line** (validity of the test)
    - → **+ve reaction** - Visible line
    - → **-ve reaction** - Not visible
  - Blood sample goes through the nitrocellulose membrane

PREVIOUS YEAR QUESTIONS

Q. Isotype class switching is seen in? (AIIMS June 2020)
A. Activated B cells
B. B cells
C. T cells
D. Cytotoxic T cells

Q. A child is suffering from recurrent chronic infection with encapsulated bacteria. Which subclass of IgG does the child has deficiency? (NEET Jan 2019)
A. IgG1
B. IgG2
C. IgG3
D. IgG4

Q. 10-year-old child Jaundice & diarrhea reported to hospital and within same period of time, many children from same school presented with same symptom. Which of the following is most probable finding? (AIIMS June 2020)
A. IgM antibody hepatitis A
B. IgG antibody hepatitis B
C. IgM antibody hepatitis B
D. IgG antibody hepatitis A

Q. In chronic allergy which Ig are more persistent in the body? (AIIMS May 2019)
A. IgG
B. IgE
C. IgA
D. IgM

Q. Classical complement activated by? (FMGE June 2019)
A. C1
B. C3 convertase
C. IgA
D. Ag–Ab complex

Q. Meningococcal meningitis is seen with which of the following deficiency? (FMGE Nov 2017)
A. C1q
B. C2
C. C4
D. C5

Q. 55-year-old patient presented with difficulty in breathing & rashes after ingestion of sea food. He has shown similar reaction in post following consumption of some food item. Which type of Hypersensitivity reaction? (FMGE Dec 2020)
A. Type 1
B. Type 2
C. Type 3
D. Type 4

Q. Type IV Hypersensitivity is due to? (AIIMS June 2020)
A. Innate immune response
B. Cell mediated immunity
C. Antibody & cell mediated immunity
D. Humoral mediated immunity

Q. T cell is associated with? (AIIMS May 2018)
A. CD4
B. CD5
C. CD8
D. First line defense against bacterial peptides

Q. An elderly male patient with some clinical scenario is undergoing kidney transport from his twin brother. What kind of graft is it? (FMGE Dec 2020)
A. Isograft
B. Allograft
C. Xenograft
D. Autograft

Q. Nude mice is not resistant to Xenograft due to absence of? (NEET Jan 2018)
A. B cell
B. T cell
C. Nk cell
D. LAK cell

Q. A person presents in the emergency because of development of allergy d/t pollen grain inhalation. Which of the following is important in pathogenesis of this condition? (FMGE Aug 2020)
A. NK cells
B. Neutrophils
C. Helper T cells
D. Cytotoxic T cell

Q. Gene not involved in SCID? (AIIMS Nov 2019)
A. BTK
B. ZAP70
C. IL2RG
D. JAK3