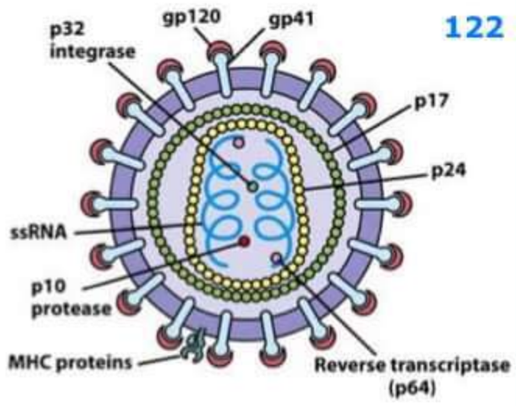
PrepNotes

HIV



HIV

→ Family → Retroviridae
Sub family → Lentivirinae

→ Prevalence in India
→ HIV 1 >>> HIV 2
→ in HIV 1 → group 'M' → subtype 'C'

→ STRUCTURAL GENES OF HIV1
gag → codes for capsid, matrix protein, p24, p17, 18
env → codes for Gp120 / Gp 41
pol → codes for p 31, 51, 61
polymerase / reverse transcriptase

→ LAB DIAGNOSIS
→ most sensitive test → ELISA
most specific test → HLV RNA PCR > Western Blot

→ MODES OF TRANSMISSION [MOT]
① Sexual → 0.1 – 1%. [mc mot in India]
② Blood Transfusion → > 90%.
③ Needle stick injury → 0.5 - 1%. [0.3 - 3%.]
④ Mother to child Transmission → 30%.

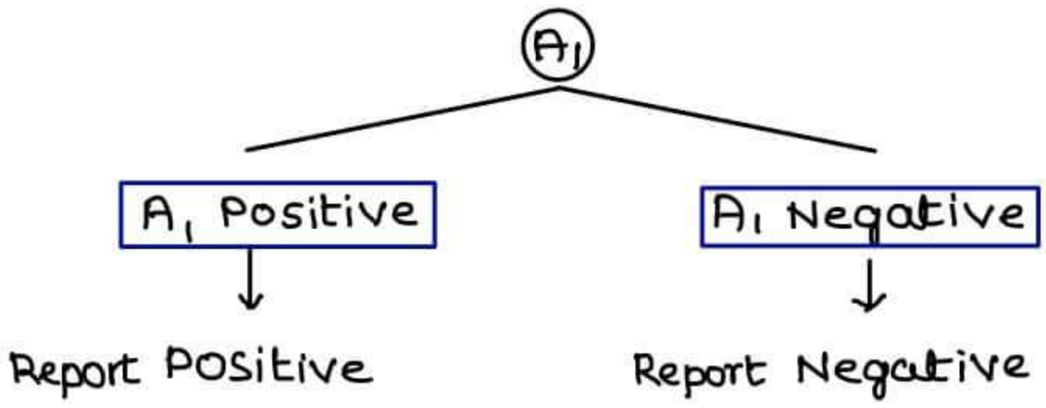
→ can ↓transmission from Mother to child
to 15%. → in developing countries
to 0-5%. → in developed countries
by even avoiding breast feed

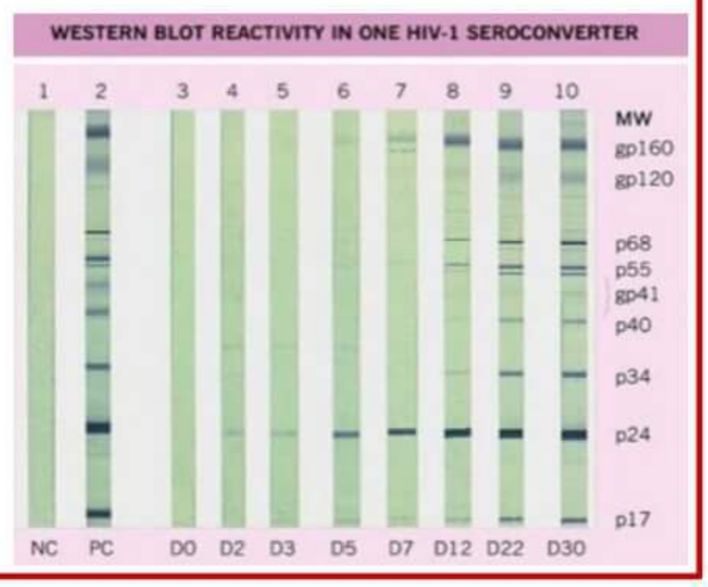
NACO STRATEGIES

STRATEGY I → done in blood banks
STRATEGY II A → done by PSM [for HIV surveillance]
II B → done in Symptomatic HIV persons
STRATEGY III → done in asymptomatic persons

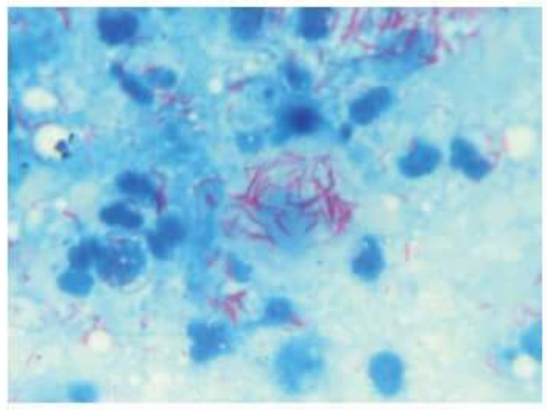
STRATEGY I / ALOGRITHM 1

→ done for transfusion & transplantation safety
→ one test kit is required





2. ACID FAST STAINING [ZIEHL NEELSEN STAINING]



ZN STAINING

- → differentiates b/w Acid fast / Non - Acid fast
- → Based on ACID FASTNESS [Ability to resist decolorisatn]
  ↓
  due to MYCOLIC ACID [Content]

- → Mycobacterium Tuberculosis → 20% Acid fast
- → Mycobacterium Leprae → 5% Acid fast
- → Nocardia → 1% Acid fast

- → Bacterial spores
- → OOcyst OF
  - ↳ cryptosporidium parvum
  - ↳ cyclospora
  - ↳ ISOspora

} 0.25 - 1% Acid fast

STEPS [for MTB]

Sputum smear
↓
carbolfuchsin
↓ Intermittent heating for fixation
20% H_2SO_4 [Decolouriser]
↓
Methylene Blue [counter stain]

KINYOUN METHOD OF STAINING

- → modification of ZN Staining
- → cold method of staining [phenol concentration is ↑sed for Fixation]

3. BIPOLAR STAINING [Safety Pin Appearance]

- → Poles are stained, remaining spared
- → Examples
  - ↳ Yersinia pestis
  - ↳ Calymatobacter [Klebsiella] granulomatis
  - ↳ Haemophylus ducreyi
  - ↳ Burkholderia mallei
  - ↳ Burkholderia pseudo mallei
  - ↳ Vibrio parahemolyticus
  - ↳ Fransciella sps.
  - ↳ Pasturella sps.

CAPSULATED ORGANISMS

P → Pneumococcus , Pseudomonas
A → Anthracis Bacillus
K → Klebsiella species
I → Influenzae Haemophilus
Y → Yersinia species
B → Bordutella species
M → Meningococci
C → Clostridium perfringes [C. welchii] , Cryptococcus neoformans
V → Vibrio parahemolyticus

Capsule

→ Virulence factor
→ Antiphagocytic
→ mainly made of polysaccharide
  Except^{n} → Bacillus anthracis capsule [made of Polypeptide]

→ Demonstrated by
  1. QUELLUNG REACTION
    → capsular swelling dlt serological react^{n}
    → seen in Pneumococcus , Haemophilus influenza

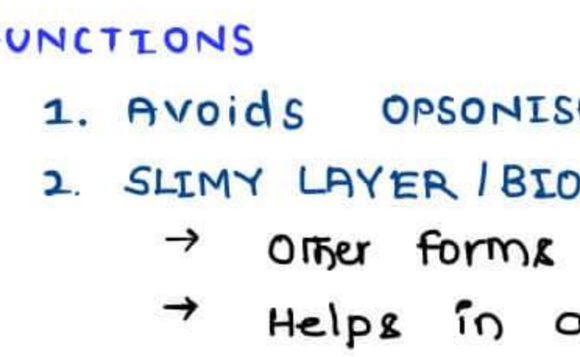
  2. Mc FADYEAN'S REACTION
    → observed in Bacillus anthracis
    → culture growth + Loffler's methylene Blue → capsule stains blue

→ FUNCTIONS
  1. Avoids OPSONISATION & Thus prevents PHAGOCYTOSIS
  2. SLIMY LAYER / BIOFILM FORMATION [MODIFICATION]
    → Other forms of capsule
    → Helps in adherence
    → BIOFILM → Antibiotic resistance can also happen

MOTILITY

→ dlt Flagella [Flagellin] → Antigenic

→ Demonstrat^{n} of Flagella



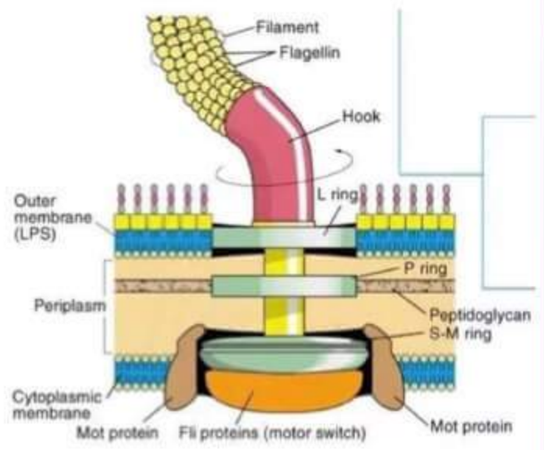
→ Monotrichous → Vibrio cholerae
→ Amphitrichous → Alcaligenes faecalis
→ Lophotrichous → Spirillum minus
→ Peritrichous → E.coli, Proteus, Listeria, Salmonella

→ MOTILITY NAMES

T → Tumbling → Listeria
D → Darting → V. cholerae
S → Swarming → Proteus

→ Falling leaf like → Giardia Lamblia [4 pairs of flagella]
→ Slow form of swarming [stately] → Clostridium
→ Gliding motility → mycoplasma

→ Spirochetes have ENDOFLAGELLA
  → Arise from periplasmic space
  → Treponema pallidum → cork screw motility
  → Borrelia → Lashing motility



METHODS TO DEMONSTRATE MOTILITY

1 Hanging drop method
2 Semi solid agar method [Mannitol motility agar] [mc used]
3 Dark Ground microscopy
4 Phase contrast microscopy

CULTURE MEDIA

CONTENTS

→ Water
→ Peptone
→ Meat or Yeast extract
→ Blood
→ Agar

AGAR

PROPERTIES OF AGAR THAT MAKES IT IDEAL SOLIDIFYING AGENT [2%] ['0' Kcal]

1. It can't be utilized / degraded by bacteria
2. Melting point → 98°C [Autoclavable]
   Solidifying point → 42°C

TYPES OF AGAR

1. JAPANESE AGAR
2. NEW ZEALAND AGAR [more jellifying property]

CLASSIFICATION

A. BASED ON NUTRITIONAL STATUS
→ Simple
→ complex
→ Synthetic

B. BASED ON CONSTITENCY
→ Liquid
→ semisolid
→ Solid

C. BASED ON O_2 TENSION
→ Aerobic media
→ Anaerobic media

D. BASED ON ORGANISMS CULTIVATED
→ media for bacteria
→ media for fungus
→ media for parasites

LIQUID MEDIA

→ used for preparing MORE GROWTH in SHORT TIME
→ used for SUGAR FERMENTATION TEST , INDOLE TEST , MR-VP TEST
→ Preparation of INOCULUMS for ANTIBIOTIC SENSITIVITY TEST
→ BUT NOT ABLE TO DEMONSTRATE COLONY MORPHOLOGY
→ Eg → PEPTONE WATER , NUTRIENT BROTH , BRAIN HEART INFUSION BROTH [BHI] etc

SEMI SOLID MEDIA

→ LIQUID MEDIA plus 0.5 - 1% AGAR
→ Eg → mannitol motility agar , Craige's tube
→ USED FOR
  ↳ demonstration of motility
  ↳ preservation of cultures

SOLID MEDIA

→ LIQUID MEDIA plus 2-3% AGAR
→ used for demonstration of COLONY MORPHOLOGY
→ ↑ concentration of 2-6% Agar → prevent swarming growth of Proteus species

SUFFIX - AGAR in a medium name indicates it is a SOLID/SEMI SOLID MEDIUM

SIMPLE MEDIA [Non - Fastidious bacteria is cultivated]

→ Nutrient agar
  Agar → Solidifying agent [2%]
  → No nutrition provided

  Nutrient → Peptone
  Meat extract

→ Nutrient Broth → Liquid culture media

PEPTONE WATER CONTAINS

1. Peptone → 1 gm
2. NACL → 0.5 gm
3. Distilled water → 100 ml

COMPLEX MEDIA

① ENRICHED MEDIA
→ Solid culture media
→ Eg: 1. Blood Agar → 5 - 10% sheep blood agar is used
2. Chocolate Agar [RBC lysis]
3. BHI Agar [Brain Heart Infusion Agar]
4. LOFFler's serum slope [used for Corynebacterium diphtheriae]


BLOOD AGAR


CHOCOLATE AGAR

BLOOD AGAR

HEMOLYSIS

1. α Hemolysis → Partial hemolysis around colonies
→ Greenish discoloration is present
→ unlysed RBCs can be seen under microscope
→ Eg → Streptococcus viridans, streptococcus pneumoniae

2. β Hemolysis → Well defined clear colorless zone of hemolysis around colonies
→ Eg → Streptococcus pyogenes

3. γ Hemolysis → no hemolysis around colonies
→ Eg → Enterococcus

CHOCOLATE AGAR [Heated Blood Agar]

→ nutrient agar is melted & cooled to 75°c in a water bath + 5 - 10% sterile defibrinated sheep blood is added & mixed
→ medium color becomes chocolate brown
→ heat ruptures RBCs [X, V released] & liberates nutrients
→ used for growing fastidious organisms
→ EXAMPLES OF ORGANISMS GROWN IN CHOCOLATE AGAR
  → Haemophilus
  → meningococci
  → Gonococci
  → Neisseria species

② ENRICHMENT MEDIA

→ always liquid culture media
  ↳ have stimulating effect on bacteria to be grown or
  ↳ inhibits its competitors [usually commensal organism]

→ EXAMPLES
  ↳ SELENITE - F - BROTH → for Salmonella, Shigella
  ↳ TETRATHIONATE BROTH → For Salmonella

③ SELECTIVE CULTURE MEDIA

→ mostly Solid
→ contains substances that inhibit all but a few types of bacteria & facilitate isolation of particular species
→ used to ISOLATE BACTERIA from a mixture of Organisms
→ EXAMPLES
  ↳ L.J. [Lowenstein Jensen] MEDIA → M. TB → malachite green [selective agent]
  ↳ DEOXYCHOLATE CITRATE AGAR → For Salmonella, Shigella
  ↳ BILE SALT AGAR → For Vibrio cholera
  ↳ TCBS AGAR for Vibrio cholerae [THIOSULPHATE CITRATE BILE SALT SUCROSE]
    → green color media, changes to Yellow When Vibrio grows
  ↳ LSS [Loeffler Serum slope] → corynebacterium diphtheriae

4. DIFFERENTIAL MEDIA

→ distinguish differing characters of bacteria
→ MAC - CONKEY - AGAR [it is also indicator & Selective media]
→ Differentiates b/w
  Lactose fermenting [E. coli, Klebsiella, Shigella Sonnei (Late lactose fermentor)
  Non lactose fermenting organisms

→ Selective d/t
  Peptone
  Lactose
  Agar
  Neutral red
  Taurocholate Sodium [Selective Agent]

5. INDICATOR MEDIA

→ contains indicator which changes color when bacterium grows in them

→ EXAMPLES

1. WILSON - BLAIR'S AGAR → Salmonella typhi produces black colonies
2. MAC - CONKEY'S AGAR → indicates Lactose fermenting colonies
3. BLOOD AGAR → Shows hemolytic organisms

CLED MEDIA [cysteine Lactose Electrocyte Deficient]

↳ Both Indicator & Differential media

6. TRANSPORT MEDIA

→ maintains the viability of organism but nobody is allowed to multiply

→ VENKATRAMAN RAMAKRISHNAN MEDIA [VR MEDIA] } For V. cholera
CARY BLAIR

STUART'S & AMIE'S → For Gonococci & Meningococci

PIKE'S MEDIA → For Streptococcus pyogenes

SACH'S BUFFERED GLYCEROL SALINE → For Shigella & Salmonella

Anaerobic media → ROBERTSON COOKED MEAT MEDIA
THIOGLYCOLATE BROTH

7. SUGAR MEDIA

→ used to study fermentation of bacteria

→ contains 1% Sugar in Peptone water Basal media,

→ Small DURHAM'S TUBE [inside larger tube in inverted fashion] to DETECT GAS PRODUCTION

→ pH Indicator → HISS Serum Sugars [3% serum]
ANDRADE'S INDICATOR

→ if color changes to Pink & Gas bubbles are seen then the sugar is fermented

→ SUGARS

- → monosaccharide → Pentose, Hexose
- → Disaccharide → Lactose
- → Polysaccharide → Starch, Inulin
- → Trisaccharide → Raffinose
- → Alcohols → Glycerol, Sorbitol
- Glucosides → Salicin, aesculin

8. ANAEROBIC CULTURE MEDIA

1. ROBERTSON'S COOKED MEAT BROTH
   - → meat turning black → Proteolytic Sps. [clostridium]
   - → meat turning pink → Sacharolytic Sps.
2. THIOGLYCOLLATE BROTH

COMPOSITE MEDIA

→ demonstrates DIFFERENT PROPERTIES using Single medium

→ Eg → TRIPLE SUGAR IRON AGAR [TSI]

→ Used to study fermentat^{n} of glucose, Lactose, Sucrose & product^{n} of H_2S & gas

STERILIZATION OF MEDIA

→ MOSTLY done by AUTOCLAVE at 121°c for 15 min at 15 Lbs/sq.inch pressure

EXCEPT For
- ↳ DORSET'S EGG MEDIUM
- ↳ LOEFFLER'S SERUM SLOPE
- ↳ LJ medium

} Sterilized by INSPISSATION METHOD [80-85°c for 20-30 min for 3 days]

→ STORAGE OF CULTURE MEDIA → b/w 4-5°c inside refrigerator

DEHYDRATED MEDIA

→ commercially available

→ reconstituted before use in distilled water

CLASSIFICATION

BASED ON O_2 REQUIREMENT

① OBLIGATE AEROBES

→ needs desperate O_2

→ MTB
Pseudomonas
Nocardia

② OBLIGATE ANAEROBES

→ Does not need O_2 at all

→ A → Actinomycetes
B → Bacteroids
C → Clostridium species

③ FACULTATIVE ANAEROBES

→ changed anaerobe

→ Aerobe + Anaerobe

→ Most of the medically important bacteria

→ Eg. Staphylococcus, Streptococcus species

④ MICROAEROPHILES

→ need 5% O_2 only

→ campylobacter
Helicobacter

⑤ CAPNOPHILES

→ CO_2 loving

→ Brucella abortus
Pneumococcus
H. influenzae

BASED ON TEMPERATURE REQUIREMENT

P → Psychrophile → − 20°C to + 20°C

M → Mesophile → 20°C to 40°C [medically important bacteria]

T → Thermophile → > 60°C

→ > 100°C [can manage]

→ Thermus aquaticus [Typical example]

→ Used in PCR

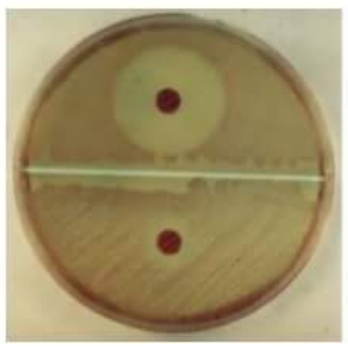
→ Father of PCR → KARRY B MULLIS

SYSTEMIC BACTERIOLOGY 1

GRAM POSITIVE COCCI

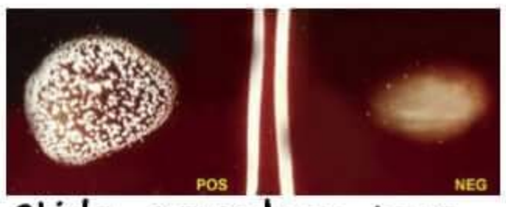
CATALASE TEST

- **Positive** → STAPHYLOCOCCUS
  - **COAGULASE TEST**
    - **Positive** → PATHOGENIC STAPH. → Staph. aureus
    - **Negative** → NON PATHOGENIC STAPH [CONS] → Staph. saprophyticus, Staph. epidermidis, Staph. lugdunensis
      - **NOVOBIOCIN TEST** →

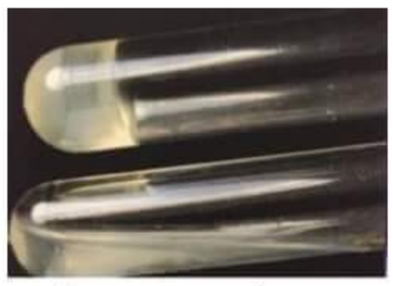


        - **Novobiocin sensitive** → Staph. epidermidis
        - **Novobiocin resistant** → Staph. saprophyticus

- **Negative** → STREPTOCOCCUS



Slide coagulase test



Tube coagulase test

- → Novobiocin test → done for antibiotic testing
- → Slide coagulase test → to determine bound form
- → Tube coagulase test → to determine free form
- → 3% H_2O_2 + Staph. colonies ⟶ Bubbles [CATALASE POSITIVE]

AST [Antibiotic Sensitivity Testing]

- → Eg: Novobiocin sensitivity test
- → Done on MHA [muller Hinton Agar]
  NA [Nutrient Agar]

- → KIRBY BAUER DISC DIFFUSION TESTING done
  - interpretation
    - ZONE OF INHIBITION → Sensitive [staph. epidermidis]
    - NO ZONE OF INHIBITION → Resistant [staph. saprophyticus]

COagulase Negative Staph(CONS)

Staph epidermidis

→ Ⓝ commensal of skin epidermidis

→ a/w INTERVENTIONS
- IV canula, CSF shunts, prosthetic valve surgery, catheters
- causes Early prosthetic valve endocarditis [<1yr]

→ BIOFILM FORMATION Seen → helps in Adherence, antibiotic resistance

→ Novobiocin sensitive → Zone of inhibitⁿ is +nt

Staph saprophyticus

→ Young female develops UTI. MC GPC involved → S. saprophyticus
Young female develops UTI. MC cause overall → E. coli

→ Novobiocin resistance → NO zone OF Inhibitⁿ

MC ORGANISMS INVOLVED IN EARLY PROSTHETIC VALVE ENDOCARDITIS → CONS

PATHOGENIC STAPH

Staph aureus

→ Gram positive cocci

→ catalase tive

→ coagulase tive

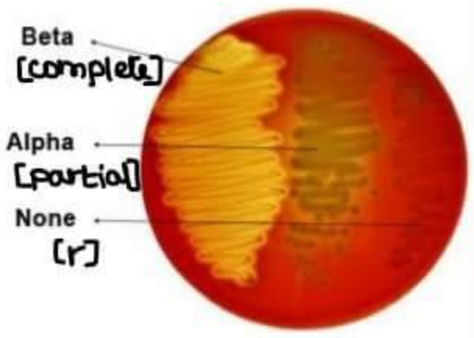
→ Gram staining → VIOLET GRAPE LIKE CLUSTERS

→ CULTURE MEDIA

① NA [Nutrient Agar] → GOLDEN YELLOW pigmentatⁿ
↓
Enhances on milk agar

BLOOD AGAR

② BA [Blood Agar]
- causes β hemolysis
- pin head size colonies



HEMOLYSIS

1. α Hemolysis → Partial hemolysis around colonies
→ Greenish discoloration is present
→ unlysed RBCs can be seen under microscope
→ Eg → Streptococcus viridans, streptococcus pneumoniae

2. β Hemolysis → Well defined clear colorless zone of hemolysis around colonies
→ Eg → Streptococcus pyogenes

3. r Hemolysis → no hemolysis around colonies
→ Eg → Enterococcus

③ MANNITOL SALT AGAR [Best selective media]

- In presence of Stap. aureus the agar changes / transforms from pink to yellow

MANNITOL SALT AGAR

④ LUDLAM'S MEDIA [2nd Best media]

⑤ SALT MILK AGAR

→ VIRULENCE FACTORS

① Thickness of peptidoglycan layer

② Teichoic Acid

③ clumping factor

④ Protein A

→ is present in COWAN - I Strain of Staph. aureus

→ plays a role in coAgglutination

→ has a property of binding ī the fc portion of IgG

Golden yellow colonies on nutrient agar

⑤ Hemolysins (α, β, γ, δ)

→ α hemolysins

→ shows PARADOXICAL ACTION

- inactivates at 70°C
- reactivates at 100°C

→ β hemolysins / sphingomyelins

→ shows HOT & COLD phenomenon

- β hemolysis better seen at 37°C
- in S. aureus better seen at 4°C

→ Gamma hemolysin + PV TOXIN [Panton valentine toxin]

→ gives SYNERGOHYMENOTROPIC action [↑ virulence of Staph. aureus]

⑥ LEUCOCIDIN [PANTON VALENTINE TOXIN]

→ Kills Leucocytes

⑦ ENZYMES

→ DNASe

→ Thermonuclease

→ Coagulase [plays the most important role in pathogenicity & virulence]

→ Hyaluronidase

→ Lipase

→ Phosphatase

→ TOXIN MEDIATED ILLNESSES

① FOOD POISONING

→ IP → 1-6 hrs

- resembles Bacillus cerus emetic strain
- S. aureus → contaminated meat & milk products
- B. emetic → contaminated chinese fast food

→ dlt Preformed toxin → Enterotoxin A
- Heat stable
- Vagomimetic act^{n} / vagal Stimulat^{n}

② SSSS [STAPHYLOCOCCAL SKIN SCALDED SYNDROME]
→ dlt Exfoliative Toxins A & B
→ aka TEN → Toxin Epidermolytic Necrolysis in adults
RITTER'S Disease in Children

③ TSS [TOXIC SHOCK SYNDROME]
→ dlt TSS toxin [ENTEROTOXIN F]
→ it is a SUPER ANTIGEN [Non-MHC restricted]

activates >20% lymphocytes
↓
massive lymphokine release
↓
Shock

→ dlt usage of contaminated vaginal tampons during menses

→ OTHER DISEASES
① CELLULITIS
② IMPETIGO
③ CARBUNCLES
④ FURUNCLES
⑤ BOTRYOMYCOSIS → mycetoma like lesion
⑥ TROPICAL PYOMYOSITIS
⑦ ACUTE OSTEOMYELITIS
⑧ ACUTE MASTITIS
⑨ NATIVE VALVE ENDOCARDITIS [original valve]
⑩ IV DRUG ABUSER → Rt SIDED VALVE ENDOCARDITIS
⑪ PNEUMATOCELE
⑫ PNEMONIA

→ Single most common cause of early prosthetic valve endocarditis → S.aureus

MRSA [Methicillin Resistant Staph. Aureus]

→ DOC → vancomycin (a/w RED MAN SYNDROME)

→ MECHANISM OF RESISTANCE

① PBP ⟶ PBP 2a [main mechanism] (PBP - Penicillin Binding Protein)

d/t MEC A gene aquisition ; PBP also known as Transpeptidase

- MEC A GENE TYPES [6 types]

I, II, III } causes NOSOCOMIAL MRSA community

IV, V, VI } causes Acquired MRSA

② Product^{n} of β lactamases

→ SCREENING TESTS

① CEFOXITIN → RESISTANT → MRSA [100%]

OXACILLIN → Resistant → Doubt [needs further testing]

→ done on MHA [MULLER - HINTON AGAR]
→ can add 4% NaCl
→ Keep temperature of incubation → < 37°C [30° - 35°]

② PCR For Mec A Gene [Best Screening test for MRSA

→ Contact isolation done for MRSA
→ Mupirocin Ointment or Bacitracin Ointment indicated for Nasal carriers
→ Best method to prevent nosocomial Spread → Hand washing

→ TRANSDUCTION

- mc method of resistance transfer in S. aureus
- Bacteriophage mediated
- PHAGE TYPING can be done
- 23 types of phages are available
- 80/81A → mc phage

STREPTOCOCCUS

CLASSIFICATION

α hemolysis	β hemolysis	γ hemolysis
→ S. pneumoniae → S. viridans	→ classified into A-V based upon carbohydrate c in the cell wall [LANCEFIELD CLASSIFICATION] → A is divided into 80 types based on M Protein [GRIFFITH TYPING] → Group A [S. pyogenes] B [S. agalactiae] D [Enterococcus]	→ Enterococcus

	GROUP A STREPTOCOCCI	GROUP B STREPTOCOCCI
BACITRACIN TEST	→ sensitive	→ Resistant
CAMP TEST	→ Negative	→ Positive
HHT	→ Negative	→ Positive

CAMP TEST → Christie Atkins munch Peterson Test

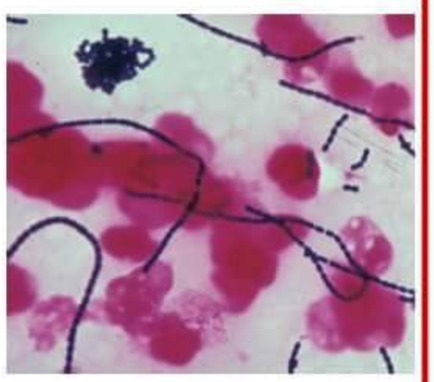
HHT → Hippurate Hydrolysis Test

DISC A - Bacitracin sensitive

GROUP A STREPTOCOCCUS

Streptococcus pyogenes

→ Gram positive cocci
→ catalase -ive
→ arranged in chains in Gram staining [violet]
- Longest chain → S. salivarius



GRAM STAINING

→ CULTURE MEDIA

1. CVBA [Crystal Violet Blood Agar]
   - CV does not allow Staph.
   - produce β hemolysis
   - PINPOINT SIZE COLONIES

PIN POINT SIZE COLONIES

2. PIKE'S MEDIA
   - Transport media

PrepLadder

→ DISEASES

① ACUTE PHARYNGITIS [Sore throat]
② CELLULTIS
③ IMPETIGO
④ ERYISEPLAS [lymphatics involved]
⑤ NECROTISING FASCITIS → FLESH EATING BACTERIA
⑥ PUEPERAL SEPSIS
⑦ SCARLET FEVER
   • Scarlet fever toxin / Erythrogenic toxin → Super antigen
⑧ NON SUPPURATIVE COMPLICATIONS

ACUTE RHEUMATIC FEVER	ACUTE GLOMERULONEPHRITIS
→ H/o sore throat	→ H/o pyodermic infect^{n}
→ Immune response marked	→ mild to moderate Immune response
→ complement Levels are Normal	→ complement → ↓ses, then Normal
→ Life long prophylaxis of Penicillin required	→ Spontaneous to come & Spontaneous to go
→ ASO Titres → > 200 + odd units	Anti DNASe B → > 300-350

GROUP B STREPTOCOCCUS

Streptococcus agalectiae

→ Normal comensal of birth canal
→ Leads to early or late neonatal meningitis
→ CAMP TEST +ive [Enhanced / Butterfly zone]
→ HHT [Hippurate Hydrolysis test] is +VE

ENHANCED / BUTTER FLY ZONE OF hemolysis

ENTEROCOCCUS

→ E. faecalis
   E. faecium

→ RESIST
   - 6.5% Nacl
   - PH = 9.4
   - 40% Bile
   - Temperature of > 55°C for minutes

→ CULTURE MEDIA
   - BILE AESCULIN AGAR → Black coloured colonies

→ On GRAM STAINING, Spectacle like appearance is seen

→ DOC → Penicillin + Aminoglycoside
Vancomycin [2nd line drug]

→ VANCOMYCIN RESISTANT ENTEROCOCCI
→ ↑ Van gene aquisition [Van A – Van H]
– Van A is most resistant
→ DOC → LINEZOLID

→ Normal commensal of gut
→ CAUSES
– Surgical wound infections
– In IV Drug abusers, Lt. sided valve endocarditis

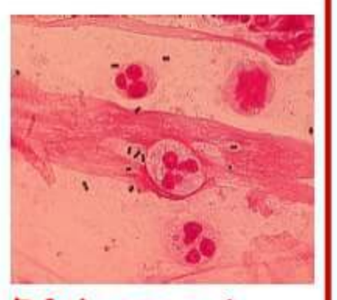
ALPHA HEMOLYTICS

→ St. viridans → Leads to SABE, Late onset prosthetic valve endocarditis
→ St. mutans → Leads to dental caries

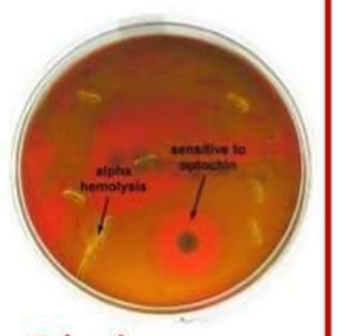
→ Early onset prosthetic valve endocarditis	→	Stap. epidermidis [<1yr]
→ Native valve endocarditis	→	Staph. aureus
→ In IV Drug abusers		
– Rt. sided valve endocarditis	→	Staph. aureus
– Lt. sided valve endocarditis	→	Enterococci
→ SABE [sub Acute Bacterial Endocarditis]	}	Strept. viridans
→ Late onset prosthetic valve endocarditis		

Streptococcus pneumoniae [Pneumococcus]

→ Gram positive cocci
→ Lanceolate [flame shaped]
→ DRAUGHTS MAN / CARROM COIN LIKE COLONIES on various culture media
→ Capsulated
– made of Polysaccharide
– demonstrated by QUELLUNG REACTION
– capsular swelling
– Serological reaction
– Capsular Antigen + Antibody → SWELLING / DELINEATION
– also seen in Haemophilus influenza


Diplococci

→ B Bile soluble
I Inulin fermenter
O Optochin Sensitive
tive



Optochin Sensitivity

– On the basis of capsular polysaccharide, divided into 90 types
SSS Typing [Specific Soluble Substance based Typing]

→ DISEASES

C → CONJUNCTIVITIS

O → OTITIS MEDIA

M → MENINGITIS [mccause of meningitis in adults]

P → PNEUMONIA [Typical / Lobar Pneumonia ; Type 3 > Type 7]

S → SINUSITIS

→ VACCINES

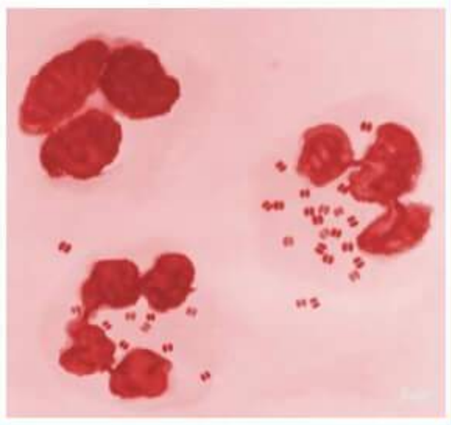
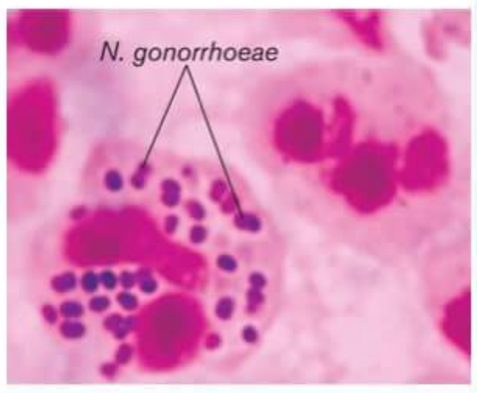
1 23 valent Polysaccharide vaccine

- used for Splenectomized individuals

2 7 valent conjugate vaccine

- For < 2yrs of child
- For cochlear implant [should be done < 2yrs]
- For CSF shunting

GRAM NEGATIVE COCCI

MENINGOCOCCI	GONOCOCCI
→ capsulated	→ Non capsulated – some are capsulated
→ Lens shaped	→ Kidney shaped
→ RCUT [Rapid carbohydrate utilizat^{n} test] – ferments Glucose & maltose	→ RCUT [Rapid carbohydrate utilizat^{n} test] – ferments only Glucose
→ Plasmid recruit absent [for resistance]	→ Plasmid recruit present
→ Extra / Intracellular 	→ mainly intracellular 

MENINGOCOCCI

→ CAPSULATED
- on the basis of capsular polysaccharides → 13 serogroups
  - A, B, C, Y, W, E → most important serogroups
  - A, B, C → most infective serogroups

→ VIRULENCE FACTORS
① capsular polysaccharide
② IgA$_1$ protease
③ Lipo oligosaccharide
④ OMP [Outer membrane Proteins]
⑤ Pili

→ 5-15% persons are normal nasopharyngeal carriers

→ Spreads by Droplet infections
↓
Lungs involved
↓
Haematogenous spread
↓
meninges [Meningitis]
- fever
- vomiting
  headache
  neck rigidity
  Rash

→ LAB DIAGNOSIS

① CSF EXAMINATION

→ 3 samples taken
- one for → Microscopy
- one for → Pathology for cytology
- one for → Biochemistry for proteins & sugars

② CULTURE

→ on MHA

THAYER MARTIN MEDIA

→ Gram staining shows

Pink colored cocci

arranged extra/ intra cellular both

→ Most dreadful complication → WATER HOUSE FRIDERICHSEN SYNDROME

– Adrenal Haemorrhagic necrosis

→ In C_{5-9} complement deficiency → Recurrent Neisserial infections

→ VACCINES

MENINGOCOCCAL CONJUGATE VACCINE

– active against A, C, Y, W

– No good vaccine is available for group B

– Group B is poorly immunogenic

→ TREATMENT

– DOC for carriers → CIPROFLOXACIN

2nd line → RIFAMPICIN

– DOC for cases → 3rd generatⁿ CEPHALOSPORINS

[NOTE: If Penicillin given as option, it will be the answer]

GONOCOCCI

→ TYPING

– Pili based typing done

– Auxo typing [Nutrient based] done

→ ICP → 2 - 7 Days

→ VIRULENCE FACTOR

① Capsular polysaccharide
② IgA$_1$ protease
③ Lipo oligosaccharide
④ OMP [Outer membrane Proteins]
⑤ Pili
⑥ Transferrin / Lactoferrin

→ DISEASES

① Gonorrhea

Males → Urethritis

Females → Cervicitis

Newborn → Ophthalmia neonatorum

→ LAB DIAGNOSIS

① SWAB

→ Males → Urethral Swab

→ Females → Endo cervical swab

High vaginal swab not taken

→ Neonates → Eye swab

CULTURE MEDIA → THAYER MARTIN MEDIA

Modified New york Agar

GRAM STAINING → Pink coloured cocci

arranged mostly intracellular

→ COMPLICATIONS

① WATER CAN PERINEUM

– occurs in males (urethritis)

② FITZ HUGH CURTIS SYNDROME

– perihepatitis

– also a/w chlamydia

③ POLY ARTHRITIS

↓

SUPPURATIVE ARTHRITIS

→ TREATMENT

– DOC → 3rd gen CEPHALOSPORINS

Plasmid recruit → ↑ Resistance against 3rd gen CEPHALOSPORINS

→ OPHTHALMIA NEONATORUM IS MORE a/w CHLAMYDIA THAN GONOCOCCUS

GRAM POSITIVE BACILLI

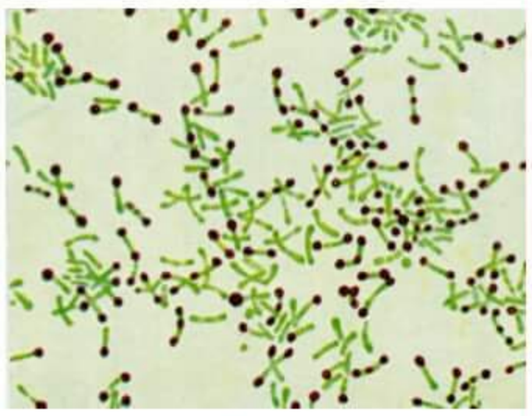
CORYNEBACTERIUM DIPHTHERIA (Klebloffler's bacillus)

→ GRANULES

① metachromatic granules
② Polymetaphosphate granules
③ volutin granules
④ Babes ernest granules

→ These are Energy deposits
→ stained by
  P → Ponders Stain
  A → Alberts Stain
  N → Neisser Stain

→ cuneiform arrangement / chinese letter pattern


Chinese letter pattern

→ CULTURE MEDIA
  → LSS [LOFFLER SERUM SLOPE]
  → Potassium tellurite Agar
  → H Hoyle's media
  → M macleod's media
  → T Tindale media

  → LSS is used for early Dx [6-8 hrs]
  → Best selective media → Potassium tellurite Agar

→ BIOTYPES

			Colonies on PTA
①	GRAVIS	→ DAISY'S	DAISY HEAD APPEARANCE
②	INTERMEDIUS	→ FROG EGGS are	FROG EGG APPEARANCE
③	MITIS	→ POACHED	POACHED EGG APPEARANCE

→ DIPTHERIA TOXIN
  → made of A + B [Bipartite toxin]
  → Strong exotoxin
  → needs Optimum iron for it's actⁿ
  → B → helps in Binding

  → A → ADP Ribosylation of Elongatⁿ Factor 2
  ↓
  Inhibition of Protein synthesis of cell
  ↓
  cell Death

→ Every Corynebacterium diphtheriae is not pathogenic,
but made pathogenic by β phage tox gene → LYSOGENIC CONVERSION

Lysogenic Phase } Part of Transductⁿ
Lytic Phase } [Bacteriophage mediatid]

→ TOXIN DEMONSTRATION

① ELEK GEL PRECIPITATION TEST
② PCR for tox gene
③ ELISA for toxin production

SCHICK TEST
determines Susceptibility of an individual to diphtheriae
If positive → needs immunizatⁿ
If negative → already immunized
neutralizatⁿ test

→ Diphtheria is TOXEMIA not Bacteraemia

→ PRESENTATION
→ PSEUDOMEMBRANE's mc location → Faucial [Tonsillar]
most dangerous location → Laryngeal [resp. obstructⁿ]

→ Neck edema +nt → BULL'S NECK

→ CHRONIC COMPLICATIONS
1 myocarditis
2 Polyneuropathies

NON CORYNEBACTERIUM DIPHTHERIAE

① C. parvum → immuno modulator
② C. jeikum → MDR Isolate
③ C. minutissimum → causes Erythrasma
coral red fluorescence is seen
④ C. pseudo tb → causes animal diseases
aka PREISZ NOCARD BACILLI

BACILLUS ANTHRACIS

→ GPB [Gram Positive Bacilli]

→ Non motile

→ capsulated

→ Spore bearing
- Certain size spores used in Category A Bioterrorism

→ CAPSULE

made of Polypeptide

Mc FADYEAN'S REACTION
- demonstrates capsule
- culture growth + LOFFLER'S METHYLE BLUE → CAPSULAR STAINING

→ APPEARANCES

GRAM STAINING → BAMBOO STICK APPEARANCE [purple to Violet]

PENICILLIN AGAR → STRING OF PEARL REACTION

GELATIN STAB CULTURE → INVERTED FIR TREE / INVERTED CHRISTMAS TREE

NORMAL AGAR MICROSCOPY → MEDUSA HEAD / MATTED WOOL LIKE / MATTED HAIR LIKE
(Hand lens)
- observed on edge of colony
- also seen in Blood Agar

→ CULTURE MEDIA

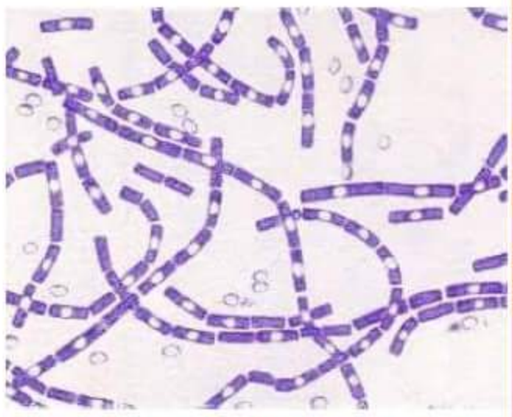
PLET MEDIA [Best selective media]

P → Polymyxin

L → Lysozyme

E → EDTA

T → Thallous acetate



Bamboo stick appearance on gram staining

→ ANTHRAX TOXIN

→ made of TRIPARTITE TOXIN

E Edema factor

P Protective factor

L Lethal factor

→ acts synergitically → ↑ cAMP

→ DISEASES

CUTANEOUS ANTHRAX [HIDE PORTER'S DISEASE]	PULMONARY ANTHRAX [WOOL SORTER'S DISEASE]
→ Eschar	→ occupational Hazard
	→ causes mediastinal hemorrhagic necrosis
→ Pustule & non pitting edema - malignant Pustule	→ Prevented by DUCKERING pre treat the wool by formaldehyde (Sporocidal)

INTESTINAL ANTHRAX
- spread through contaminated wild animal meat ingestion

→ LAB DIAGNOSIS

① ASCOLI'S THERMO PRECIPITATION TEST [Ascoli → Anthrax antigen]

② PCR for Bacillus anthracis

BACILLUS CEREUS

EMETIC STRAIN	DIARRHOEAL STRAIN
→ IP → 1 to 6 hrs	→ IP → 8-16hrs
→ causes food poisoning – dit chinese food	
→ resembles Heat stable toxin of E.coli in action → ↑ cGMP	→ resembles Heat labile toxin of E.coli in action → ↑ cAMP

→ CULTURE MEDIA

→ MYPA [Mannitol Yolksac Polymyxin Phenol Red Agar] MEDIA

Obligate Anaerobes

CLOSTRIDIUM

→ GPB

→ Non capsulated

→ motile → STATELY [Slow form of swarming]

Except^{n} → cl. perfringes [capsulated, non motile]

ROBERTSON COOKED MEAT MEDIUM

CULTURE MEDIA

ROBERTSON COOKED MEAT MEDIA

→ if meat pieces turn PINK → saccholytic → cl. perfringens

→ if meat pieces turn BLACK → Proteolytic → cl. tetani

SPORE ARRANGEMENT

→ Terminal & round → DRUMSTICK APPEARANCE → cl. tetani

→ Sub terminal spore → most clostridia

→ Central spore → cl. bifermentans

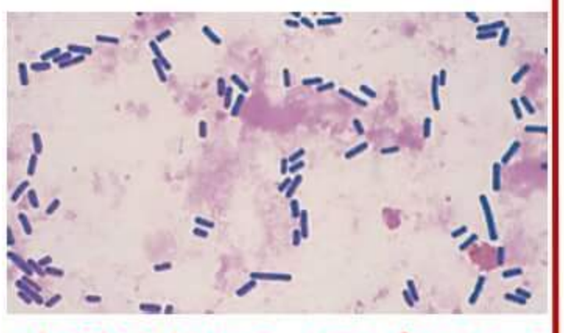
Clostridium perfringens/welchii

→ 13 major & minor toxins are present

α, β, epsilon, theta, iota

Type A – E

most infections are caused by Type A/α toxin


clostridium perfringens

DISEASES

① GAS GANGRENE / CLOSTRIDIAL MYONECROSIS

→ IP → 1-2 days

→ C/F → Pain

crepitus [Gas in the deep compartment]

→ TREATMENT

→ For Initial lesion

→ Surgical debridment & Antibiotics

→ For Advanced case

→ Amputation of the limb + Antibiotics

→ GAS GANGRENE CAUSED BY

P → Cl. perfringens

N → Cl. novyii

S → Cl. Septicum

② FOOD POISONING

→ dlt Contaminated meat products

→ IP → 8-24 hrs

→ involves Heat labile enterotoxin

→ but spores are Heat Resistant

③ PIGBEL

→ dlt uncooked pork consumption

→ causes necrotising enteritis

→ dlt β toxin TYPE C

REACTIONS

① NAGLER'S REACTION

② STORMY FERMENTATION IN LITMUS MILK

③ TARGET HEMOLYSIS [Double zone of hemolysis]

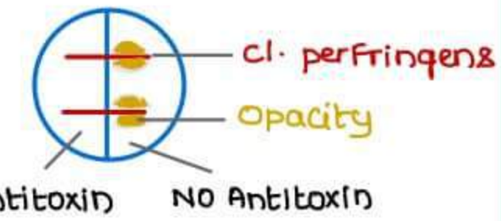
④ REVERSE CAMP TEST

① NAGLER'S REACTION

→ dlt α-Lecithinase

→ it is a neutralizatn Reaction

→ done on Egg Yolk Agar



② STORMY FERMENTATION IN LITMUS MILK

→ immense fermentation of colored milk


TARGET ZONE HEMOLYSIS


STORMY FERMENTATION

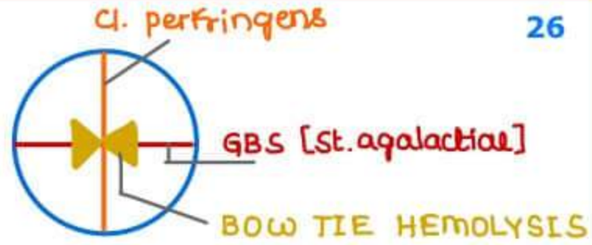
③ TARGET HEMOLYSIS [DOUBLE ZONE HEMOLYSIS]

→ Inner zone → dlt θ toxin

Outer zone → dlt α toxin

④ REVERSE CAMP REACTION

→ BOW TIE HEMOLYSIS ī St. agalactiae



REVERSE CAMP REACTION

Clostridium tetani

TOXINS

① TETANOLYSIN

② TETANOSPASMIN

→ more dangerous

→ causes presynaptic inhibition of Glycine/GABA [inhibitory neuro-transmitters]

→ constant stimulation

↓

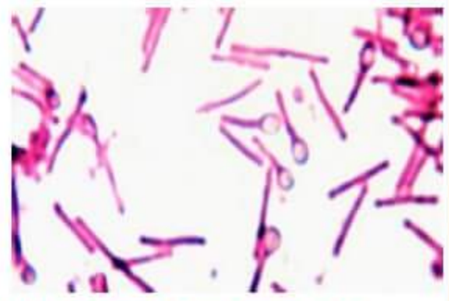
LOCK JAW [TRISMUS]

↓

OPISTHOTONUS POSTURE

↓

↑ Deep Tendon Reflexes


DRUMSTICK APPEARANCE OF Cl. tetani

IP → 8 - 10 days

→ if IP → < 6days → carries poor prognosis

NEONATAL TETANUS

→ India is free of neonatal tetanus d/t

→ Clean practices

→ Antenatal immunization

→ Hospital deliveries

Clostridium botulinum

TOXINS

① Neurotoxins [main] → most potent toxin on earth

② C_2 [Enterotoxin] → a/w canned food poisoning

CANNED FOOD POISONING

CLINICAL FEATURES

→ Dysphagia

→ Diplopia

→ Dysarythria

→ CONSTIPATION [d/t ↓ Ach]

→ ↓ DTR

INFANT BOTULISM

→ a/w Sporulated honey ingestion

→ CLINICAL FEATURES

→ Flaccid paralysis → FLOPPY INFANT SYNDROME

NEUROTOXIN USES

① Botox treatment → to remove wrinkles

② Eye Surgeries

→ Blepharospasm

→ Strabismus

DIAGNOSIS

① PCR for cl. botulinum

② culture for cl. botulinum

Clostridium difficle

→ difficult to isolate

→ CLOSTRIDIUM DIFFICLE ASSOCIATED DIARRHOEA [CDAD]

→ IF not Rx in time, leads to Pseudomembranous colitis

→ Ⓝ commensals of gut [cl. difficle]

→ ↑ irrational broad spectrum antibiotic usage.

Normal Flora destroys

↓

cl. difficles attaches to gut

↓

Diarrhoea

LAB DIAGNOSIS

① TOXIN DEMONSTRATION [Entero toxin [A] + Cyto toxin [B]]

Demonstrated on Hep 2 cell lines [commonly used for viral isolatⁿ]

② PCR for tox gene

③ ELISA for toxin production

④ SIGMOIDOSCOPY to visualise any ulcer

⑤ CULTURE for cl. difficle

Mycobacterium tuberculosis

→ ACID FAST
  → ability to resist decolorization
  → depends on Mycolic Acid content

→ VIRULENCE FACTORS
  ① Cord factor
  ② Lipo arabinomannan

→ In children, Lower lobes are involved
  → GHON'S FOCUS + Hilar lymphadenopathy → GHON'S COMPLEX
  → calcified Hilar lymphadenopathy → RANKE'S COMPLEX

→ POST PRIMARY TB , Upper lobes are involved
  → Subpleural focus → SIMON'S FOCUS
  → B/L infraclavicular lesions → ASSMAN'S FOCUS

→ SKIN TB
  MC primary lesion → LUPUS VULGARIS [Apple jelly nodules]
  and mc lesion → SCROFULODERMA

→ GI TB
  mc site → Ileo caecal junction

→ Bone TB → POTT'S SPINE

LAB DIAGNOSIS

① SPUTUM EXAMINATION
  → 2 sputum samples taken
    ① Onspot sample
    ② Early morning sample
  → concentratn method → PETROFF'S METHOD
  → Thick sputum + NaOH / Hcl → Liquified sputum
  @ ZN STAINING

Sputum smear

↓

carbol fuschin

↓

intermittent heating

↓

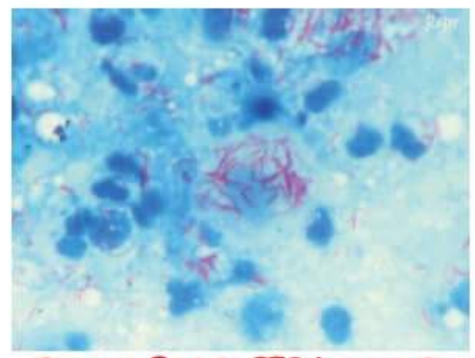
20% H_2SO_4 [Decolouriser]

↓

Methylene Blue

↓

Blue background; Pinkish to red coloured Bacilli



ACID FAST STAINING
ZN STAINING

⑥ LJ MEDIA [LOWEN STEIN JENSEN MEDIA]

- → Eggs are used for Solidification
- → Selective media agent → Malachite green
- → Green colored media
- → Rough, Tough, BUFF Colonies
  - → Rough → wrinkled apparance
  - → Tough → difficult to remove
  - → BUFF → Yellowish Brown

LJ MEDIA

② MTB PCR METHODS

③ Gene Xpert rif [rif - detection of Rifampicin resistance]

④ CBNAAT [cartridge Based Nucleic Acid Amplification Test]

⑤ Line Probe Assays

⑥ MTB PCR + multi Drug Resistance detection

- → can give the results in 2 hrs
- → Drawback of PCR → Does not differtiated b/co Active and latent TB

⑦ QUANTIFERON TB GOLD ASSAY [IGRA - Interferon Gamma Release Assay]

Mycobacterial Antigens

↓

Stimulati

BLOOD sensitised T lymphocytes

↓

measure Interferon γ activity

[Activity OF sensitised T lymphocytes]

↓

Prior exposure will be known

→ Drawback → Does not differtiated b/co Active and latent TB

⑧ AURAMINE - RHODAMINE STAINING

- → Fluorescent staining
- → Done in cases of more load
- → Drawback → false positive Ratis are high

⑨ TUBERCULIN TEST / MONTOUX TEST

- → 0.1 ml PPD [Purified Protein Derivative]
- → into Flexor aspect of fore arm
- → After 3 days, measure Induration [Hardness Diameter]
  - < 5 mm → negative
  - 5 - 10 mm → Equivocal
  - > 10 mm → +ive

→ False Positive

- recent BCG vaccinat^{n}
- Atypical mycobacteria infection

→ False negative

- HIV +ve [Advanced stage]
- Miliary TB
- Malignancy
- Immunosupression

→ Delayed hypersensitivity reaction

BCG [Bacillus calmette Guerin] VACCINE

→ derived from Danish 1331 strain of Mycobacterium bovis
→ Intra dermal
→ Diluent → Normal saline
→ Efficacy → 0 - 80%

→ TB is a Notifiable Disease
→ Serological tests [MTB card tests, MTB ELISA] are banned in India

Atypical Mycobacteria

RUNYON'S CLASSIFICATION

PHOTO CHROMOGENS	M A S K
SCOTO CHROMOGENS	S S G
NON CHROMOGENS	MAC, M. xenopi, M. ulcerans
RAPID GROWERS	M. cheloni, M. fortuitum

PHOTO CHROMOGENS

→ Grow in light

→ M	Mycobacterium	marinum
A	Mycobacterium	asiaticum
S	Mycobacterium	simiae
K	Mycobacterium	kansasii

SCOTO CHROMOGENS

→ Grow in darkness

→ S	Mycobacterium	szulgai
S	Mycobacterium	scrofulaceaum
G	Mycobacterium	gordanae

NON CHROMOGENS

→ neither light nor darkness required
→ MAC → Mycobacterium avium Complex / Mycobacterium intracellulare
aka → BATTEY'S BACILLUS
→ M. xenopi
→ M. ulcerans

RAPID GROWERS

→ M. cheloni
→ M. fortuitum
→ M. Phlei
→ M. Smegmatis

DISEASES

① SWIMMING POOL GRANULOMA / FISH TANK GRANULOMA → d/t M. marinum
② BURULI ULCER → d/t m. ulcerans
③ POST INJECTION ABSCESS → d/t RAPID GROWERS
④ most Atypical bacteria causes → CUTANEOUS INFECTIONS
LYMPHADENOPATHY

Atypical Mycobacteria which mimics M.TB in Lung involvement → M. Kansassi

Mycobacterium leprae

→ Causes HANSEN'S DISEASE
→ Not cultured in pure culture media [does n't follow KOCH uncle's Postulates]
Grows in FOOT PAD of ARMADILLO [Nine Banded]
Grows in FOOT PAD OF MICE

Nine banded Armadillo

LEPROMIN TEST

→ Type IV HSR
→ Lepromin Test is a PROGNOSTIC TEST, not a diagnostic test

EARLY LEPROMIN REACTION / FERNANDEZ REACTION	LATE LEPROMIN REACTION / MITSUDA REACTION
→ read after 3 days	→ reads after 3 wks
→ measures Indurat^{n} Diameter	→ measures Nodular Diameter

LEPRA REACTIONS

TYPE 1 LEPRA REACTION	TYPE 2 LEPRA REACITON
→ Down grading reaction	→ Erythema Nodosum Leprosum
→ Type IV hypersensitivity	→ Type III hypersensitivity reaction
→ mc feature → edema	→ immune complex mediated reaction
→ mc nerve involved → Ulnar	→ crops of macules & nodules over the skin [d/t TNF α]

→ can occur spontaneously or after the treatment

→ Lepra Reactⁿ is a case of emergency

→ DOC → Gluco corticoids

LAB DIAGNOSIS

SLIT SKIN SMEAR EXAMINATION

→ Taken from

→ B/L ear lobes

→ Forehead

→ chin

→ Buttocks

→ ⊕ Nasal mucosal swabs

→ ZN Staining

→ add carbol fuschin , do intermittent heating

→ add 5% H_2SO_4

→ add Methylene Blue

→ Blue Background

→ cigar bundle shape / Globi like arrangement of leprae seen

TREATMENT

① PAUCI BACILLARY → D + R

→ Dapsone

Rifampicin

→ For 6 months

② MULTI BACILLARY → D + C + R

→ DAPSONE

CLOFAZIMINE

RIFAMPICIN

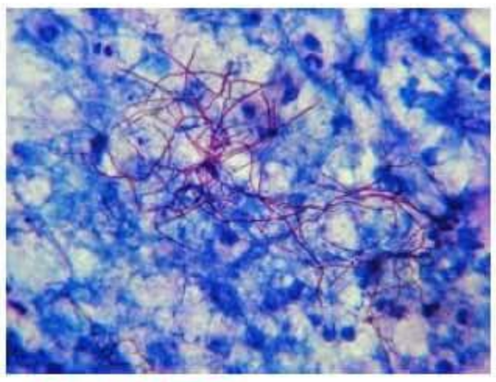
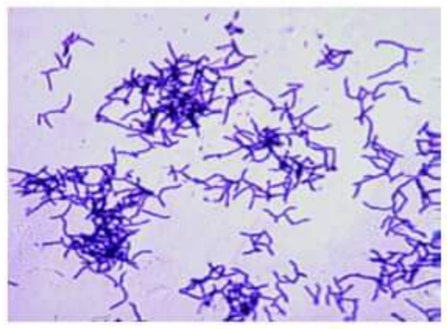
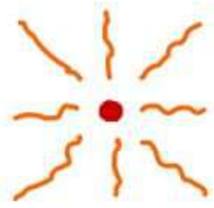
→ For 1 year

→ For Prognosis of leprosy

→ MORPHOLOGICAL INDEX → Better

– measures % of solid stained bacilli [Live bacilli]

Nocardia & Actinomycetes

NOCARDIA	ACTINOMYCETES
→ Obligate Aerobe	→ Anaerobe
→ 1% Acid fast	→ mostly non Acid fast but some species are acid fast
→ causes PULMONARY NOCARDIOSIS  NOCARDIA  Mycetoma  • antler like branching [filamentous branching] • ACTINOMYCETES  Sulphur granule	→ causes ORAL ACTINOMYCOSIS → sinus swelling sulphur granules } TRIAD → mc site → cervico facial SULPHUR GRANULES → crush & see under microscope → SUN RAY APPEARANCE Rays → organisms
	→ ACTINOMYCES ISRAELII → causes PID → Acid fast in nature
Both Nocardia & Actinomyces combine to cause → ACTINOMYCETOMA → Nocardia caviae → Nocardia asteroides → Actinomadura → Actinomadurae	
→ CULTURE OF NOCARDIA → PARAFFIN BAIT TECHNIQUE	→ CULTURE OF ACTINOMYCES → mostly non cultured → But some can be cultured
→ DOC → COTRIMOXAZOLE	→ DOC → PENICILLIN

Listeria monocytogenes

→ Gram positive cocci Bacillus
→ Tumbling motility [Peritrichous flagella]
→ CULTURE
  → Blood Agar
  → Chocolate Agar
  → PALCAM Agar

  → COLD ENRICHMENT METHOD [at 4°C]

L. monocytogenes

→ ANTON TEST Positive
→ CAMP TEST Positive
→ CATALASE TEST Positive

ANTON TEST → Putting the Listeria culture into eyes of Rabbit → Keratoconjunctivitis

DISEASES

① GRANULOMATOSIS INFANTISEPTICA [in neonates]

② MENINGITIS [children (mainly) , Adults]

③ CHORIOAMNIONITIS [in pregnancy] → PROM

- Preterm Births
- Abortions

④ SEPSIS [in immunocompromised]

→ DOC → AMPICILLIN

→ Listeria shows DIFFERENTIAL MOTILITY based on temperature

- 37°C → Non motile
- 20 - 25°C → Motile

ENTEROBACTERIACAE

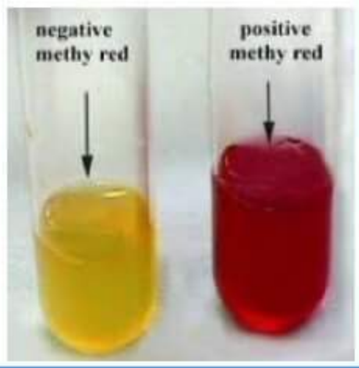
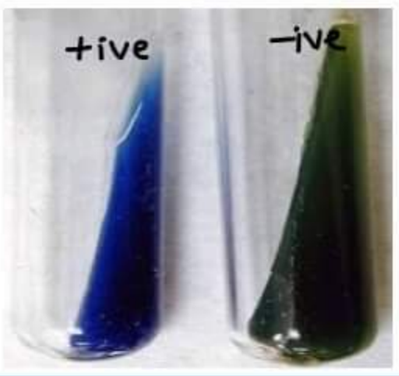
Lactose Fermenters | NON Lactose Fermenters

LACTOSE FERMENTERS
E.coli
Klebsiella
Shigella sonnei

CRITERIA

① Gram Negative Bacteria
② Catalase Positive
③ Oxidase Negative
④ Nitrate to Nitrite Reduction Test Positive
⑤ motile
⑥ Ferment sugar & Produce Acid with gas

E.coli

	I	M	Vi	C
	INDOLE	METHYL RED	VOGES PROSKAUER	CITRATE
E.coli	Positive	Positive	Negative	Negative
Klebsiella	Negative	Negative	positive	positive
	 Kovac's reagent			

→ color change in citrate Test → Green to Blue ["Gram Bacillus"]

DISEASES

① UTI
② DIARRHEA
③ NEONATAL MENINGITIS
④ EMPHYSEMATOUS PYELONEPHRITIS
⑤ FOURNIER'S GANGRENE
⑥ PRIMARY BACTERIAL PERITONITIS
⑦ SCROMBOID FISH POISONING [E.coli > Morganella]
⑧ Histamine Production +nt

UTI

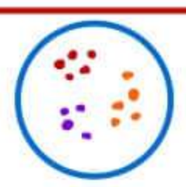
'P' Fimbriae possessing E.coli attach to uroepithelial cells
$> 10^5$ E.coli/ml of urine → SIGNIFICANT BACTERIURIA

→ Criteria for diagnosis of UTI

① $>$ 100 colonies $\times 10^3$ → $> 10^5$ / ml
② $>$ 10 colonies $\times 10^3$ → $> 10^4$/ml
⊕ Symptoms / signs of UTI [Dysuria, burning micturition]
③ Even 1 bacteria / ml in Supra pubic Aspirate

[10^3 - Dilution Factor]

→ > 2 types of colonies on a culture plate → CONTAMINATION



→ then repeat the sample

→ mid stream urine sample taken

DIARRHOEA CAUSING E. Coli

EPEC → Entero Pathogenic E. Coli

EIEC → Entero Invasive E. coli

ETEC → Entero Toxigenic E. coli

EHEC → Entero Haemorrhagic E. coli

EAEC → Entero Aggregative E. coli

EPEC

→ causes Pediatric age group Diarrhoea

→ attach to Enterocytes & Damage them

- causes attaching & effacing lesions

EIEC

→ resembles SHIGELLOSIS

→ dit VMA [Invasiveness]

→ SERENY TEST Positive

ETEC

→ causes Travelers Diarrhoea

→ TOXINS

Heat Stable Toxin → acts by ↑ cGMP

Heat Labile Toxin → acts by ↑ cAMP

EHEC

→ O157 : H7

O → Somatic antigen

H → Flagellar antigen

→ Causes Hemolytic Uremic Syndrome in children

→ TOXINS

Verotoxin 1 & 2 [VERO TOXIGENIC E.coli] → SHIGA LIKE TOXIN

→ Cuture media → SMAC [Sorbital Mac - conkey Agar]

Does not ferment Sorbital

EAEC

→ causes Persistent type of Diarrhoea

causes Traveler's diarrhoea too [few cases]

→ STACKED BRICK APPEARANCE on Hep 2 cell lines

NEONATAL MENINGITIS

common causes of Neonatal meningitis :

→ E. coli [↑ K1 antigen]

→ Klebsiella

→ GBS [Streptococcus agalactiae]

SCROMBOID FISH POISONING

→ a/w Histamine production

→ caused by E.coli > Morganella

[NOTE - As per new data → Morganella > Proteus > E.coli]

- Morganella
  - belongs to family PROTEA
  - Proteus is another member of the family

KLEBSIELLA

→

I	M	Vi	C
−	−	+	+

→ Gram Negative Bacillus

→ Capsulated

→ non - motile

→ Lactose fermenter

→ Urease Positive [Yellow → Pink]

UREASE POSITIVE ORGANISMS		
Staph	→	Staph . aureus
klub	→	Klebsiella
main	→	Morganella
Urease	→	Ureaplasma urealyticum
Programme	→	Proteus
Hai	→	H. pylori [max. urease producer]
Chaloe	→	Cryptococcus neoformans

Klebsiella pneumoniae [FRIED LANDER'S BACILLI]

→ causes Typical pneumonia

→ a/w RED CURRENT JELLY SPUTUM

→ a/w BULGING FISSURE SIGN on CXR

Klebsiella ozaenae

→ a/w Ozaena [atrophic rhinitis]

→ Roomy nose

→ immense foul smell

→ R/ by $NaHCO_3$ douches

MODIFIED YOUNG'S OPERATION

Klebsiella rhinoscleromatis

→ causes Rhinoscleroma [WOODY NOSE]

→ MIKULIZ CELLS
RUSSEL BODIES
} Seen

Klebsiella granulomatis

→ causes Granuloma inguinale or Donovanosis

→ DONOVAN BODIES are seen

→ BIPOLAR SAFETY PIN APPEARANCE

→ aka SUPER BUG

→ K. pneumoniae strain

→ resistant to all most all the drugs

→ dlt metallic enzyme → Metallic Action → ↑sed virulence

→ now termed as METALLO BETA LACTAMASE 1

PROTEUS

→ GNB

→ SWARMS

→ inhibit swarming by

- ↑ % of Agar from 2% to 6%
- instill some chemicals [Boric acid & Chloral Hydrate]

swarming

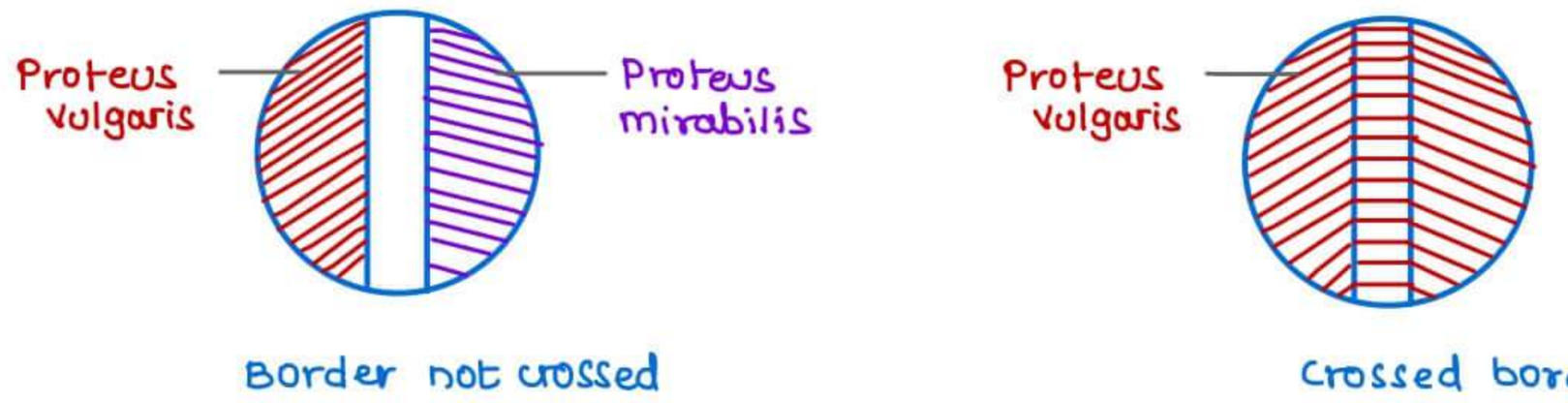
→ can be cultured on

Mac - conkey Agar → produce Non Lactose fermenting [NLF] colonies

Phenyl Pyruvic Acid Agar

FISHY SMELL / SEMINAL SMELL produced on both media

→ DIENELS PHENOMENON



Border not crossed

Crossed borders

→ PROTEUS ANTIGEN INTERACTIONS

→ OX 19, 2 & K → cross reacts ē Rickettsial family members

→ Gives WEIL - FELIX REACTION [Heterophile agglutinatn test]

→ OX K is strongly positive for Scrub typhus

→ Proteus a/w chronic UTI

Leads to Struvite | Triple Phosphate stones / Staghorn calculus

SALMONELLA

→ GNB

→ motile mostly

non motile → S. gallinarum
S. pullorum

→ KAUFMANN & WHITE SCHEME is used to classify salmonella

Salmonella typhi

→ causes TYPHOD FEVER

→ Enteric Fever caused by

- S. typhi
- S. paratyphi A, B & C

→ transmitted by Faeco oral contaminatn

→ Stepladder pattern fever

→ ROSE SPOTS seen

→ Longitudinal ulcers seen

→ PEA SOUP DIARRHOEA seen

ROSE SPOTS

LAB DIAGNOSIS

B → Blood culture → 1 st Week
A → Agglutination Test → 2 nd Week
S → Stool culture → 3 rd Week
U → Urine culture → 4 th Week

BLOOD CULTURE

→ overall Best

→ Blood : culture fluid → 1 : 10 [Eg. 5 ml : 50 ml]

- Bile broth or Glucose broth can be used

→ Add SPS [Sodium Polyanethol Sulfonate]

- to remove antibiotic effect

WIDAL TEST

→ Highly sensitive but Poorly specific

- also Positive in BFP - Biological false Positive
  - Infectious mono nucleosis
  - Malaria
  - SLE [auto immune Diseases]

WIDAL TEST

→ Anti Body titre against O → > 1:100
H → > 1:200
} POSITIVE

Flagellar antigen is more immunogenic

→ Paired testing should be done, after 2 weeks of initial testing
2nd Test - 4 fold ↑ in titre should be present → Positive

→ Antibody titre values are not fixed, they changes according to locality

→ TYPES

① SLIDE WIDAL TEST

② TUBE WIDAL TEST

- serial dilutⁿ ī Normal saline
- PROZONE PHENOMENON is avoided
  - Prozone phenomenon → Antibody excess

STOOL & URINE CULTURE

→ Enrichment media [SELENITE F BROTH] is used

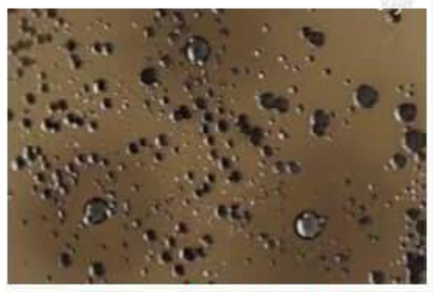
→ Selective media

- DCA [Deoxy Cholate Agar]
- XLD [Xylose Lysine Deoxycholate]
- SS [Salmonella Shigella Agar]
- HE [Hektoen enteric agar]

ABOVE MEDIAS ARE ALSO USED FOR SHIGELLA

Except WILSON & BLAIR MEDIA

- Best media for S. typhi
- Jet Black colonies are produced



Salmonella typhi colonies in WB - medium

Vi Antigen

→ Vi mostly covering up 'O'

→ Vi phage typing can be used for epidemiological studies

NON SALMONELLA TYPHI

→ S. typhi murium
S. choleraesuis

→ S. typhimurium, S. enteridis } causes Gastro enteritis

→ S. typhimurium
- a/ω Contaminated Poultry Products
- can enter the intact egg shell

DOC

→ 3rd Generatⁿ CEPHALOSPORINS

SHIGELLA

→ GNB
→ non - motile
→ Mannitol fermentatⁿ Based classificatⁿ used
  - non mannitol fermenter → shigella dysentriae

→ catalase -ive → S. dysentriae type 1
→ VMA [virulence Marker Antigen] → responsible invasiveness
→ Secrets Endo & Exotoxins
→ SHIGA TOXIN
  - Exotoxin
  - by S. dysentriae

→ mcc of bacillary dysentry in India → S. flexneri
mcc of bacillary dysentry in World → S. sonnei
  - S. sonnei → Late Lactose fermenter [LLF]

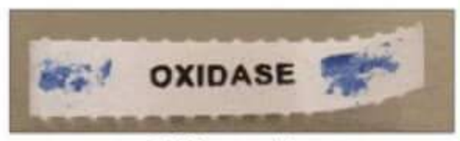
→ CULTURE MEDIA
  - same as Salmonella

Transport media → Sach's Buffered Glycerol saline

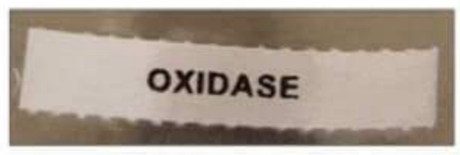
Non fermenters - Doesn't ferment sugars

① Pseudomonas
② Burkholderia mallei
③ Burkholderia pseudo mallei
④ Acinetobacter baumanii

PSEUDOMONAS



Oxidase positive



Oxidase negative

→ GNB
→ catalase +ive
→ OXIDASE +ive
  Oxidase reagent
  - Tetra methyl Para Phenylene diamine di hydro chloride
  - comes as White Disc
  - change of color from White to blue in 10sec → Positive

→ VIRULENCE FACTORS
① Capsular Polysaccharide
② Alginic Acid
③ Pigments
  a. Pyocyanin → Blue
  b. Pyorubrin → Red
  c. Pyomelanin → Brown Black


MULLER HILTON AGAR

d. Pyoverdin → Green
   - mc
   - mostly found in Pseudomas aeruginosa [verdigris]

These pigments enhance on KING'S media

→ Best selective media → cetrimide Agar

DISEASES

1 SHANGHAI FEVER
2 MALIGNANT OTITIS EXTERNA
3 ECYTHMA GANGRENOSUM
4 VENTILATOR ASSOCIATED PNEUMONIA
5 BURN WOUND INFECTIONS
6 MENINGITIS
7 UTI


Pseudomanas infectn

→ ANTI PSEUDOMONAL DRUGS
1 POLYMYXIN - B
2 COLISTIN
} no resistance reported uptill

BURKHOLDERIA MALLEI

→ causes GLANDER'S DISEASES
   - animal disease [Horses]
→ Non - motile

BURKHOLDERIA PSEUDO MALLEI

→ causes MELIOIDOSIS
→ aka WHITMORE'S BACILLI
   VEITNAM BOMB
→ Both Burkholderia are Bipolar stained

ACINETOBACTER BAUMANII

→ ICU isolate
→ MDR [multi Drug Resistant] isolate
→ Oxidase negative

VIBRIO CHOLERAE

→ on the basis of "O1" antigen, vibrio classified into

	classical	Eltor
Polymyxin B sensitivity	sensitive	Resistant
Mukherjee Phage Ⅳ susceptibility	susceptible	Resistant
Mukherjee Phage Ⅴ susceptibility	Negative	Positive
VP [voges Proskauer]	Negative	Positive
CAMP	Negative	Positive
Chick Erythrocyte Agglutinatn Test	Negative	Positive

→ SUB DIVISIONS
- → classical → ogawa, inaba, Hikojima
- → Eltor → ogawa, inaba, Hikojima

→ First 6 pandemics caused by → classical
7th pandemic caused by → Eltor

→ Eltor
- Place in Saudi → Haj
- Quarantine station

→ NON O1 → O2 - O139
NAG → non agglutinable vibrios

→ CHOLERA TOXIN
- → A → ADP ribosylatn of GTP → ↑ in adenylate cyclase activity
  ⟶ ↑ cAMP
- → B → Binds to GM_1 ganglioside receptor
- → intracellular water comes to Lumen resulting in massive diarrhoea
- → Massive Diarrhoea
  - Non inflammatory diarrhoea
  - RICE WATERY STOOLS
  - resembles Arsenic metal poisoning

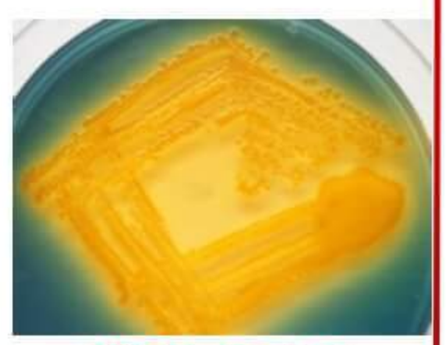

RICE WATERY STOOLS

→ CULTURE MEDIA

Transport media [maintain viability, no multiplicatn]
① VenkatRaman RamaKrishan Media
② Cary Blair media

Enrichment Media → Alkaline Peptone water

Selective media
① TCBS [Thiosulphate Citrate Bile Salt Sucrose]
→ Green → Yellow [dlt Sucrose lysis]
→ Best selective media


TCBS MEDIA

HALOPHILIC VIBRIOS

① V. parahemolyticus [7-8% halophilic]
② V. vulnificus [8% halophilic]
③ V. alginolyticus [10% halophilic]

V. PARA HEMOLYTICUS

→ causes SEA FOOD POISONING
→ also KANAGAWA PHENOMENON on WAGATSUMA AGAR [enhanced β hemolysis]

VIBRIO CHOLERA

C → catalase positive
O → Oxidase positive
I → Indole positive
N → Nitrates to Nitrites Reduction Test positive
S → Sucrose lysis
→ String Test positive

→ On Hanging Drop, DARTING MOTILITY seen

STRING TEST POSITIVE

→ STRING TEST
- Sodium de oxy cholate [Bile salt] is added to Stool Sample
- Try to lift a loop → String is formed

HAEMOPHILUS

HAEMOPHILUS DUCREYI

→ causes CHANCROID [Soft sore]
- painful genital ulcer

→ Grows on Chocolate agar → CULTURE MEDIA
→ TISSUE BIOPSY + STAINING → TRAMTRACK APPEARANCE or SCHOOL OF FISH APPEARANCE

HAEMOPHILUS INFLUENZAE

CAPSULATED	NON CAPSULATED
→ causes invasive diseases - Meningitis - Epiglottitis	→ Non invasive Diseases - Otitis media - Sinusitis - LRTI

→ Shows SATELLITISM

H. AEGYPTI CUS

→ causes

① PINK EYES

② BRAZILIAN PURPURIC FEVER

NORMAL MOUTH COMMENSALS

H → H. parainfluenzae , H. aprophilus , H. para aprophilus

A → Aggregatibacter [Actinobacillus mycetic omitans]

C → Cardiobacterium hominis

E → Eikenella coordens [a/w human bites]

K → Kingella Kingae

They can cause culture negative Endocarditis in immunocompromised

BORDETELLA PERTUSSIS

→ causes PERTUSSIS / WHOOPING COUGH / 100 DAY COUGH

→ CULTURE MEDIA

1 BORDET GENGOU MEDIA

2 REGAN LOWE MEDIA

→ COUGH PLATE METHOD obsolete now

→ SAR [Secondary Attack Rate → > 90%

→ STAGES

① CATARRHAL → most infectious

② PAROXYSOMAL → Whoops +nt

③ CONVALESCENT → Recovery phase

→ IP → 1 - 2 WKS

→ LAB DIAGNOSIS

① Naso Pharyngeal swabs

ⓐ Culture media

→ On Bordet Gengou media → BISECTED PEARL APPEARANCE

ⓑ PCR for B. pertussis

→ ACELLULAR PERTUSSIS VACCINE

→ used now [equal in efficacy ≀ cellular vaccine]

→ made up of

- Pertussis Toxid
- Fimbrial Haemagglutinin
- Pertactin
- Agglutinogens 1, 2 & 3

BRUCELLOSIS

→ Zoonotic Disease

→ CAUSES

Brucella abortus	→	infects cattles
B. melitensis	→	infects Sheep, Goat, camel
B. suis	→	infects Pigs
B. canis	→	infects Dogs

→ MOT → by eating & drinking contaminated food & milk products

B. MELITENSIS

→ Causes ACUTE BRUCELLOSIS / MALTA / MEDITTERANEAN / UNDULENT FEVER
- Typhoid like illness

→ LAB DIAGNOSIS

① Blood culture
- ratio OF blood & culture fluid → 1:10
- Glucose Broth is taken as culture fluid
- Add ERYTHRITOL → stimulate Brucella growth
- carried by CASTANEDA METHOD
  - Biphasic media [Solid & liquid culture media]
  - use – minimizes contaminat^{n} in sub cultures

② catalase positive
Oxidase positive
Urease positive

③ TBLISI PHAGE TYPING is done

④ STANDARD AGGLUTINATION TEST [SAT] is done

⑤ PCR for Brucella species

⑥ TESTS FOR CONTAMINATED MILK FOR BRUCELLA
- ⓐ ROSE BENGAL CARD TEST
- ⓑ MILK RING TEST
- ⓒ WHEY AGGLUTINATION TEST

→ DOC → DOXYCYCLINE + RIFAMPIN

PLAGUE

→ caused by Yersinia pestis

→ TYPES

① B → Bubonic → IP → 2-7 Days

② S → Septicaemic → IP → 2-7 Days

③ P → Pneumonic → IP → 1-3 Days [most Dangerous]

→ CULTURE MEDIA

GHEE BROTH – STALACTITE TYPE OF GROWTH

→ Ratflea index → Cheopis index is used

→ LAB DIAGNOSIS

① Buboes smear
② Blood smear
} + WAYSON STAIN → BIPOLAR STAINED APPEARANCE

→ DOC → STREPTOMYCIN

→ Last major epidemic in India → Surat ; Gujarat

LEGIONELLA

LEGIONELLA PNEUMOPHILA

→ most common

→ MOT

① Aerosol route from centralized AC plants

② Drinking contaminated water ∴ Legionella

→ DISEASES

① PONTAIC FEVER

② LEGGIONARE'S PNEUMONIA

→ Atypical pneumonia

→ complicates ∴ Encephalitis & Diarrhoea } LEGIONNARE'S DISEASES – a/w mortality

→ CULTURE MEDIA → Buffered charcoal Yeast Extract [BCYE]

→ PCR for Legionella species

→ Legionella micadedi is Acid fast in nature

SPIROCHETES

→ Spiral in shape

→ DARK FIELD MICROSCOPY is done
- background is dark
- reflected light is used

Dark Field microscopy

→ FONTANA & LEVADITI STAINING done
- Sliver impregnation method
- makes them thick

TREPONEMA PALLIDUM

→ causes SYPHILIS

→ IP → 9-90 Days

→ STAGES

① PRIMARY → Hard, painless chancres seen

② SECONDARY → Rashes over the palms & soles are present

→ condyloma lata lesions are seen
- most infectious lesion

③ LATENT

④ TERTIARY → GUMMAS [skin lesions] seen

→ Complications

Neuro Psyphilis
- presents i GPI [General Paralysis of Insane]
Tabes dorsalis

Cardiovascular Psyphilis
- presents i Aneurysm of Aorta

→ TREATMENT

→ DOC for Primary, secondary & Latent stages of Syphilis → BENZATHINE PENICILLIN

→ DOC for Neurosyphilis → PENICILLIN G > PROCAINE PENICILLIN

→ LAB DIAGNOSIS

① NON TREPONEMAL TESTS

@ VDRL [veneral Disease Research Lab]

ⓑ RPR [Rapid Plasma Reagent]

VDRL	RPR
→ needs a microscope → Fluid is to be prepared & to be used in 24hrs → done i CSF [neuro syphilis] Best test to assess prognosis	→ Better Test - card test - no fluid is prepared → in CSF sample, RPR fails

② TREPONEMAL TESTS

ⓐ FTA - ABS [Fluorescent Treponemal Antibody Assay]
ⓑ TPI [Treponema pallidum Immobolizatⁿ Assay]
ⓒ TPHA [Treponema pallidum Haemagglutinatⁿ Assay]
ⓓ TPPA [Treponema pallidum Particulate agglutinatⁿ Assay]

MOST SENSITIVE → FTA - ABS

MOST SPECIFIC → TPI
- but routinely not done
- d/t involvement of live Treponemes

→ TPPA
- 2nd most Specific Test

MC DONE → TPHA

CONGENITAL SYPHILIS

→ IgM FTA ABS is done

T. Pertunal	→	causes	YAWS	NOT STDs
T. endemicum	→	causes	BEJEL [endemic Syphilis]	
T. carateum	→	causes	PINTA	

BORRELIA

→ EPIDEMIC LOUSE BORN RELAPSING FEVER → caused by B. recurrentis
→ ENDEMIC TICK BORN RELAPSING FEVER → caused by B. duttoni & B. hermesii

→ Relapses are d/t ANTIGENIC VARIATIONS

B. BURGDORFERI

→ transmitted by Ticks
→ causes LYME'S DISEASE
- characteristic lesion → ERYTHEMA CHRONICUM MIGRANS

→ CULTURE MEDIA → BARBOUR STANNOUR KELLY MEDIA

B. VINCENTI & FUSIFORMIS

→ causes VINCENT ANGINA / TRENCH MOUTH

LAB DIAGNOSIS

① CULTURE
② PCR for Borrelia
③ Dark Field microscopy

LEPTOSPIRA

LEPTOSPIRA INTERROGENS

→ causes WEIL'S DISEASE [ICTERO HAEMORRHAGIC FEVER]

→ CULTURE MEDIA

① EMJH

② FLETCHER

③ KORTAOKOFF

→ LAB DIAGNOSIS

① SEROLOGICAL TEST → MAT

→ Microscopic Agglutinatⁿ Test [preferred]

→ Macroscopic Agglutinatⁿ Test

② PCR for Leptospira species

→ R → Rat urine
R → Rice [Paddy field workers]
R → Rainy water
} a/w LEPTOSPIROSIS

Rat urine contaminates Rainy water, a/w Paddy field workers

→ National Referal centre for Leptospira located at → Port Blair

Q A 20 yr old male who works as truck driver c a genital ulcer, painless for last 10 days. He had unprotected contact with a commercial sex worker about 2 weeks previously, O/E ulcer was circumscribed, indurated & partially healed. The inguinal lymph nodes enlarged. Cause ?

(A) HIV
(B) HPV
(C) Treponema pallidum
(D) HSV-2

RICKETTSIAE

→ Obligate intracellular organisms
→ Arthropod borne transmission
→ non culturable

	CAUSED BY	ARTHOPODS INVOLVED
EPIDEMIC TYPHUS	R. prowazekii	LOUSE
ENDEMIC TYPHUS	R. typhi	Flea
RMSF	R. rickettsi	Tick
INDIAN TICK TYPHUS	R. conori	Tick
R. POX	R. akari	Mite
SCRUB TYPHUS	Orientia tsutsugamushi	Mite

→ Presents ī fever, rash & lymphadenopathy
→ DOC → DOXYCYCLIN
→ OXK is strongly positive in Scrub typhus
→ Epidemic VS Endemic Typhus

TUNICA REACTION [NEIL MOOSER'S REACTION]

Scrotal necrosis & inflammation of gunie pig → Endemic typhus
No such Reaction & inflammatⁿ in guinea pig → Epidemic typhus

QUERY FEVER I Q FEVER

→ caused by Coxiella burnetti
→ contaminates milK
Killed by Flash method of pasteurisation
→ MOT → Aerosol transmission [no arthopod transmission]
→ No Rash occurs
→ a/co culture negative endocarditis

BARTONELLA

→ can be cultured on normal culture media
→ B. bacilliformis → causes OROYA FEVER
B. henselae → causes CAT SCRATCH DISEASE
→ causes BACILLARY ANGIOMATOSIS [Common in HIV+]
B. quintana → causes TRENCH FEVER
causes BACILLARY ANGIOMATOSIS [Fewer cases]

EHRLICHIA CHAFFENENSIS → causes Human monocytic ehrlichiosis
EHRLICHIA PHAGOCYTOPHILA → causes Human granulocytic ehrlichiosis

CHLAMYDIA (ATP PARASITE)

→ FORMS

Extra cellular / Elementary Body → Infectious

Intra cellular / Reticulate Body → Replicating

C. TRACHOMATOSIS

→	Serogroups A, B, Ba, C	→	causes TRACHOMA

Mx Strategy

S → Surgery

A → Antibiotics

F → Face clean

E → Environmental clean

→	Serogroups D - K	→	causes INCLUSION CONJUCTIVITIS INFANTILE PNEUMONIA GENITAL CHLAMYDIASIS
→	Serotypes L_1, L_2, L_3	→	causes LYMPHO GRANULOMA VENERUM [$L_2 > L_1$]

C. PSITTACI

→ causes BIRD PNEUMONIA

- mainly to Parrots
  - helps in aerosol transmission

C. PNEUMONIAE

→ causes ADULT PNEUMONIA

→ a/w Atherosclerosis

→ a/w Asthma [to be confirmed]

INCLUSION BODIES		
Trachoma	→	HP Bodies [HALBERDT PROWAZEK]
Molluscum contagiosum	→	HP Bodies [HENDERSON PETERSON]
Psittacosis	→	LEVINTHAL COLE LILEE BODIES

→ CULTURE

- on Mcoy cell line [continuous cell line]
- on Yolk sac of eggs [Hen's]

→ IOC → NAAT [Nucleic Acid Amplification Test] → ≅ PCR

LGV

→ painless ulcer

→ Painful lymph nodes → Para rectal LN involved

→ FRIE'S SKIN TEST is done

→ Complicatn → ESTHIOMENE → Rectal & Vulval Strictures

MYCOPLASMA

→ smallest bacteria
→ No cell wall
→ Cell membrane is present → made of sterol
→ can pass through bacterial filters
→ CULTURE MEDIA
PPLO Agar → FRIED EGG COLONIES
stained by DIENE'S METHOD
[DIENE'S PHENOMENON seen in PROTEUS]


FRIED EGG COLONIES

M. PNEUMONIAE / EATON'S AGENT

→ mc bacterial cause OF Atypical pneumonia
→ aka WALKING PNEUMONIA
→ LAB DIAGNOSIS
1 CAT [Cold Agglutinatn Test] → type of Heterophile aggutinatn test
2 Streptococcus MG Agglutinatn Test → type of Heterophile agglutinatn test
3 PCR for M. pneumoniae

M. genitalium, M. hominis } causes NON GONOCOCCAL URETHRITIS

NON GONOCOCCAL URETHRITIS CAUSES
① Chlamydia trachomatis
② Ureaplasma urealyticum
③ Mycoplasma genitalium
④ Mycoplasma hominis

→ Diagnosed by PCR

MISCELLANEOUS

CAMPYLOBACTER JEJUNI

→ GNB
→ micro aerophile [5% O_2 required]
→ motile
→ GULL WING SHAPED BACTERIA
→ a/w Poultry bird contaminatn
→ causes Inflammatory Diarrhoea
- Fecal leucocytes are +nt

→ In children, Diarrhoeal episodes —Followed By→ GULLIAN BARRE SYNDROME

→ LAB DIAGNOSIS

① STOOL SAMPLE

→ Gram staining → Pink coloured Gull wing shaped bacteria
→ Fecal leucocytes + nt
→ Culture done on charcoal based medias
→ charcoal based medias
→ CAMPY BAP
→ BUTZLER
→ SKIRROW

→ incubate at 42°C

② PCR for campylobacter jejuni

HELICOBACTER PYLORI

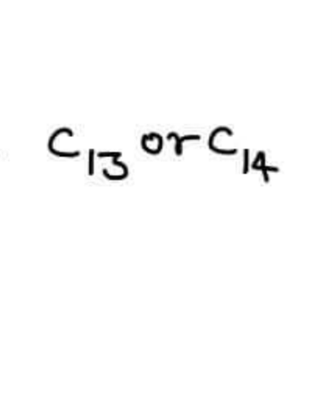
→ GNB
→ micro aurophile
→ motile
→ Strongly urease positive [yellow → Pink]
→ DISEASES
PUD [PEPTIC ULCER DISEASE]
MALTOMA
CA STOMACH
→ LAB DIAGNOSIS
NON INVASIVE TESIS → Urea Breath Test ī radio labelled C_{13} or C_{14}
INVASIVE TEST → Endoscopic Biopsy
- Stain ī WARTHIN STARRY SILVER STAIN
- Demonstrate H. pylori
- Bx tissue + urease media → Yellow → Pink

→ Rx → HELIBACT / PYLO KITS for eradicatⁿ of H. pylori
→ Cag & vac → ↑ses virulence of H. pylori

BACTERIAL VAGINOSIS

caused by

→ Gardanella vaginalis
→ Mobiluncus
→ Provetella



→ AMSEL'S CRITERIA → PH > 4.5
→ WHIFF TEST → Discharge + 10% KOH → fishy odour
→ CLUE CELLS → Epithelial cells ī bacteria
→ NUGEN'S CRITERIA → for Bacterial vaginosis
→ Grading of replacement of normal flora of vagina by Pathogenic organisms

MISCELLANEOUS IMP POINTS

STREPTOBACILLUS MONILIFORMIS

→ causes HAVERHILL FEVER
→ a/w Rodents [Rat bite fever]

SPIRILLUM MINUS → causes RAT BITE FEVER

BACTEROIDES SPECIES

→ Obligate anaerobes
→ GNB
→ normal commensal of gut
→ produce mild potent endotoxins

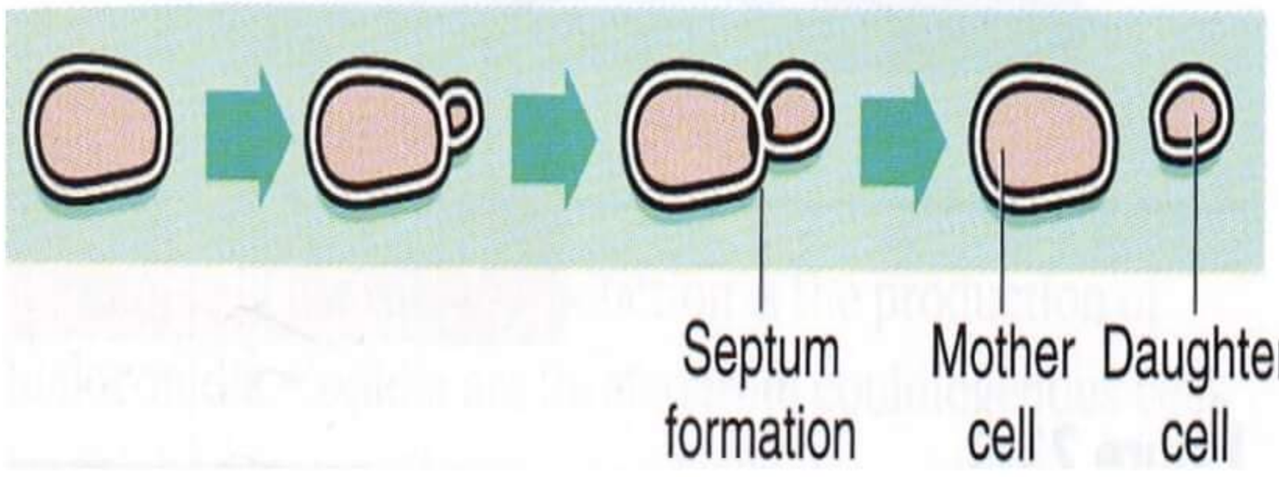
HUMAN BITES

→ a/w Eikenella corrdens
a/w anaerobic Streptococci

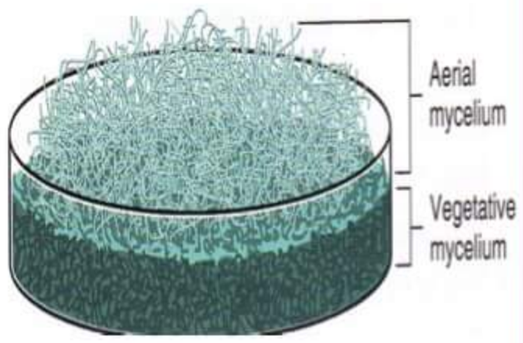
CAT & DOG BITES → a/w Pasturella multocida

SCROMBOID FISH POISONING a/w → E.coli > Morganella
[NOTE - As per new data → Morganella > Proteus > E.coli]

FUNGI → Eukaryotic protista
CELL WALLS → Chitin, mannan & polysaccharides
CYTOPLASMIC MEMBRANES → Sterols
POSSESS true nuclei, nuclear membrane & paired chromosomes
SIMPLEST TYPE OF FUNGUS → unicellular Budding yeast



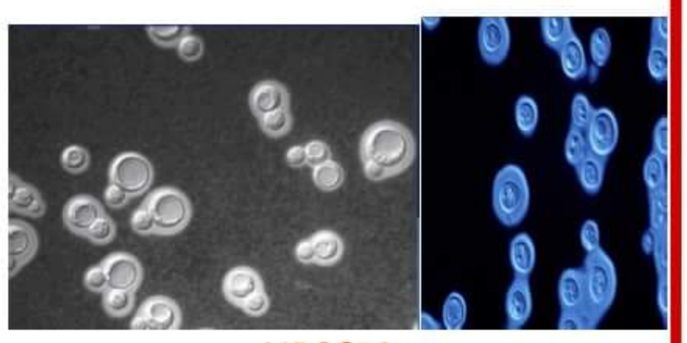
HYPHA → Elongated tubular structure
MYCELIUM → Tangled mass of hyphae
HYPHAE → Septate or aseptate
MYCELIUM
→ VEGETATIVE → Grows into medium
→ AERIAL → projects from surface



CLASSIFICATION

DEPENDING ON CELL MORPHOLOGY

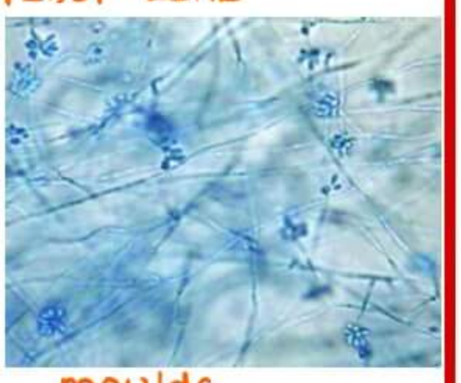
1. YEAST / TRUE YEAST
   → Spherical or ellipsoidal cells
   → reproduce by budding
   → Ex: Cryptococcus neoformans


YEASTS

2. YEAST LIKE
   → Partly as yeast
   → partly as elongated cells: PSEUDOHYPHAE
   → Ex: Candida albicans
      ↳ can show true hyphae / pseudohyphae
      ↳ can show yeast / yeast like [main form]


YEAST LIKE

3. MOULDS / MOLDS
   → True mycelia
   → reproduced by Spores
   → Ex: Rhizopus, Aspergillus


moulds

4. DIMORPHIC FUNGI
   → In host tissue or cultures at 37°C → Yeasts
   → In soil & cultures at 22°C / 25°C → moulds / molds
   → Ex:

H	→	Histoplasmosis	C	→	Coccidiodomycosis
S	→	Sporotrichosis	P2	→	Para coccidiodomycosis
B	→	Blastomycosis		→	Penicilliosis

BASED ON SEXUAL SPORE FORMATION

1. PHYCOMYCETES / ZYGOMYCETES

→ non - septate hyphae
→ obtuse angled
→ Forms asexual spores : Sporangia
→ Sexual spores → zygospores

2. ASCOMYCETES

→ Sexual spores → Ascospores in an Ascus

3. BASIDIOMYCETES

→ Sexual spores on a basidium
→ Seen in Cryptococcus neoformans

4. FUNGI IMPERFECTI [DEUTEROMYCETES]

→ no sexual phases

SEXUAL SPORES			ASEXUAL SPORES		
Z	→	Zygospore	A	→	Arthrospore
A	→	Ascospore	B	→	Blastospore
B	→	Basidiospore	C	→	Chlamydospore

LAB DIAGNOSIS

By microscopic examination of materials

1. Wet mounts ī 10% KOH → can see fungal elements
2. Tease mounts ī Lactophenol cotton blue [LPCB]
   - Calcoflour white staining [Fungal Fluorescent staining]
3. Slide culture
4. Periodic Acid Schiff [PAS]
5. Gomori methanamine Silver stains
   → used for Pneumocystis carnii / jiroveci
   → Stains green
   → Trophozoites stained green
   cysts stained black

   → PNEMOCYSTIS CARNII
   ↳ causes Pneumocystis carnii Pneumonia in HIV +ive ī CD4 < 200
   ↳ DOC → Cotrimoxazole

CULTURE MEDIA

1. SABOURAUD'S DEXTROSE AGAR [SDA] [PH = 5.6 (5.4 - 5.6)]
2. CZAPEK - DOX MEDIUM
3. CORN MEAL AGAR

→ Cycloheximide → Selective agent
→ INCUBATION TIME & TEMPERATURE
↳ at room temp. (22°C) → Weeks
↳ at 37°C → 4 days

→ IDENTIFICATION

1. By colony & fungal morphology
2. Rapidity of growth, color
3. morphology of the colony → obverse
4. Pigmentation → reverse

→ TEASE MOUNT or SLIDE CULTURE

↳ hyphal diameter, septa & special structures are important

→ SPECIAL HYPHAE

1. Spiral hyphae → Spring like helical coils
2. Racquet hyphae → resembling tennis racquets
3. Favic chandelier → numerous short branches appearing at the ends of hyphae

→ CONIDIA

1. microconidia → small, single celled
2. macroconidia → Large, single or multi celled

→ SPORES

1. Blastospores → budding
2. Chlamydospores → thick walled resting spores formed by rounding up & thickening of hyphae
   → Eg: Candida albicans on corn meal agar
3. Arthrospores → Along the hyphae by segmentation & condensatn
   → rectangular
   → Eg: coccidiomycosis

CLASSIFICATION BASED ON LOCATION

MYCOSES

1. Superficial mycoses → Superficial layer of skin involved
   Subcutaneous mycoses → Skin & Subcutaneous tissue tissue involved
2. Deep mycoses / Systemic mycoses
3. Opportunistic mycoses

CLINICAL CLASSIFICATION OF MYCOTIC INFECTIONS

Area of Predominant involvement	MYCOSIS	ETIOLOGY
1. SUPERFICIAL	Pityriasis versicolor	Malassezia furfur Malassezia globosa [skin]
	Tinea nigra	Phaeoannellomyces werneckii / Exophila werneckii
	white piedra	Trichosporon begelii
	Black piedra	Piedraia hortae

Area of Predominant involvement	MYCOSIS	ETIOLOGY
2. CUTANEOUS	Dermatophytosis	microsporum species Trichophyton species & Epidermophyton floccosum
	Candidiasis of skin, mucosa or nails	candida albicans, other candida species
3. SUB CUTANEOUS	Sporotrichosis	Sporothrix schenckii
	Chromomycosis	Philaphora verrucosa Fonsecaea pedrosoi & others
	mycetoma	Pseudallescheria boydii, madurella mycetomatis & others
	Mneumonic M - mycetoma R - Rhinosporidiosis C - chromomycosis S - Sporotrichosis	
4. SYSTEMIC MYCOSES [HSBC P²]		
PRIMARY MYCOSES	Coccidioidomycosis	coccidioides immitis
	Histoplasmosis	Histoplasma capsulatum
	Blastomycosis	Blastomyces dermatitidis
	Paracoccidiomycosis	Paracoccidioides brasiliensis
OPPORTUNISTIC MYCOSES	candidoidomycosis	candida albicans, other candida sp.
	Cryptococcosis	cryptococcus neoformans
	Aspergillosis	Aspergillus fumigatus, other asp. Sp.
	mucormycosis	Species of Rhizopus, Absidia, mucor & others

SUPERFICIAL MYCOSES

Include

A. SURFACE INFECTIONS

B. CUTANEOUS INFECTIONS

Tinea versicolor

SURFACE INFECTIONS

1. TINEA VERSICOLOR

→ caused by malassezia furfur

→ presents ĩ discrete hyperpigmented or hypopigmented macules | scales on chest or upper back

→ colony → yeast like consistency

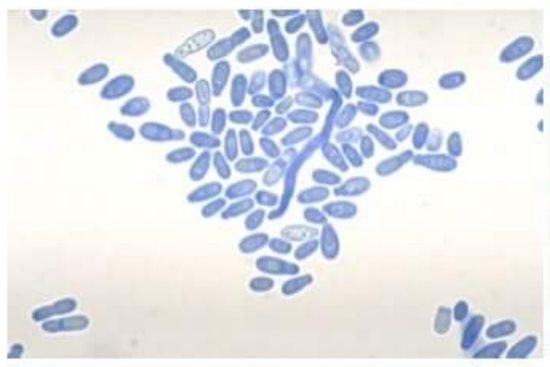
→ microscopy → spherical, oval or cylindrical cells

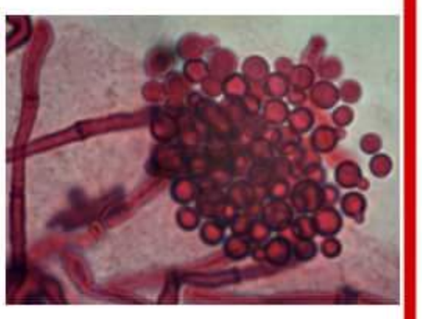
Tinea versicolor

Tinea versicolor

CLINICAL FEATURES

1. Higher incidence in tropics
2. PREDISPOSING FACTORS
   → perspiration
   → corticosteroids
   → malnutrition
   → heredity
   → Immunosuppression





DIAGNOSIS

1. MICROSCOPIC EXAMINATION
   → SKIN SCRAPINGS
      ↳ scales + 10% KOH → SPAGHETTI & MEATBALL APPEARANCE
      ↳ scales + Wood lamp → YELLOW FLUORESCENCE
      ↳ scales + SDA & olive oil overlay [Lipophilic fungus] → FRIED EGG COLONIES

TREATMENT

→ Topical application of
   1. Selenium sulphide for 10 min daily for 7 days for 1 year
   2. miconazole, ketoconazole

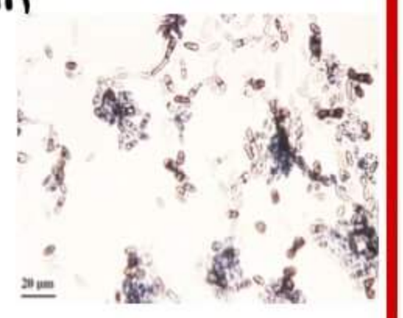
2 TINEA NIGRA

→ Superficial, chronic & asymptomatic infection of stratum corneum

→ caused by Cladosporum wernickii or Exophila Wernikii

→ Saprophyte & dimatiaceous [pigmented hyphae]

→ microscopically → Budding yeasts & chains of yeast cells



→ LESIONS
   ↳ solitary, innocuous maculae ĩ brownish color at the advancing periphery
   ↳ found in palms, face & fingers

→ DIAGNOSIS
   ↳ by direct KOH & culture of skin scrapings from periphery of the lesion

→ TREATMENT
   ↳ topical keratolytic solutions of SULPHUR SALICYLIC ACID or TINCTURE of IODINE

3. PIEDRA

→ Fungal infection of hair by appearance of firm, irregular nodules along the hair shaft

→ BLACK PIEDRA → Piedra hortae
WHITE PIEDRA → Trichosporon beigelii

→ TREATMENT
↳ Removal of hair & application of topical antifungal agent

White piedra

Black piedra

DERMATOPHYTES / CUTANEOUS INFECTIONS

→ Group of filamentous fungi that infect only superficial keratinized tissues - skin, hair & nails
→ Clinical condition → Tinea or ringworm
→ They secrete : Keratinases, proteolytic enzyme that digest keratin of hair, nails & epidermis
→ attack rate is higher in institutions & overcrowded population

CLASSIFICATION

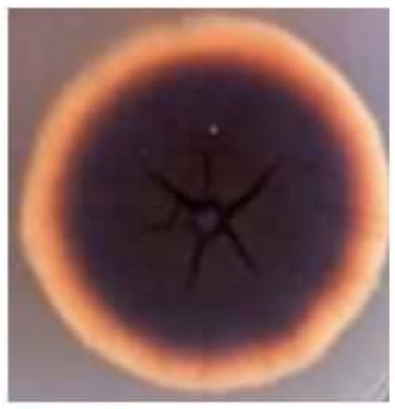
3 GENERA

1. Trichophyton → Skin, hair, nails
2. microsporum → Skin, hair
3. Epidermophyton → Skin, nails

MORPHOLOGY → Identification by colonical appearance & microscopy

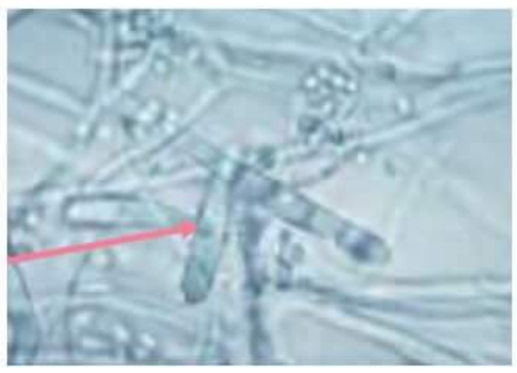
TRICHOPHYTON

→ cylindrical / Pencil shaped, smooth walled macro conidia & microconidia



T. rubrum

microconidia



macroconidia

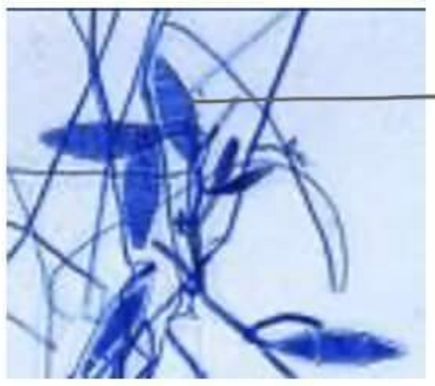
TRICHOPHYTON MENTAGROPHYTES

TINEA VIOLACEUM

MICROSPORUM

→ multicellular macroconidia [Spindle shaped] ī rough walls & few microconidia

M. gypseum

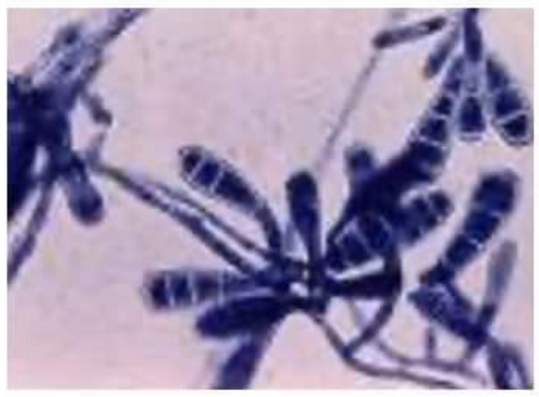


→ Spindle shaped macroconidia

M. canis

EPIDERMOPHYTON

→ only macroconidia which are smooth walled & clavate / club shaped / Boat shaped



PATHOGENESIS & IMMUNITY

→ By contact ī dermatophytes in soil, animals or by direct contact or fomites

→ Host susceptibility increased by : Moisture, warmth, genetic predisposition & Skin composition of Sebum

EPIDEMIOLOGY

→ Anthropophilic
→ zoophilic
→ Geophilic

CLINICAL FEATURES

→ Erroneously termed : Ringworm or tinea because of raised circular lesion

Majocchi's Granuloma

TINEA CORPORIS

→ Lesions an annular, sharply marginated ī raised border

→ This pustular well circumscribed, elevated, crusted lesions is called as MAJOCCHI'S GRANULOMA

→ caused by → T. rubrum

TINEA CAPITIS

→ Injection of the shaft of hair of scalp & may lead to alopecia & scarring

INFLAMMATORY → KERION, FAVUS

a. KERION (honey comb)

→ painful boggy swelling over the scalp

→ painful severe form of inflammatory reaction

→ caused by Trichophyton verrucosum, T. mentagrophytes

b. FAVUS

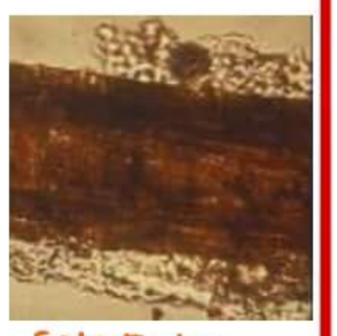
→ dense crust formation on scalp

→ caused by Trichophyton Schoenleinii

ECTOTHRIX

→ sheaths of spores around the shaft

→ caused by microsporum canis, M. audonii



Ectothrix

ENDOTHRIX

→ Arthroconidia within the hair shaft

→ caused by

T → Trichophyton tonsurans

V → Trichophyton Violacaeum

S → Trichophyton Schoenleinii

Endothrix

LAB DIAGNOSIS

→ SKIN SCRAPINGS, NAIL CLIPPINGS, INFECTED HAIR → ⊕ 10% KOH → FUNGAL ELEMENTS OBSERVED

→ DIRECT EXAMINATION by WOOD'S LAMP

→ Tinea capitis → infected hair exhibits greenish fluorescence

TINEA BARBAE [BARBER'S ITCH] → infection of beard area of the face

TINEA CRURIS | DHOBI ITCH | JOCK ITCH

→ infection of groin seen in men

→ involves perineum, scrotum, perianal regions

TINEA MANUUM

→ infection of skin of hands

→ diffuse keratotis of palms & fingers by E. floccosum, T. rubrum, T. mentagrophytes

TINEA UNGUIUM → infection of nail plates at the free edge of the nail

TINEA PEDIS [ATHLETE'S FOOT]

→ Toe web scaling fissuring, maceration

→ also known as sandal ringworm

T. Barbae

T. cruris

T. mannum

T. unguium

T. Pedis

Id REACTION

→ Allergic response to fungal antigens

→ Infection in feet elicits the allergic reaction on the hands [always away from site]

→ Ex: Tinea pedis elicits allergic reaction on the hands

LAB DIAGNOSIS

A. SKIN SCRAPINGS
NAIL CLIPPINGS
INFECTED HAIR
→ ⊕ 10% KOH → FUNGAL ELEMENTS OBSERVED

B. Skin scrapings, infected nails, hairs + SDA | DIM [dermatophyte Identificat° media] → culture growth [may take 2-3 days | weeks | months]

C. Culture Growth + LPCB & Observe

	Macro conidia	Micro conidia
Trichophyton	→ cylindrical / pencil shape	→ numerous
Microsporum	→ spindle shape	→ less
Epidermophyton	→ club / boat shape	→ absent

SUB CUTANEOUS MYCOSIS

→ Involves Skin & Sub cutaneous tissues

→ Eg: M → Mycetoma
R → Rhinosporidiosis
C → Chromoblastomycosis
S → Sporotrichosis [dimorphic]

MYCETOMA / MADURA FOOT

ACTINOMYCETOMA	EUMYCETOMA
→ Bacterial	→ Fungal
→ numerous sinus & swelling	→ Single sinus & swelling
→ Purulent discharge	→ serous discharge
→ White / yellow granules • Actinomadura madurae • Nocardia caviae • Nocardia asteroides • Nocardia brasilensis	→ Black granules • Madurella grisea • Madurella mycetomatis
→ Red granules • Actinomadurae pelletri	→ White granules • Acremonium • Pseudoallescheria boydii
→ Crush granules & see under microscope → <2μ bacilli	→ Crush granules & see under microscope → >2μ hyphae/ >2μ Fungal colonies
→ On x ray → osteolytic lesions	→ On x ray → Osteosclerotic lesions
→ Responds well to antibiotics	→ Responds very poorly to antifungals [amputatⁿ may required]

→ BOTRYOMYCOSIS → mycetoma like lesion
→ mainly caused by Staph. aureus

RHINOSPORIDIOSIS

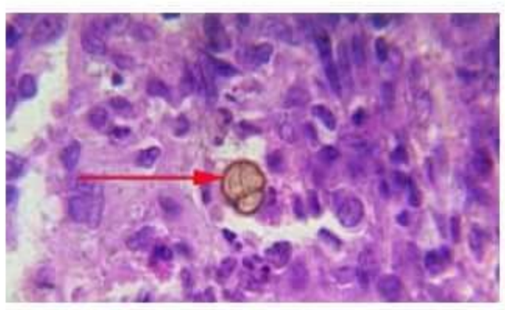
→ caused by Rhinosporidium seeberi [AQUATIC PROTOZOA]
→ presents ↄ B/L Nasal polyps [mc presentatⁿ]
→ POLYP BIOPSY → shows SPHERULE ↄ endospores
→ Rx → Sx Polypectomy [Best]
→ Non cultured on SDA

SPHERULE ↄ endospores

CHROMOBLASTOMYCOSIS | DERMATIACEOUS FUNGUS | PIGMENTED FUNGUS

→ involves Skin & Subcutaneous tissues
→ THORN PRICK / WOOD PRICK
↓
VERRUCCOUS LESION / WARTY LESION

→ BIOPSY OF WARTY LESION + H&E SHOWS
→ Brown colored globose bodies
→ SCLEROTIC BODIES / MEDLAR BODIES
→ show COPPER PENNY APPEARANCE

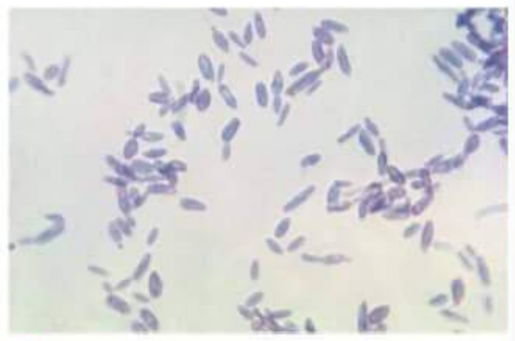


MEDLAR/SCLEROTIC BODY

→ Examples →
1. Alternaria
2. Bipolaris
3. Curvularia
4. Phialophora
5. Cladosporium
6. Exophilia

SPOROTRICHOSIS | ROSE GARDENER'S DISEASE

→ caused by Sporothrix schenkii
→ spread by thorn prick [Gardener's disease]
→ ↑ed incidence in & around valentine day
→ lympho cutaneous spread is seen
→ Dimorphic fungus [at 22-25°C - moulds, at 37°C - yeast]
  → mold → Flower like sporulation
  → yeast → Cigar shaped yeast cells or
  → narrow based budding yeast cells


Sporothrix schenkii

→ ASTEROID BODIES are seen
  → Basophilic material is surrounded by Eosinophilic material →
  SPLENDORE - HOEPELLI PHENOMENON

DEEP MYCOSIS

All are Dimorphic Fungus Except Sporotrichosis

HISTOPLASMOSIS [DARLING'S DISEASE]

→ Systemic, granulomatous, intracellular infection
→ Affects reticuloendothelial system
→ caused by Histoplasma capsulatum [Dimorphic fungi]
→ Source → Soil droppings from pigeon droppings & bat droppings
→ MODE OF TRANSMISSION → Aerosol transmission
→ IP = 10 - 16 days
→ Lung involvement → Blood → viscera

CLINICAL FEATURES

1. PULMONARY DISEASE

→ Acute form
  → influenza like disease
  → resolves within 2-3 weeks
  → shows lymphocyte rich infiltrate

→ Chronic form
  → haemoptysis
  → apical cavities
  → calcification
  } resembles TB

2. CUTANEOUS & SUB CUTANEOUS DISEASE → Petechial lesions

3. DISSEMINATED DISEASE
  → Anemia
  → Leucopenia
  → Hepatosplenomegaly
  → multiple lymphadenopathy

LAB DIAGNOSIS

SAMPLES
1. Sputum
2. Sternal Bone marrow
3. Lymph node

MICROSCOPY

→ Giemsa stain

→ Gram's stain

→ We find - intracellular, oval, budding yeast cells

CULTURE

→ SDA ī Actidione

↳ White cottony mycelial growth ī tuberculate spores/macroconidia [MOLD]

→ Potato dextrose agar

SEROLOGY

→ Immunodiffusion

→ Latex agglutination

→ complement fixation test

→ Histoplasmin skin test - DTH [delayed type hypersensitivity]

MOLD → TUBERCULATE MACROCONIDIA

YEAST → NARROW BASED BUDDING OF YEAST CELL

BLASTOMYCOSIS | NORTH AMERICAN BLASTOMYCOSIS | CHICAGO | GILCHRIST DISEASE

→ caused by Blastomyces dermatitidis

→ infection by inhalation of conidia

→ Pyo - granulomatous fungal infection of respiratory tract

→ Dissemination to skin, bone & genito urinary system

CLINICAL FEATURES

1. CUTANEOUS BLASTOMYCOSIS

→ commonest form

→ trauma

→ Papule → secondary nodules → coalesce to form ulcers

2. PULMONARY BLASTOMYCOSIS

→ resemble Pulmonary TB, carcinoma

3. DISSEMINATED BLASTOMYCOSIS

→ Hematogenous spread

→ Bones

→ Genito urinary tract

LAB DIAGNOSIS

SAMPLES

→ Pus

→ Sputum

→ Tissues

→ Bronchoalveolar lavage [BAL]

1. MICROSCOPY

→ Gms staining → multiple budding , calcofluor white

2. CULTURE

SDA at 25°C

→ Fluffy white to tan colored

→ Fine branched filaments ī conidia on terminals

On BHI at 37°C

→ waxy & heaped mass

YEAST FORM → Broad Based budding yeast cell

[at 37°C] → Figure of 8 appearance

COCCIDIOIDOMYCOSIS

→ caused by coccidioides immitis

→ Pathogenic dimorphic fungi

→ In culture, it produces arthroconidia within the mycelia at 25°C

→ In tissue, it produces Spherules ī endospores at 37°C

→ Found in soil

↓

Grows as a mold under dry conditions

↓

Arthroconidia break out of mycelia

↓

Become airborne

→ Incubation Period → 1 - 4 WEEKS

CLINICAL FEATURES

1. PULMONARY INFECTION : Primary type

→ San Joaquin valley fever or Desert Rheumatism

→ dlt Inhalation of conidiae

→ Acute influenza - like Self limiting illness

→ Bronchopneumonia happens rarely

2. PRIMARY CUTANEOUS TYPE

→ Nodule ⟶ ulceration [DD - syphilitic, TB ulcer]

→ Lymphangitis

→ Lymphadenopathy

3. DISSEMINATED TYPE → rare

→ Fatal if occurs [granulomas in internal organs]

→ meninges, bones, joints are involved

LAB DIAGNOSIS

SAMPLES

- → Skin scrapings
- → Sputum
- → Bronchial washings
- → CSF
- → Pleural fluid & Blood etc

1. DIRECT MICROSCOPY OF SKIN SCRAPINGS
   - → 10% KOH & Parker ink
   - → Sporangia of coccidioides immitis
   - → It is diagnostic

- → TISSUE SECTION → Spherules of coccidioides Immitis ī endospores

2. CULTURE
   - → SDA [at 25°c]
   - → Brain Heart Infusion agar ī 5% Sheep Blood
     - → colonies are initially moist & glabrous but rapidly becomes greyish white in color ī a tan reverse

MICROSCOPY MORPHOLOGY
- → Single celled, Hyaline
- → rectangular to barrel shaped, alternate arthroconidia

At 25°C → Arthroconidial arrangement [MOLD]
At 37°C → Spherule ī endospore [Yeast]

3. SKIN TEST → coccidioidin
4. SEROLOGICAL TESTS
   - → Immunodiffusion test
   - → complement fixation test

PARACOCCIDIOIDOMYCOSIS | SOUTH AMERICAN DISEASE | LUTZ MYCOSES
- → caused by Paracoccidioides brasiliensis
- → dimorphic fungi
- → Common in Coffee growing belt
- → Favourable conditions
  - → ↑ humidity
  - → ↓ temperature [23°c]

CLINICAL FEATURES

1. MUCOCUTANEOUS FORMS → ulcerated lesion
2. LYMPHATIC FORM → cervical lymphadenopathy, Bull neck appearance
3. VISCERAL FORM
   - → Spreads by lymphatic or hematogenous route
   - → Lung (90% of cases)
   - → CNS, Bones
   - → BIL patchy pneumonia ī nodular miliary shadows

LAB DIAGNOSIS

SAMPLES

- → Sputum
- → Bronchoalveolar lavage
- → Pus

1. MICROSCOPY → multipolar budding yeast cells
2. CULTURE
   - → YEAST → mickey mouse appearance / mariner's wheel appearance / Pilot wheel appearance
   - → MOLD → mycelial form

3. SEROLOGY
   - → Antibody detection
   - → Latex agglutination test
   - → complement fixation test
   - → Immunodiffusion

4. RADIODIAGNOSIS
   - → CT Scan
   - → MRI

Fungus	In vitro (25°C)	In vivo (37°C)
Blastomyces	Mold	Yeast
Coccidioides	Mold	Spherule
Histoplasma	Mold	Yeast
Paracoccidioides	Mold	Yeast
Sporothrix	Mold	Yeast

CANDIDA [YEAST LIKE]

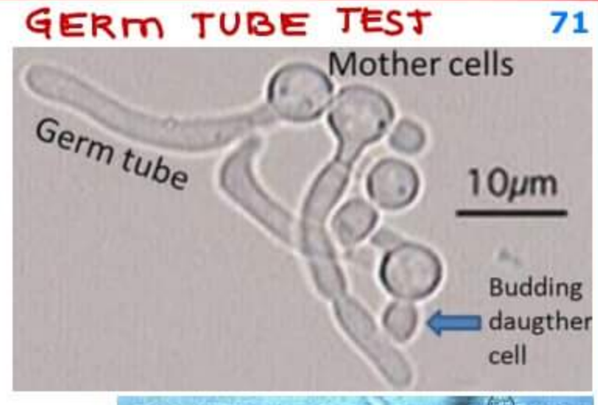
CANDIDA ALBICANS

→ Shows

① REYNOLD BRAUDE PHENOMENON [Germ tube formation]

② CHLAMYDOSPORE FORMATⁿ ON CORN MEAL AGAR

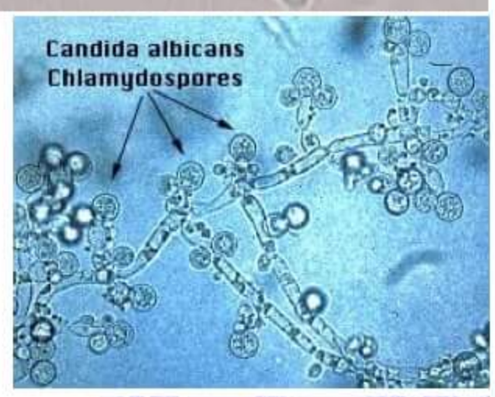
GERM TUBE TEST 71



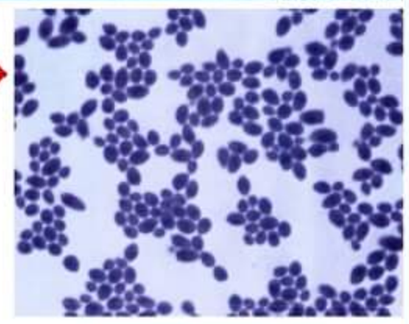
→ REYNOLD BRAUDE PHENOMENON [Cx growth + human plasma]

→ shows germ tube formation [GERM TUBE TEST]

→ on corn meal agar (nutrient deficient medium), Chlamydospores are formed [at 20°C]



candida budding →



NON - CANDIDA ALBICANS [NCA]

→ C. krusei → naturally resistant to Azoles

→ C. kefyr

→ C. glabrata

→ C. stellatoidea

→ C. guilliermondii

→ C. tropicalis

→ C. vishwanathi

→ C. aureus → caused worldwide infections mostly in immunodeficient

oral thrush

CANDIDA ALBICANS - DISEASES

① ORAL THRUSH

② OESOPHAGEAL CANDIDIASIS

③ VAGINAL CANDIDIASIS

④ CANDIDAEMIA [candida yeast cells in blood]

⑤ CANDIDURIA [candida yeast cells in urine]

⑥ ONYCHOMYCOSIS [nail folds are involved]

⑦ UTI / RTI

⑧ MENINGITIS

CANDIDA INFECTIONS ARE COMMON IN

→ neutropenic individuals

→ CMI ↓↓↓

- AIDS
- malignancies
- chronic muco cutaneous candidiasis [T-cell disorder]

LAB DIAGNOSIS

→ Tissue Biopsy ; samples

→ Gram stain → yeast cells are seen

→ Cx on SDA → creamy, pasty colonies

→ Chromagar method → colourful agar

→ used for CA / NCA identification

chromagar

→ SUGAR FERMENTATION METHODS

→ β GLUCAN TESTING FOR INVASIVE CANDIDIASIS

→ PCR for candida species

on SDA

CRYPTOCOCCUS NEOFORMANS

→ present in pigeon faeces → Aerosol transmission

→ LUNG INVOLVEMENT

↓ Haematogenous spread

MENINGES INVOLVED [meningitis]

} NEUROTROPISM [for HIV +ve individuals]

→ mc cause of Meningitis in HIV +ve → Cryptococcus neoformans

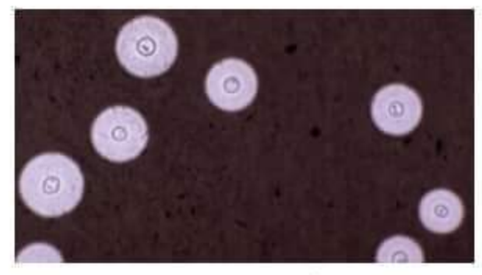
LAB DIAGNOSIS

① CSF SAMPLE

ⓐ Indian Ink staining

→ negative staining

→ back ground is dark



Indian Ink Staining

ⓑ culture

→ on SDA → mucoid, smooth

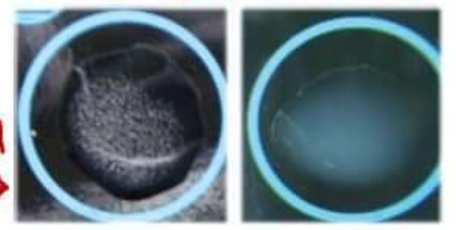
→ on Niger seed agar → Blackish Brownish colonies

Cryptococcus on SDA

② LATEX CARD AGGLUTINATION TEST for Cryptococcal antigens

③ PCR for Cryptococcus neoformans

Latex card test →



→ DOC for Cryptococcal meningitis → AMPHOTERICIN B + FLUCYTOSINE

ZYGOMYCETES

→ Rhizopus

→ mucor

→ Absidia

Urea hydrolysis test [+ive] for Cryptococcus

RHINO CEREBRAL ZYGOMYCOSIS

→ caused by Rhizopus species

→ fulminant disease

→ Tissue Biopsy + H&E staining → Aseptate hyphae Obtuse angled

Tissue Biopsy + SDA

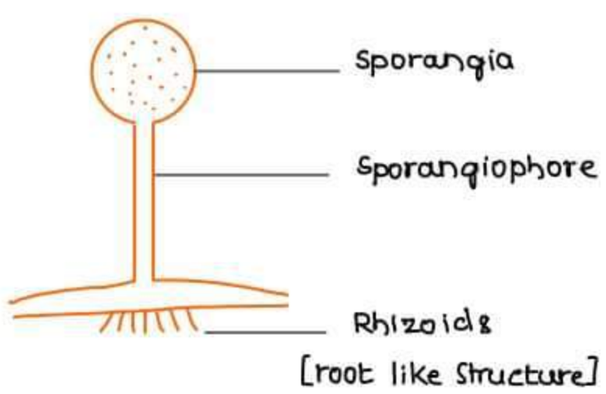
↓

culture growth

↓

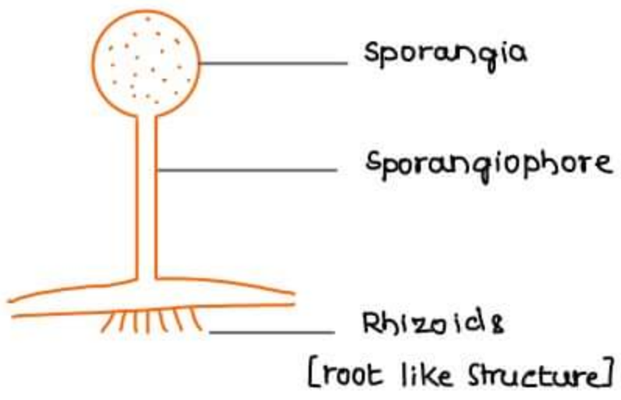
LPCB mount [Lactophenol cotton blue mount]

↓

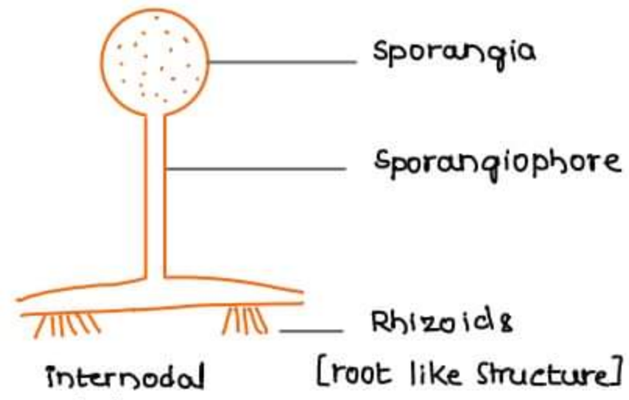


PrepLadder

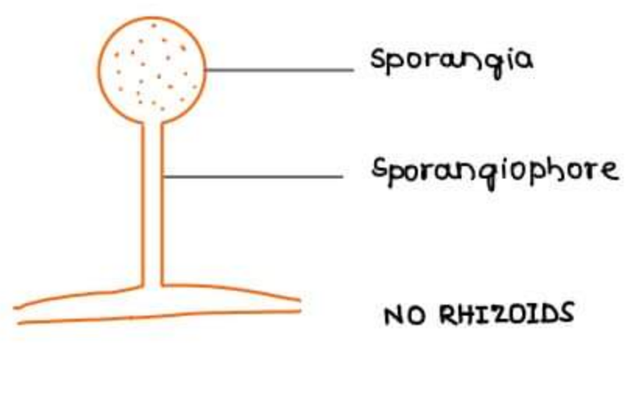
DIFFERENT ZYGOMYCETES



RHIZOPUS



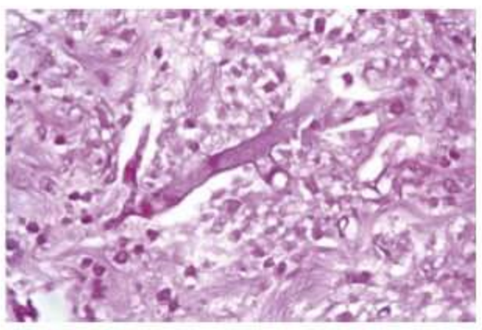
ABSIDIA



MUCOR

MUCOR MYCOSIS

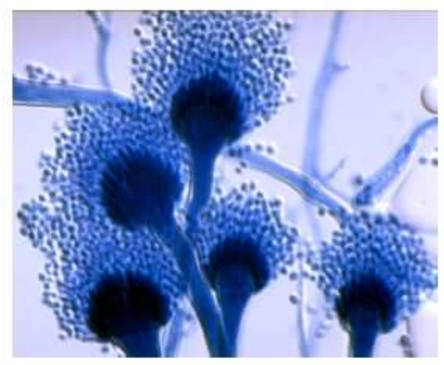
→ Predisposes in DM
Frequent blood transfusions
chronic dialysis



mucor

ASPERGILLUS

A. Fumigatus → causes ABPA (Allergic Broncho Pulmonary Aspergillosis)
Aspergilloma (fungal ball)
Oculomycosis
Otomycosis



Aspergillus

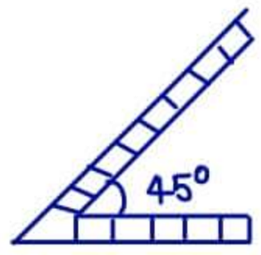
A. niger → causes Otomycosis
Blackish crust is seen

A. clavatus → causes Maltster's Lung
common in Barley / malt factory workers
occupational disease

A. flavus → produces Aflatoxin
Aflatoxin a/w HCC (Hepato cellular Carcinoma)

LAB DIAGNOSIS

Tissue Biopsy + H & E Staining → Septate hyphae
dichotomous branching
acute angled
(mostly 45°)



ERGOTOXICOSIS [ERGOT POISONING]

→ Edibles + clavicepS purpurea seeds → Ergotoxicosis
→ consumptⁿ of contaminated edibles leads to
① St. Vitus Dance &
② St. Anthony's fire
→ Gastric irritation occurs
↓
Restlessness [St. vitus Dance]

PNEUMOCYSTIS CARNII / PNEUMOCYSTIS JIROVECII

→ Protozoa → cyst / Trophozoite + nt
Fungus → chromosomal studies favours fungus

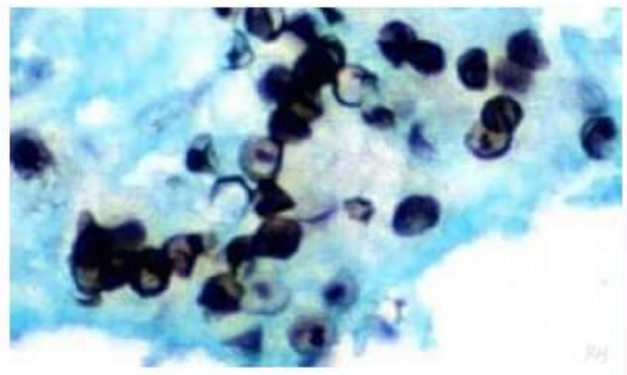
→ Pneumocystis carnii pneumonia
- occurs in persons ↓ CD_4 → < 200 [HIV +ve]
- DOC → COTRIMOXAZOLE
- LAB DIAGNOSIS
  ① BAL [Broncho Alveolar Lavage] Fluid
  ② Gastric washings (children)

↓

GMS [Gomorii Methanamine Silver]

↓

Trophozoites → Green
Cysts → Black



Pneumocystis carinii
Cyst

→ Non cultured on SDA

OCCUPATIONAL DISEASES

FARMER'S LUNG

→ dlt Hay dust
caused by Micromonospora / micropolyspora faenii, faenia rectivirgula
Thermo actinomyces vulgaris

→ Type III hypersensitivity

BAGGASSOSIS

→ dlt sugarcane dust
caused by Thermo actinomyces Sacchari

MALTSTER'S LUNG

→ dlt Barley & malt → caused by Aspergillus clavatus

CHEESE WASHER'S LUNG

→ a/w cheese factor workers
→ caused by Penicillium caseocolum

MAPLE BARK STRIPPER'S LUNG

→ dlt maple tree bark
→ caused by cryptostroma corticale

SEQUOIOSIS

→ dlt redwood saw dust
→ caused by aureobasidium pullulans

SUBEROSIS

→ dlt cork

→ caused by Penicillium frequentans

WOOD PULP WORKERS

→ dlt wood pulp

→ caused by alternering species

HUMIDIFIER'S LUNG

→ dlt humidifier

→ caused by T. vulgaris , T. candidus

PROTOZOA

Classification

I AMOEBAE	II CILIATES
a Free living – Naegleria fowleri – Acanthmoeba balamuthia b Intestinal – Entamoeba histolytica – E. coli	Balantidium coli
III FLAGELLATES	**IV SPOROZOA**
a Intestinal – Giardia lamblia b Vaginal – Trichomonas vaginalis c Blood & Tissue – Leishmania – Trypanosoma	a Blood Species – Plasmodium – Babesia b Tissue species – Toxoplasma c Intestinal species – Isospora – cyclospora

Entamoeba hystolytica

QUADRI NUCLEATE CYST [Infective form]

↓

ingested ī contaminated food & water

↓

Bypasses gastric acidity

↓

Excystation in small intestine | large intestines

↓

TROPHOZOITES

- active feeding & growing stage
- 8 Metacystic trophozoites are formed

↓

favourable conditions

① 85% ASYMPTOMATIC

② 5-15% INTESTINAL AMOEBIASIS

③ EIA [Extra Intestinal Amoebiasis]

unfavourable conditions

↓ Encystation

Uninucleate cyst

↓ Binary fission

Binucleate cyst

↓ Binary fission

Quadrinucleate cyst (Faeces)

Favourable conditions

③ EIA [EXTRA INESTINAL AMOEBIASIS]

↓

Trophozoites Secretes cysteine proteinase

↓

Erode intestinal layers

↓

FLASK SHAPED ULCERS

↓ via portal circulath

Liver [Extra Intestinal Amoebiasis]

→ mc site for EIA → Liver > Lungs > Brain
→ chocolate pus / Ancovy sauce pus formed due to damage to hepatocytes
→ Right lobe, posterio superior portion is involved
→ IOC for EIA → ELISA

INTESTINAL AMOEBIASIS

LAB DIAGNOSIS

① STOOL EXAMINATION

ⓐ Saline mount for trophozoite

ⓑ Iodine mount for cysts

ⓐ Stool sample + Normal Saline → Trophozoites seen under microscope
→ motile by pseudopodia
→ Eccentric nucleus ī central karyosome is present
→ ERYTHROPHAGOCYTOSIS seen
→ Engulfed RBCs
→ seen in fresh stool samples
→ characteristic feature of E. hystolitica

ⓑ Stool sample + Iodine mount → cyst is seen under microscope
→ 4 nuclei cyst (mostly)
→ uninucleate or binucleate cysts ī chromatoidal bars & Glycogen
→ vacuoles also seen some time

② CULTURE

media used are

P → PHILIPS MEDIA
C → CRAIGE'S MEDIA
R → ROBINSON MEDIA

③ PCR for Entamoeba hystolytica

NAEGLARIA FOWLERI [Brain Eating Amoeba]

→ Infective form → Amoeboid form ⇌ Trophozoite form

Amoeboid form > Trophozoite form

↓

Enters Nares

↓

Erodes cribriform plate & enters into brain

↓

PAM [Primary Amoebic Meningoencephalitis]

→ H/O swimming in pond + nt

LAB DIAGNOSIS

CSF EXAMINATION

→ Trophozoites are observed

→ Neutrophiles are observed

ACANTHAMOEBA SPECIES

→ causes contact lens Keratitis

→ Aerosal route

↓

Lungs

↓ hematogenous spread

Brain

↓

Granulomatous Encephalitis

Even through optic route can occur

LAB DIAGNOSIS

CSF EXAMINATION

→ cysts & trophozoites are seen

→ lymphocytes are seen

FREE LIVING AMOEBAE	
Naegleria fowleri Acanthamoeba Balamuthia	} causes Encephalitis

INTESTINAL FLAGELLATES

GIARDIA LAMBLIA

→ Quadri nucleate cyst [Infective form]

↓

ingested ī contaminated food & water

↓

Bypass gastric acidity

↓

Excystat^{n} mostly in duodenum

↓

Trophozoites attach to intestinal surfaces

↓

Malabsorption syndrome

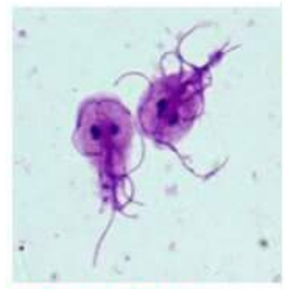
Steatorrhoea

Fat in stools

Bulky stools

TROPHOZOITE

→ TENNIS RAQUET APPEARANCE or
MONKEY FACE APPEARANCE or
TEAR DROP APPEARANCE



GIARDIA TROPHOZOITE

→ Axostyles are +nt
4 pairs of flagella → FALLING LEAF LIKE MOTILITY
Sucking disc → helps in adherence to intestine (virulence factor)

CYST

→ 4 nuclei +nt
→ have Axostyle

GIARDIA CYST

→ ENTERO TEST / STRING TEST done to demonstrates trophozoites
→ Giardia lamblia infect^{n} a/w CVID [common variable Immuno Deficiency]

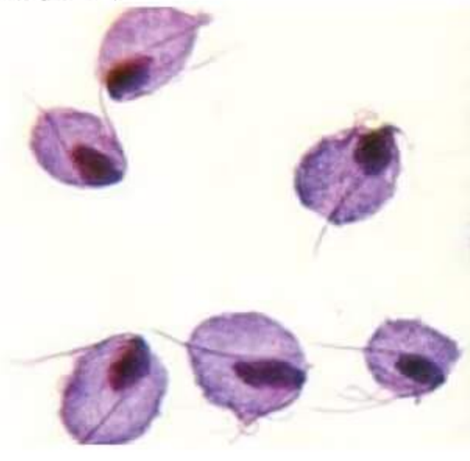
VAGINAL FLAGELLATE

TRICHOMONAS VAGINALIS

→ No cystic stage
→ only Trophozoite stage is present
– 5 pairs of Flagella +nt → TWITCHING MOTILITY

→ causes TRICHOMONIASIS

FEATURES
→ pH > 4.5
→ Greenish frothy discharge
→ Colpitis macularis / strawberry vagina



TRICHOMONAS VAGINALIS TROPHOZOITE

LAB DIAGNOSIS

① URINE EXAMINATIONS → motile trophozoites are found

② CULTURE → on BUSHLEY'S JOHNSON & TRUSSEL MEDIA

→ DOC → METROGYL to both partners

HAEMO FLAGELLATES

① LEISHMANIA → Promastigote [infective form]

② TRYPANOSOMA → Metacyclic trypomastigote [infective form]

LEISHMANIA

→ L. donovani → causes Kala Azar (Phlebotomous (Sandfly) transmits)

→ L. tropica → causes Oriental sore / Delhi or Baghdad boil / Aleppo button

→ L. brasilensis → causes Espundia [muco cutaneous leishmaniasis]

KALA AZAR

→ hyper pigmentation + Fever + hepatosplenomegaly seen

→ KA & HIV +ve → hepatosplenomegaly absent (atypical presentatn)

→ Anemia, thrombocytopenia, Leucopenia seen

→ Hypergammaglobunemia seen

→ LAB DIAGNOSIS

① BLOOD SMEAR

→ A → Anemia
L → Leucopenia
T → Thrombocytopenia
} demonstrated

→ Hypergammaglobunemia demonstrated by

→ NAPIER'S ALDEHYDE TEST

→ CHOPRA'S ANTIMONY TEST

② SPLEENIC BIOPSY vs BONE MARROW BIOPSY

Spleenic Biopsy is more sensitive

more Amastigote forms are observed

AFlagellar forms

aka LD BODIES [LEISHMAN DONOVAN BODIES]

③ rK-39 ANTIGEN TESTING using CARD METHOD

④ MONTENEGRO TEST [skin Testing]

⑤ CULTURE on NNN [Novy Neil Nicholle] media

– Promastigote forms (Flagellar forms) demonstrated

⑥ SEROLOGICAL TESTS are available

⑦ PCR for L. donovani is available

→ DOC → AMPHOTERICIN B

KALA AZAR

LD BODY

NNN Media

PROMASTIGOTES

PKDL [POST KALA AZAR DERMAL LEISHMANIASIS]

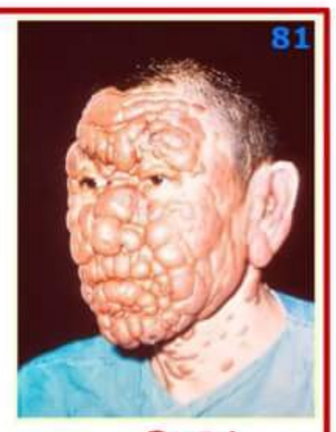
↓

After 2 yrs of successful Rx

↓

Suddenly develops crops of nodules / macules over the skin

→ dlt dermotropic conversion



PKDL

TRYPANOSOMA BRUCEI

→ Gambiense → causes West African Sleeping Sickness

Winter bottom sign seen (B/L cervical lymphadenopathy)

→ Rhodesiense → East African Sleeping sickness

→ TSE TSE FLY [Glossina Species] transmits Sleeping Sickness

→ AFRICAN TRYPANOSOMIASIS = sleeping sickness

T. CRUZI

→ transmitted by Reduvid bug

→ causes CHAGAS DISEASE / AMERICAN TRYPANOSOMIASIS

FEATURES

→ CHAGOMA → entry site edema

→ ROMANA SIGN → eye lid swelling

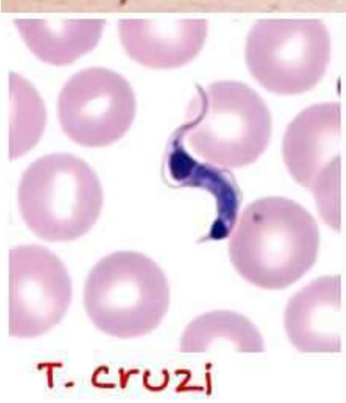
→ M → Mega oesophagus

→ M → Mega colon

→ M → Myocarditis

→ M → Meningo encephalitis

REDUVID BUG



T. cruzi

COCCIDIAN PARASITES

	Oocyst size
Cryptosporidium parvum	4-6 μ
Cyclospora	8-10 μ
Isospora belli	23-36 μ

cryptosporidium parvum oocyst

→ All are Acid fast

→ If Stool Examinatn shows coccidian parasites

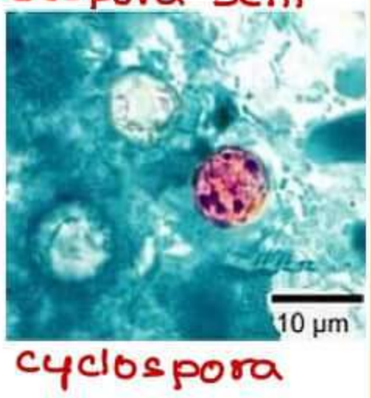
↓

Check HIV Status

+ ive → Admit
- IV Fluids
- Antibiotics

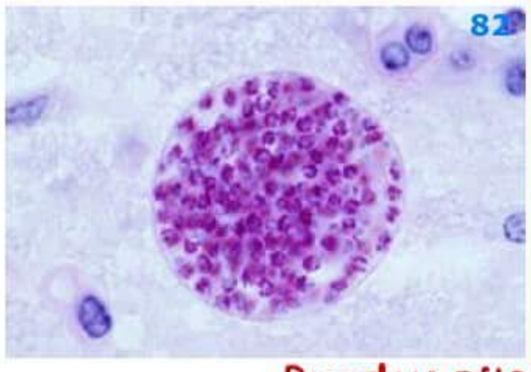
- ive → Self limiting diarrhoea

isospora belli



cyclospora

TOXOPLASMA GONDII



Bradyzoite

→ causes TOXOPLASMOSIS

→ Definite Host → cat
Intermediate Host → Rat / man

→ cat → Enteric cyclic occurs → oocyst formed
Rat / Man → Exoenteric cyclic occurs → No oocyst formatⁿ

→ Infective forms
① Sporulated oocyst
② Tachyzoites
③ Bradyzoites


Tachyzoites

→ LAB DIAGNOSIS

① BLOOD SMEAR +GEIMSASTAIN	② TISSUE SMEAR +GEIMSASTAIN
Tachyzoites – rapidly dividing forms – sickle shaped	Bradyzoites / Tissue cysts – Slowly dividing forms

② SABIN - FELDMAN DYE TEST → Compliment fixation test
③ PCR for Toxoplasma gondii
④ ELISA in a pregnant woman ∴ Toxoplasmosis
→ if she have IgM antibodies → cause for concern
→ if she have IgG antibodies → no cause for concern

→ Toxoplasmosis in Ⓝ individual → mild cervical lymphadepathy
→ Toxoplasmosis in immunodeficiency (HIV+ve) → Seizures
→ Toxoplasmosis in pregnancy at 1st Trimester → max damage to fetus
at 3rd Trimester max chance of transmisⁿ

PLASMODIUM SPECIES

→ P. vivax
→ P. ovale
→ P. Falciparum
→ P. malariae
→ P. knowlesi

LIFE CYCLE

Anopheles

SPOROZOITES

↓

Bite of female anopheles

↓

Sporozoites move towards liver

d/t presence of circumsporozoite Proteins in liver

↓

Pre Erythrocytic Schizogony

↓

Merozoites

↓

some change to HYPNOZOITES

- → resting forms
- → resides in liver
- → responsible for relapse
- → seen in P. vivax, P. ovale
- → DOC → PRIMAQUINE

some capture RBC

↓

Erythrocytic schizogony

↓

Trophozoites

↓

Schizont

↓

Merozoites

↓

Gametocytes

(micro / macro)

↓

Female Anopheles bites man & take Blood meal

↓

mature gametes

Female + Male

↓

Zygote

↓

Ookinete

- needle like structure
- penetrate gut of mosquito

↓

Oocyst

↓

moves to salivary glands

↓

sporozoites

↓

Probosis of mosquito

CONDITIONS PREVENTING P. falciparum infection

G → G6PDeficiency
S → Sickle cell anemia
T → Thalassemic trait
Excess of fetal Hb

CONDITION PREVENTING P. vivax infectn → Duffy negative RBC

LAB DIAGNOSIS

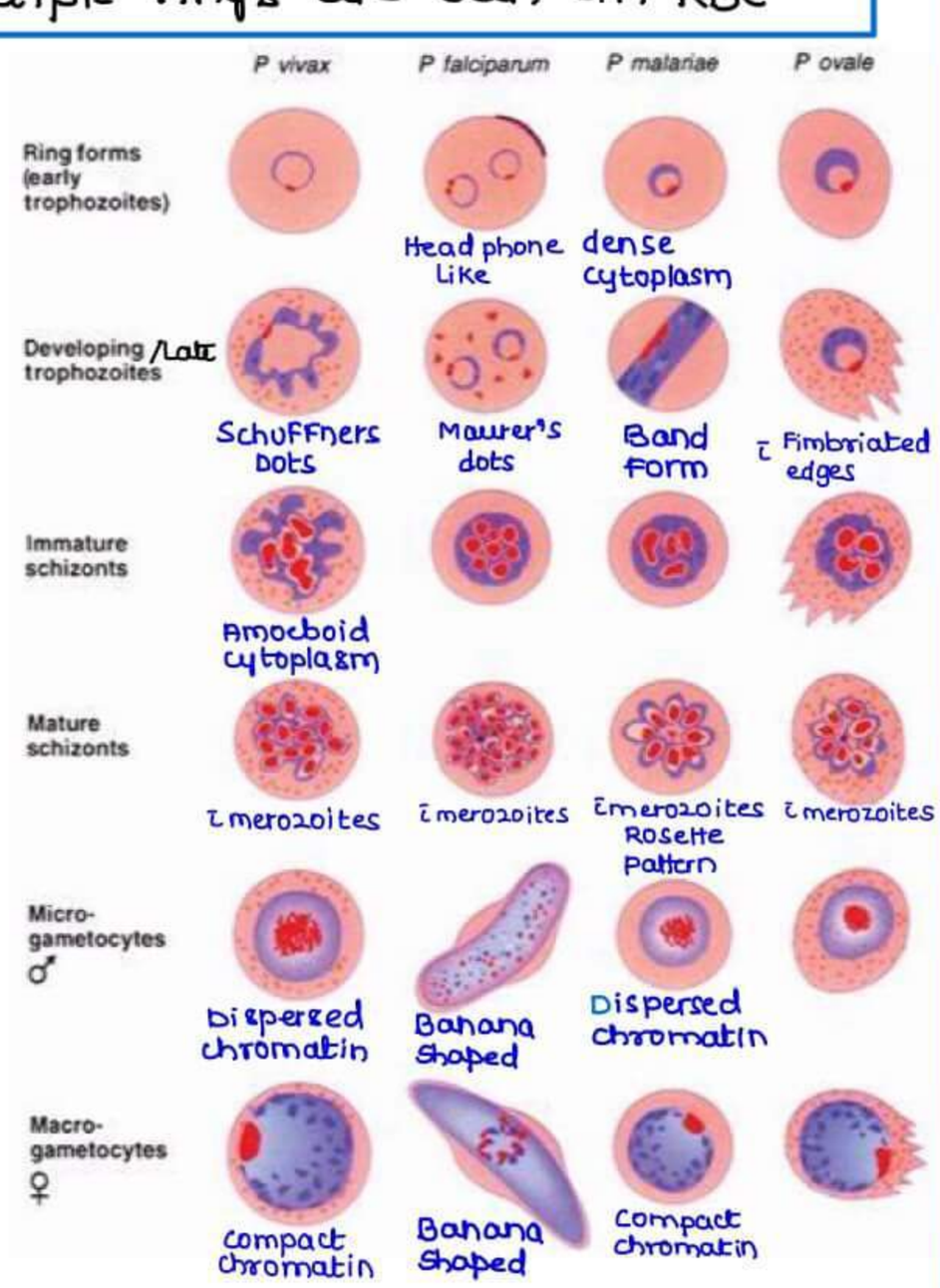
① Blood smear EXAMINATION
   ⓐ Thick Smear → to know prevalence of parasite
   ⓑ Thin Smear → to know speciation

P. vivax	P. falciparum
young RBCs affected	All age RBCs affected
RBC Size ↑ses	Ⓝ Size RBCs
SCHUFFNER'S DOTS seen	MAURER'S DOTS seen
ACCOLE FORMS −nt	ACCOLE FORMS +nt
SCHIZONT STAGE +nt	SCHIZONT STAGE −nt
GAMETOCYTE no proper shape	banana shaped
RELAPSE Seen d/t hypnozoites	RECRUDESCENCE Seen d/t incomplete Rx
Single large ring in RBC	multiple rings are seen in RBC

parasite
RBC

P. malariae
Z → Ziemann's dot
O → Old age RBC
O →
M → Max IP
I
N → Nephrotic syndrome

BAND FORMS → P. MALARIAE



② BUFFY COAT EXAMINATION
→ using Acridine orange & using fluorescent microscopy

③ CULTURE on RPMI - 1640 media

④ MALARIA CARD TEST
→ HRP-2 based Kits [Histidine Rich Protein - HRP] for P. falciparum
→ LDH & Aldolase based Kits for other plasmodium species

Malaria card Test


BUFFY COAT EXAMINATN [P. Falciparam]

⑤ PCR for Malarial Parasites

BABESIA MICROTI

- → Intra erythrocytic protozoa
- → causes BABESIOSIS
- → transmitted by TICK
- → MALTESE CROSS ARRANGEMENT in RBC
- → resembles P. falciparum gametocyte stage

CROSS LIKE APPEARANCE

BALANTIDIUM COLI

- → Ciliate
- → contains
  - Peristome
  - cytostome
  - cytopharynx
  - micro & macro nucleus
- → largest intestinal protozoa
- → Rotatory motility + nt
- → causes Diarrhoeal Episodes
- → DOC → TETRACYCLIN

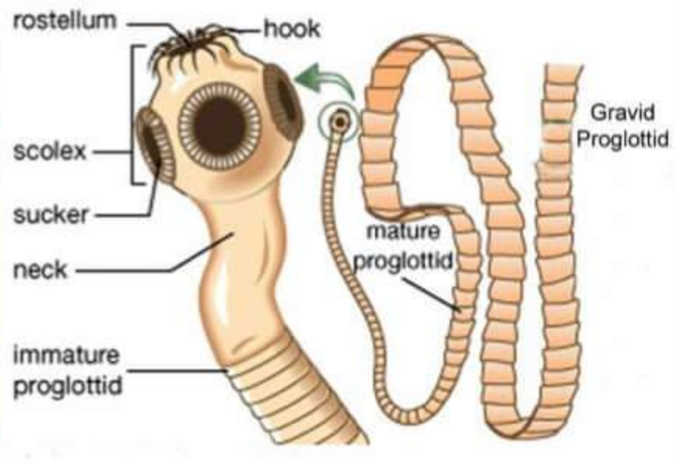
HELMINTHOLOGY

CESTODES	TREMATODES	NEMATODES
→ tape like monoecious	→ leaf like monoecious except Schistosomes (dioecious)	→ cylindrical → dioecious
→ suckers & hooks	→ suckers & hooks	→ have buccal capsule
→ absent alimentary canal	→ poorly developed alimentary canal	→ well developed alimentary canal
→ Body cavity absent	→ Body cavity absent	→ Body cavity present

CESTODES

BASIC STRUCTURE

SCOLEX	NECK	STROBILA
consists of suckers rostellum hooklets		contains proglattids



CLINICALLY IMPORTANT CESTODES

	Definite Host	Intermediate Host
Taenia solium	man	Pig
Taenia saginata	man	Cattle
Echinococcus granulosus	Dog	Sheep / man
H. nana	man	man
Diphyllobothrium latum	man	1st → Cyclops 2nd → Fish

DIPHYLLOBOTHRIUM LATUM / FISH TAPE WORM

Plerocercoid larva [L_3 larvae] [Infective form] present in fish

↓

Man eats infected fish

↓

larvae released

↓

changes to Adult forms

↓

settle up in small & large intestine

↓

↓ Vit B 12 absorption → Megaloblastic Anaemia

↓

Adult forms

↓

produce eggs

↓

Goes into water

↓

hatch into Coracidium larvae [L_1 Larvae]

↓

reaches cyclops & in cyclops it changes to Procercoid [L_2 larvae]

↓

Eaten by Fish

↓

in fish it changes into Plerocercoid larvae [L_3 larvae]

→ Egg of Diphyllobothrium latum → Operculated egg

H. nana / Dwarf Tape worm

Eggs are ingested ī contaminated food & water [infective form]

↓

Hatch up in intestine

↓

Cysticercoid larvae released

↓

attach to the intestinal layers

↓

changes to adult form

↓

produce eggs

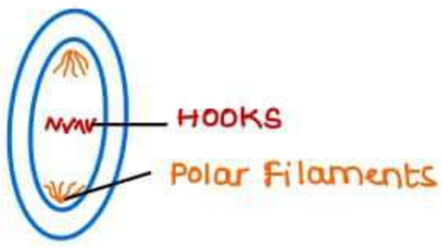
- passed out in faeces
- Hatch ĩn lumen
  ↓
  releases Hexacanth embryo
  ↓
  penetrates back into circulation
  ↓
  Auto infection

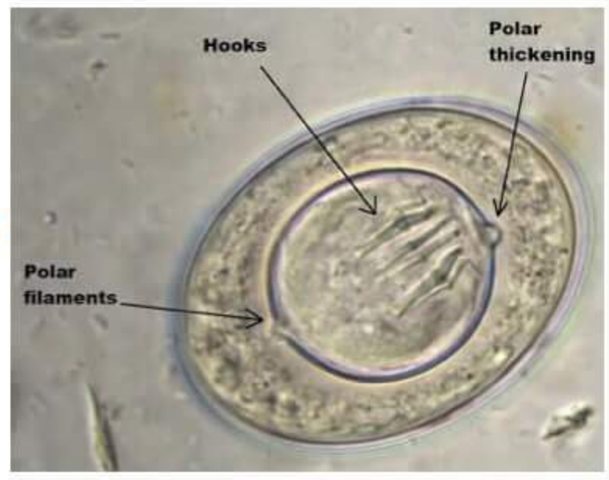
NON BILE STAINED EGGS		
N	→	Necator americanus
E	→	Enterobius vermicularis
H	→	H. nana
A	→	Ancylostoma duodenale

EGG

→ contains → polar filaments
central hooks

→ non bile stained Egg





TREATMENT → DOC → PRAZIQUANTEL

ECHINOCOCCUS GRANULOSUS / DOG TAPEWORM

Eggs of E. granulosus ingested ĩ contaminatid food & water [Infective form]

↓

Hatch up in duodenum

↓

releases Hexacanth embryo

↓

penetrates the intestinal layer

↓

through portal circulation

↓

reaches Liver

↓

Forms Hydatid cyst

HYDATID CYST

- consists of
  - Pericyst
  - Ectocyst
  - Endocyst (Germinal epithelium)
    - most active layer
    - secretes hydatid fluid
    - alw Brood capsule
      protoscolices

HYDATID CYST

Hydatid Fluid

- Casoni's Test
  - Type 1 hypersensitivity
    - immediate wheal & flare reaction
  - obsolete now

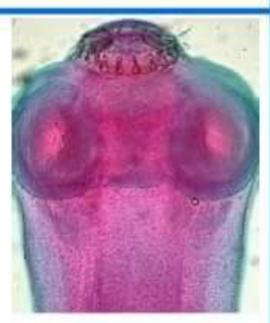
- E. multilocularis → causes Malignant Hydatid Diseases

TREATMENT OF HYDATID CYST

① mild to moderate cyst

PAIR THERAPY → Percutaneous Aspirat^{n} Inject^{n} Reaspirat^{n} Therapy using scolicidal agent

② Large size cyst → Surgical Removal

Taenia solium	Taenia saginata
→ Shorter → Armed tapeworm [rostellum & hooklets +nt] 	→ Longer [in metres] → Unarmed tapeworm [No rostellum & hooklets] 
→ Eggs of both look like same DD → T. saginata eggs hooklets are acid fast	
→ Proglattids number → <1000 Proglattids → male & female ill defined organs	→ Proglattids number → >1000
→ eaten uncooked pork ∴ Cesticercus cellulosae ↓ TAENIASIS [diarrhoeal episodes] CYSTICERCOSIS [few cases] dlt ingestion of eggs	→ eaten uncooked beef ∴ Cesticercus bovis ↓ TAENIASIS [diarrhoeal episodes] → nothing happens if eggs are eaten

CYSTICERCOSIS

Portal of entry for cysticercosis

↓

Eggs ī contaminated food & water

↓ can lead to

NEUROCYSTICERCOSIS

→ mc site → Subarachnoid > Parenchymal
→ presents ī atypical seizures
→ IOC → CT / MRI Scan
→ DOC → ALBENDAZOLE


Egg of Taenia Sp.

TREMATODES / FLUKES

PROPERTIES

① Leaf like
② Monoecious
③ have two Intermediate hosts
1st → Snail
2nd → Aquatic vegetation
Fish
Crab | cray water fish

④ Infective form → Meta cercariae (cyst like structure)
⑤ All have Operculated Egg [Lid]

EXCEPTION → SCHISTOSOMES
- Dioecious
- Single Intermediate host → Snail
- infective form → cercariae
- have non operculated eggs

SCHISTOSOMES

SCHISTOSOMA HAEMATOBIUM

→ resides in vesicle plexus
→ causes squamous cell carcinoma of Urinary bladder > Transitional cell carcinoma
→ causes terminal hematuria
→ Egg has terminal spine

Egg ī terminal spine

S.MANSONI

→ resides in inferior mesentoric plexus
→ causes Swimmer's Itch
Katayama fever


Egg ī lateral spine

S.Japonicum

→ resides in superior mesentric plexus

→ causes Katayama fever
Eosinophilic diarrhoea

Egg ī Lateral knob

Fasciola Hepatica / Sheep liver fluke

→ 1st IH → Snail
and IH → Aquatic vegetatⁿ
[ī metacercarial]

↓

Man eats this aquatic vegetatⁿ

↓

Metacercaria is ingested

↓

in duodenum excystation occurs

↓

Larvae released

↓

Penetrates intestinal layers

↓

Penetrates Liver capsule

↓

penetrates bile ducts

↓

matures to adult forms

↓

produce Eggs

↓

comes in contact ī water

↓

Eggs → Miracidium [In SNAIL]

↓

Sporocyst

↓

Radiae [1st generatⁿ & 2nd generatⁿ]

↓

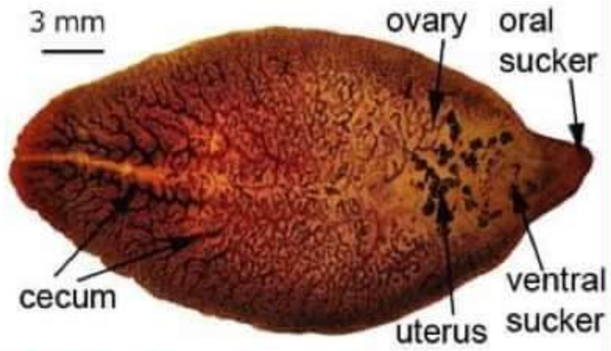
cercariae

↓

Metacercariae [cyst like structure]

↓

Goes & settles on aquatic vegetation



Fasciola hepatica

Fasciola hepatica egg
[operculated]

PrepLadder

Clonorchis sinensis / oriental / chinese liver fluke

→ 1st IH → Snail

2nd IH → Fish

[↓ metacercariae]

↓

Eaten by man

↓

Excystation

↓

Larvae released

↓

Penetrates intestinal layers

↓

Penetrates Liver capsule

↓

penetrates bile ducts

↓

matures to adult forms &

causes constant inflammation

↓

causes cholangiocarcinoma [Bile duct carcinoma]

Paragonimus westimonii [Lung Fluke]

→ 1st IH → Snail

2nd IH → crab / cray water fish

↓ metacercaria

↓

Eaten by man

↓

Excystation

↓

Larvae released

↓

Penetrates intestinal layers

↓

Penetrates diaphragm

↓

Penetrates Rt. lung capsule

↓

enters into Rt. lung

↓

cysts → Erosions

Red Brown Sputum [Endemic hemoptysis]

NEMATODES

SMALL INTESTINAL NEMATODES	TISSUE NEMATODES
Ascaris	Resides in Lymphatics
Hook worm	Wuchereria bancrofti
Necator americanus	Brugyia malayi
strongyloides stercolaris	Brugyia timori
LARGE INTESTINAL NEMATODES	Resides in Subcutaneous Sites
Trichuris trichiura	Loa loa
Enterobius vermicularis	Onchocerca volvulus
MISCELLANEOUS NEMATODES	Resides in Serous Cavities
Dracunculus medinensis	Mansonella ozzardi
Trichinella spiralis	Mansonella perstans

TRANSIMISSION BY

EATING EGGS	PENETRAT^{n} OF SOLE OF FOOT [Filariform larvae]
E → Enterobius vermicularis	A → Ancylostoma duodenale
A → Ascaris	N → Necator americanus
T → Trichiuris Trichiura	S → Strongyloides stercolaris

LUNG FOR MATURATION	
A^{2} →	Ancylostoma duodenale
	Ascaris
N →	Necator americanus
S →	Strongyloides stercolaris

LARGE INTESTINAL NEMATODES

TRICHURIS TRICHIURA / WHIP WORM

Eggs are ingested ī contaminated food & water

↓

Hatch up in duodenum or caecum

↓

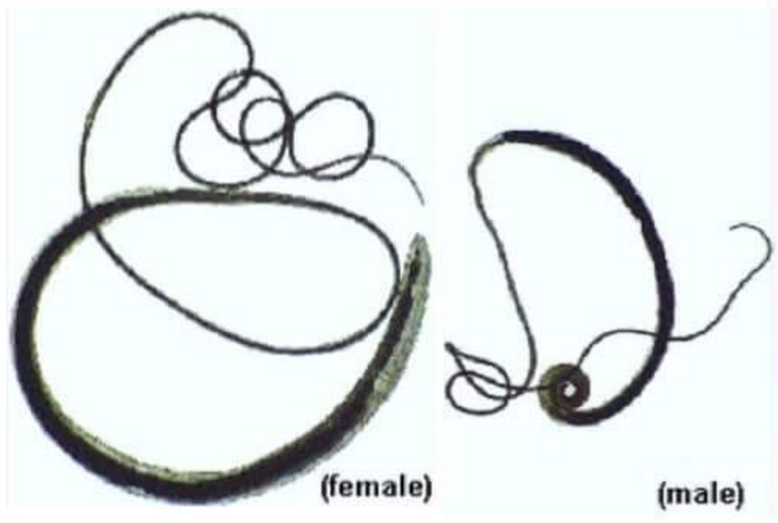
Larvae released

Male ← → Female

⊕ Fertilise

↓

Eggs



EGG

→ Barrel / dumb bell shaped egg

→ have mucous plugs on both sides

Egg ī mucous plugs

→ causes Fe deficiency anaemia

→ causes Frequent Rectal prolapse in children

COCONUT CAKE LIKE RECTUM

Growth retardation

ENTEROBIUS VERMICULARIS / PIN WORM / THREAD WORM / SEAT WORM / OXYURIASIS

→ presents ↓ Perianal pruritis

Eggs deposit in nail beds

↓

child putting up fingers in mouth

↓

eggs are ingested

↓

hatch up in SI/LI

↓

Larvae released

Male — female

↓

After fertilizat^{n} male dies

Female goes in night time & lays eggs in perianal region

↓

Autoinfection

AUTO INFECTION SEEN IN

C^{2} → Capillaria phillipensis

Cryptosporidium parvum [Sporozoa]

H → H. nana

E → E. vermicularis

S → Strongyloides stercolaris

T → Tania solium

EGG

→ Plano convex egg

→ having a tadpole like larvae

→ non bile stained egg

NIH SWAB

→ National Institute Health swab

→ made of glass rod & cellophane tape

SMALL INTESTINAL NEMATODES

ANCYLOSTOMA DUODENALE / HOOK WORM

Penetration of filariform larvae in sole of foot

↓

causes Ground itch / Maculo papular dermatitis

↓

Penetrates all skin layers

↓

Gets into Cutaneous Circulatⁿ

↓

Lymphatics

↓

Venous circulatⁿ

↓

Portal circulatⁿ

↓

Liver

↓

Rt. side of heart

↓

Lungs

↓

After slight maturatⁿ

↓

RETROGRADE JOURNEY

↓

Penetrates pulmonary capillaries

↓

Alveoli → Bronchiole

↓ ↓

Larynx ← Trachea

↓

Pharynx

↓

Swallowed ∈ sputum

↓

In the esophagus, 3rd moulting happens

↓

In duodenum, 4th moulting happens

↓

Mature larvae

Male | Female

Male → Females

↓

Eggs

↓

Faeces

↓

Soil → Eggs

↓

Rhabditiform larvae

[2 moultings (1st & 2nd)]

↓

Filariform larvae [Needle like]

[Infective form]

Rhabditiform larva

A. brasilensis

animal worm

↓

can not penetrate all skin layers

↓

trapped b/w skin layers

↓

Cutaneous larvae migrans


Cutaneous larvae migrans

A. duodenale

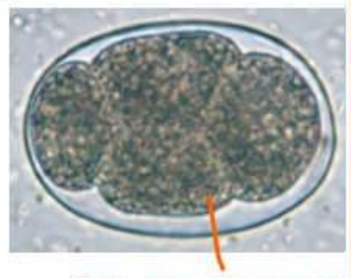
→ causes Fe deficiency anaemia [0·2ml/Day/worm Blood sucked]

→ EGG

→ segmentation is seen [Blastomeres]

→ CHANDLER'S INDEX → >300 eggs/gm of stool

— Social Problem


Blastomeres

S. stercolaris

→ a/w HIV +ive

→ ovo - viviparous

→ undergo auto infection

changes to HYPER INFECTION SYNDROME in HIV +ive

- CNS & viscera involved
- carries some mortality

→ undergoes PARTHENOGENETIC DEVELOPMENT

- Females directly lay eggs tout male

→ also undergoes cutaneous larvae migrans → LARVAE CURRENS

- moves faster under skin

ASCARIS

Eggs are ingested ī contaminated food & water

↓

Hatch up in duodenum

↓

Larvae released

↓

Penetrates intestinal layers

↓

Gets into portal circulatⁿ

↓

Liver

↓

Rt. side of heart

↓

Lungs [2nd & 3rd moulting happens]

↓

RETROGRADE JOURNEY

↓ (penetrating pulmonary capillaries)

Alveoli → Bronchiole

↓

Larynx ← Trachea

↓

Pharynx

↓

Swallowed ī sputum

↓

in duodenum, 4th moulting occurs

Male / Female

Male → Eggs

↓

Faeces → Soil

↓

Eggs [1st moulting]

↓

Rhabditiform larvae ī egg

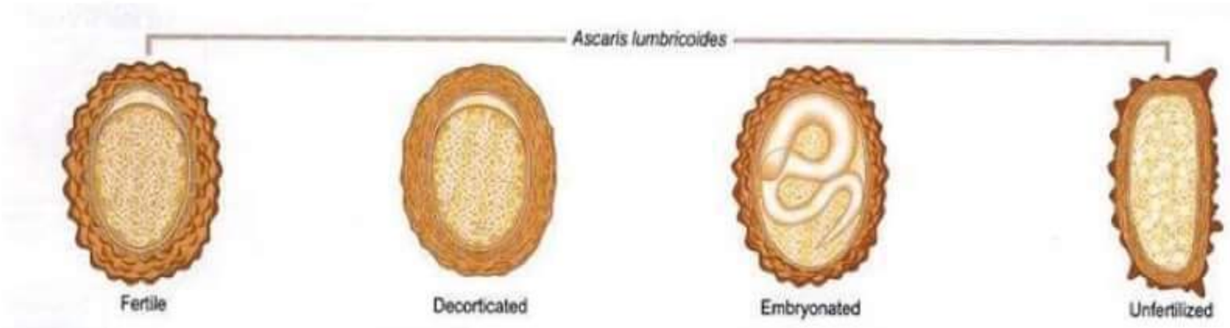
↓

Eaten by man

→ causes Intussusceptⁿ → intestinal obstructⁿ

→ a/w Loeffler's syndrome [strong association] → massive eosinophilia

EGG OF ASCARIS



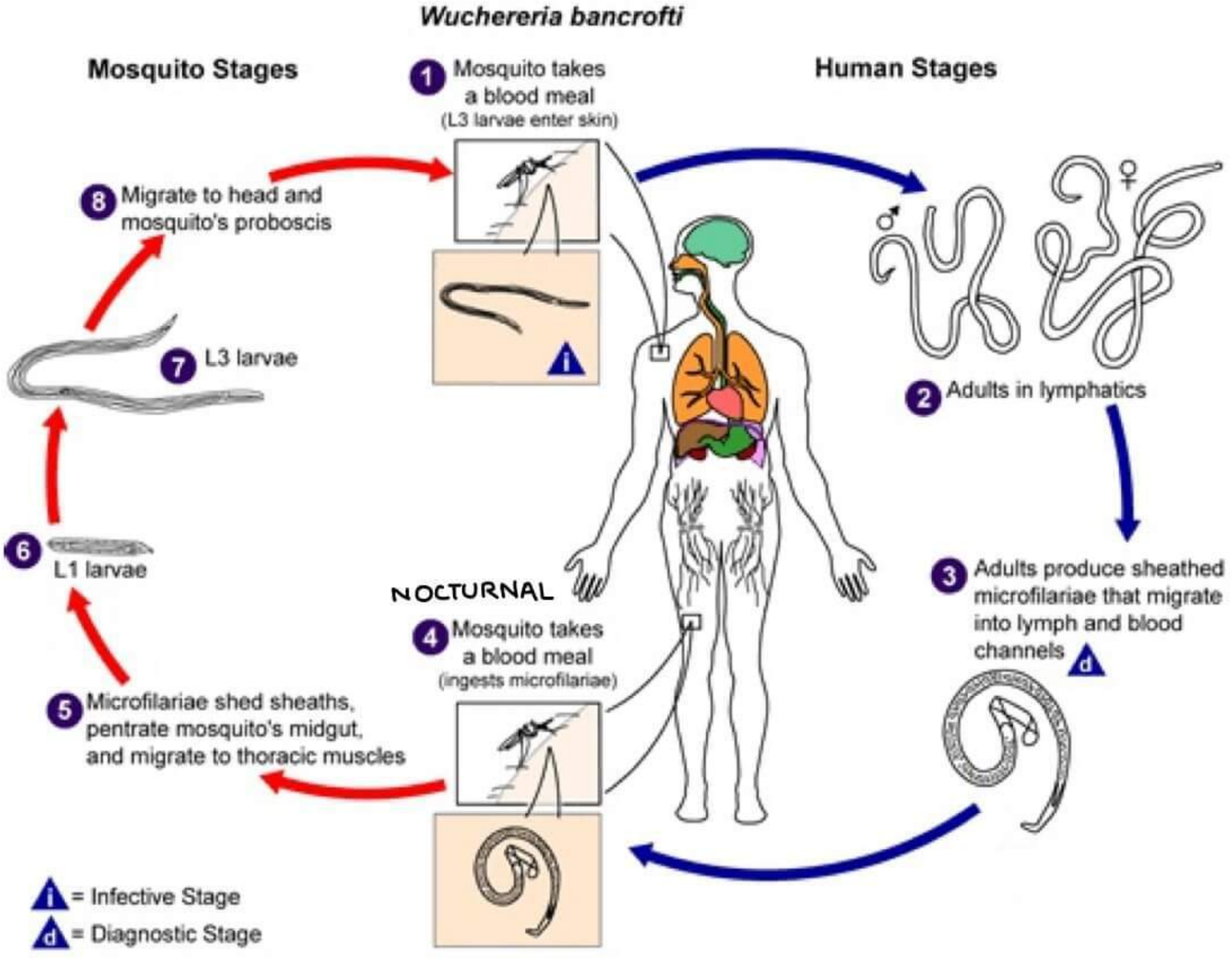
EGGS WHICH FLOAT ON SATURATED SALT SOLUTION

F → Fertilised egg of Ascaris
A → Ancylostoma duodenale
T → Trichuris trichiura
E → Enterobius vermicularis
H → H. nana

TISSUE NEMATODES

WUCHERIA BANCROFTI

→ Infective form → L_3 Filariform larvae
- transmitted by
  - culex [main]
  - Ades
  - Anopheles



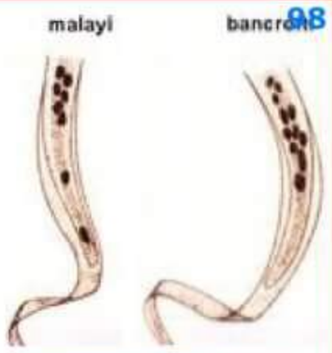
ACUTE FILARIASIS FEATURES → Fever, lymphadenitis, lymphangitis

CHRONIC FILARIASIS FEATURES → Elephantiasis, hydrocele, granuloma breast
Kidney LN block up → Chyluria
Albuminuria

LAB DIAGNOSIS

① BLOOD SMEAR EXAMINATION

→ taken at night time d/t nocturnal periodicity



② DEC PROVOCATIVE TEST


ELEPHANTIASIS

TAIL TIP

→ For identificatⁿ Terminal Tail Tip is examined

No nuclei seen in W. bancrofti

→ having terminal 2 nuclei → microfilariae of Brugyia malayi

③ RADIOLOGICAL

→ X Ray

→ CT Scan

→ USG of Scrotum → DANCING WORM SIGN Seen


Hydrocele of scrotum

④ PCR for W. bancrofti

→ even picograms of DNA will be detected

→ Best investigation

SUB CUTANEOUS SITES

ONCHOCERCA VOLVOLUS

Skin Disease	Eye Disease
ONCHOCERCOMA	RIVER BLINDNESS
[sub cutaneous nodules]	[by Simulium fly / Black fly] deposit in eyes of people residing near the river bed


Oncocercoma

LOA LOA

Skin	Eye
CALABAR SWELLINGS	LOASIS
– migratory swellings	[by Chrysops fly]

MISC

DRACUNCULUS MEDINENSIS [Guinea worm]

→ Eradicated from India

Last case reported from Rajasthan


Match stick method

VIROLOGY

VIRUSES

→ viruses are obligate intracellular organisms
→ contains either DNA or RNA
→ Genome is surrounded by capsid
→ capsid is surrounded by Envelope

IMPORTANT PROPERTIES

1. smaller in size
2. Lack the enzyme necessary for protein & nucleic acid synthesis
3. do not have cellular organisation or ribosome
4. do not divide by binary fission
5. do not grow in any culture media
6. contain either DNA or RNA
7. resistant to antibiotics
8. Host specificity present
    1. ANIMAL VIRUS
    2. PLANT VIRUS
    3. BACTERIAL VIRUS [Bacteriophage]

MORPHOLOGY OF VIRUS

→ size → 20 - 400 nm

STRUCTURE

→ central core of nucleic acid of a virion is called as genome
→ Genome is surrounded by a protein coat called capsid [made of capsomeres]
→ Genome & surrounded capsid → NUCLEOCAPSID

FUNCTIONS OF CASPID

1. impermeable shell around genome
2. introduction of viral genome into host cell by absorbing on cell surface
3. capsid envelope → Bilayered lipoprotein
    - virus codes glycoprotein subunits on the surface of envelope [PEPLOMERS]
    - PEPLOMERS - H & N [in influenza virus]

SYMMETRY

→ ICOSAHEDRAL [mc] → Polygonal i 12 vertices / corner, 20 facets / sides [each facet is a equilateral triangle]

→ HELICAL → Some RNA viruses
→ COMPLEX → POX virus

SHAPES		
Bullet shaped	→	Rabies virus
Rod shaped	→	Tobacco mosaic virus
Brick shaped	→	Pox virus
Space vehicle	→	Adeno virus
filamentous	→	Marburg & Ebola virus

CHEMICAL PROPERTIES

→ inactivated at 56°c x 30 min
→ Stable & can be stored at −40 to −70°c by lyophilization
→ Radiation → UV rays, X Rays, Gamma rays inactivate the virus
→ Lipid solvent → Chloroform, ether & bile salts destroy easily enveloped viruses
→ Disinfectants → Like Oxidizing agents of chlorine, iodine & H_2O_2 Kills most viruses
→ Enteroviruses are resistant to phenol & chlorination

Replication

STEPS

① Adsorption
② Penetration
③ uncoating
④ Biosynthesis / Assembly
⑤ Maturation
⑥ Release

"A PUB MR" → mneumonic

ADSORPTION

→ comes in contact by RANDOM COLLISION
→ depends on presence of SPECIFIC RECEPTORS
→ Eg: 1. HIV → Envelope proteins gp 120 / gp 41
↓
Binds to CD 4 T cell receptors

2. Influenza virus A
→ H → Hemagglutinin [adsorption on pharyngeal cells]
→ N → Neuraminidase

VIROPEXIS / PENETRATION

→ VIROPEXIS → after attachment the virus particle is taken inside the cell
→ in enveloped viruses, envelop fuses ∑ plasma membrane of host cell
– releasing the nucleocapsid into the cytoplasm

UNCOATING

→ physical seperat^ of nucleic acid from capsid occurs
→ Enveloped viruses enter cell by receptor mediated endocytosis & lose the outer covering by lysosomal enzymes

→ Viral genome directs, the biosynthesis machinery of the host to synthesize components of "new progeny virus"

→ Viral proteins are synthesized mostly in cytoplasm

→ most of the DNA viruses replicate in the nucleus except Pox virus

→ most of the RNA viruses replicate in the cytoplasm except Influenza & retro virus

→ most of DNA viruses are DS except Parvo virus

→ most of RNA viruses are SS except Reo virus

→ In case of SS DNA virus → Viral nucleic acid utilizes cellular DNA polymerase for making DNA double stranded

→ Double stranded DNA virus → Viral nucleic acid enters the nucleus of host cell

→ Transcription of Parental DNA
↓
Early mRNA
↓ Translation
Early non-structural proteins

→ Early non-structural proteins help parental DNA
↓
Shut host cell machinery
↓
Progeny DNA

→ Progeny DNA undergo transcription
↓
Late mRNA
↓
Translated to Late structural proteins
[capsid & Envelope proteins]

→ In the case of RNA VIRUS

SS RNA viruses classified into 2 categories

① The positive sense RNA [Plus strand]
↓
acts as mRNA
↓
Early proteins
↓
RNA Polymerase activity
direct replication of (+) SS RNA
↓
-ive SS RNA → + SS RNA → Late proteins are produced

- Eg. Picorna & togavirus

② The Negative sense [minus strand]

↓

posses their own RNA polymerase for mRNA transcript^{n}

↓

tive sense RNA

↓ Transcription

m RNA

↓

Proteins

- Eg. Rhabdo, orthomyxo, paramyxoviridiae

→ In DS RNA VIRUS [Reovirus] have segmented genome
Each segment codes for 1 polypeptide

→ + strand RNA can act as mRNA

↓ Translation

Proteins

→ Both ⊕ & ⊖ strands serves as templates for synthesis of complementary strands to form a duplex

→ In case of RETRO VIRUS,

SS RNA genome

↓ viral reverse transcriptase [RNA dependent DNA polymerase]

SS DNA

↓

DNA : RNA Hybrid

→ Reverse transcriptase enzyme has ribonuclease activity also by which it digest RNA from DNA : RNA Hybrid

- SS DNA ⟶ (using DNA polymerase of same reverse transcriptase enzyme) DS DNA → integrated into the host cell chromosome [nucleus] by viral integrase

- integrated DNA serves as a template to produce mRNA
- m RNA ⟶ Early & Late proteins

ASSEMBLY

→ Viral nucleic acid & proteins are packaged to form progeny viruses [Nucleocapsid]
→ Assembly may takes place in host cell nucleus & cytoplasm
→ DNA viruses mostly assembled in nucleus except Pox & Hepadnavirus
→ RNA viruses mostly assemble in cytoplasm

MATURATION

→ may takes place in nucleus or cytoplasm

Eg. in nucleus → Herpes, Adeno virus
in cytoplasm → Picorna, Pox virus

→ in case of enveloped virus, the envelope is derived from nuclear membrane [if they assemble in nucleus] or from plasma membrane during the process budding [if they assemble in cytoplasm]

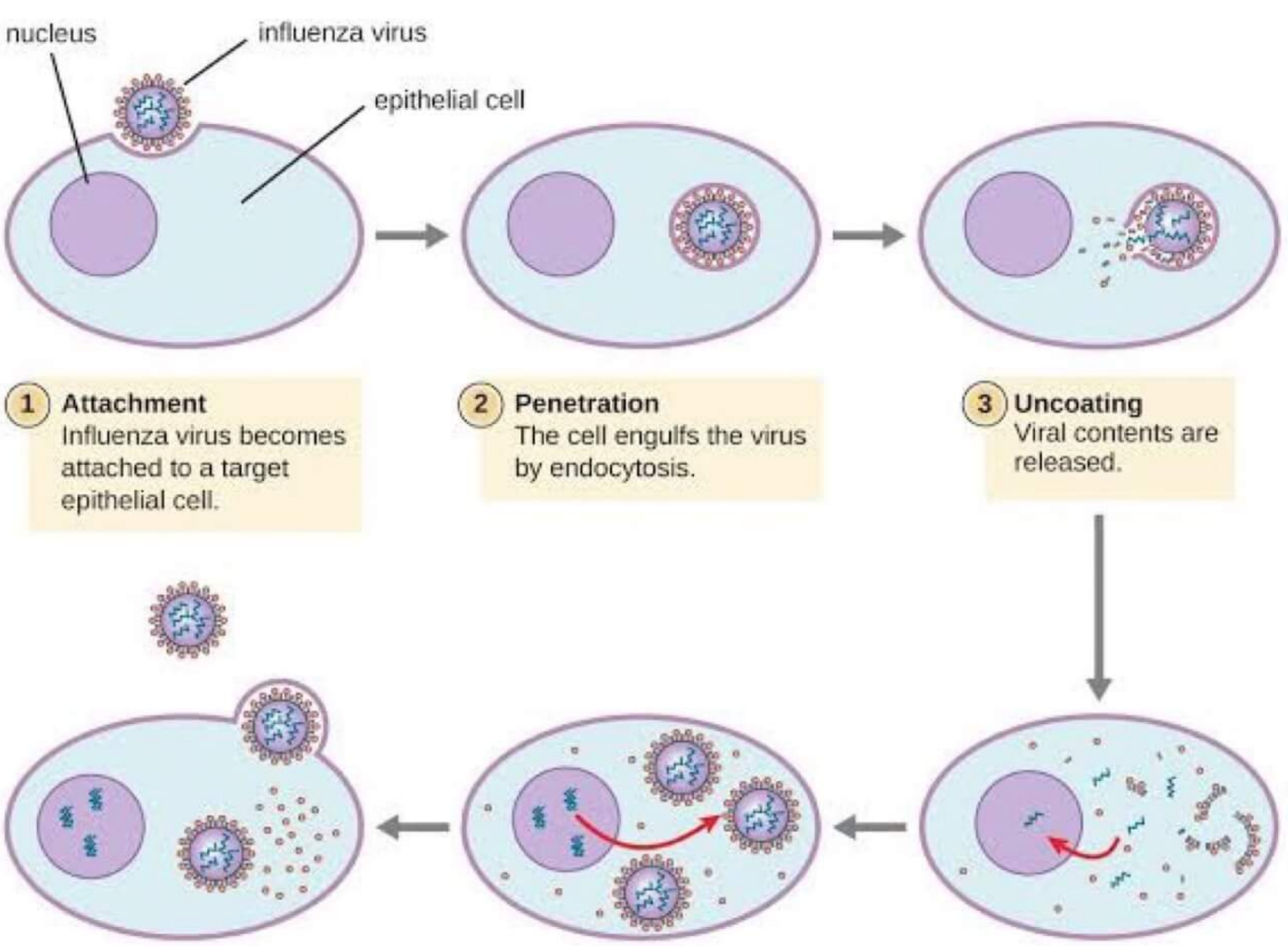
6. RELEASE BY

1. LYSIS → Lysis of host cells is done by non-enveloped viruses & Bacteriophages

2. BUDDING → happens in enveloped virus - they acquire a part of host cell membrane to form lipid part of envelope

→ Eg: Envelope is acquired in influenza virus from plasma membrane & for Herpes virus from nucleus membrane

→ viral glycoproteins are inserted into the envelopes
→ excess viral glycoproteins are synthesized to saturate cell receptors
→ So that virus will not stick to host cells following release



ABNORMAL REPLICATIVE CYCLES

INCOMPLETE VIRUSES

→ results from defective assembly [proteins are assembled tout nucleic acid]
→ Eg. VON MAGNUS PHENOMENON [Influenza virus]
→ High haemagglutination titer but low infectivity

PSEUDO VIRUSES - During replication of viruses, capsid occassionally encloses [Host cell nucleic acid] instead of viral nucleic acid. They are neither infective nor replicate

ABORTIVE INFECTION → dlt wrong selection of host cells by virus

MUTATION

→ occurs at 10^{-4} to 10^{-10} per base pair per generation
→ mutation can happen spontaneously or induced by 5-fluorouracil, UV light

CONDITIONAL LETHAL MUTANTS

→ can grow only in some permissive conditions
→ Eg: Temperature sensitive mutants can grow at 28-31°c but not at 37°c and are used in making influenza vaccines

REASSORTMENT

→ happen in influenza virus A
→ Two strains of influenza infect a host cell, Gene exchange blw them happens

VIRAL INTERFERENCE

→ Two viruses infect a host cell [one virus leads to inhibition of other virus]
→ one virus blocks the host cell receptor & other virus destroys host cell receptor
→ The 1st virus may compete t 2nd virus for replication apparatus & induce interferon production to Kill second virus
→ Eg. Rubella | Togaviridae
Polio | Picornaviridae

CULTIVATION OF VIRUS

① HEN'S EGG INOCULATION
② ANIMAL INOCULATION
③ CELL CULTURE | TISSUE CULTURE

1. HEN'S EGG INOCULATION [CANDLING]

→ CAM → Chorio Allantoic Membrane → variola/ vaccinia [pox virus]

→ Allantoic cavity → vaccine product^{n} → Y → Yellow fever [IFD]
I → Influenza
R → Rabies

→ Amniotic sac → primarily used for isolat^{n} of influenza virus

→ Yolk sac → C → Chlamydia
A → Arbo Virus
R → Rickettsiae

2. ANIMAL INOCULATION

→ New Born Suckling mice [<48hr] → used for Arbo virus, Coxsackie Virus
→ to study viral oncogenesis → Hamsters, guinea pigs, Rabbits, monkeys are used

→ ROUTES OF INOCULATION
  → Intracerebral
  → Intranasal
  → Intra peritoneal
  → Sub cutaneous

→ Animals are observed for the signs of illness & stained for inclusion bodies

3. CELL CULTURE / TISSUE CULTURE

1. Organ culture → small bits of organs [Tracheal ring - corona virus]
2. Explant culture → fragmented minced tissue
3. Cell culture → mostly done now a days

CELL CULTURE LINES [Growth medium]

1. PRIMARY CELL LINES
  → Obtained from fresh organs of animals or humans
  → allow only 5-10 divisions
  → isolation of virus done
  → Eg. Rhesus monkey Kidney cell lines
    Chick embryo fibroblast cell lines

2. SEMI CONTINUOUS / DIPLOID CELL LINES
  → usually fibroblasts
  → allow 10-50 divisions
  → Eg. Human fibroblast cell lines [WI-38]

3. CONTINUOUS / CANCER CELL LINES
  → Serially subcultured
  → Unlimited divisions
  → Eg. Hela → derived from cervical cancer of a lady
    Hep2 → derived from human epithelioma of larynx
    BHK → derived from baby hamster kidney
    vero → derived from vervet monkey kidney cell line

CYTOPATHIC EFFECT → helps in identification of virus

1. multinucleate Giant cell formation → Measles virus / RSV
2. Cell necrosis / lysis → Enteroviruses
3. cell clumping [grape like clusters] → Adenovirus
4. Cytoplasmic vacuolation → SV-40

INCLUSION BODIES

→ replicating aggregates of the virus either intra nuclear or intracytoplasmic
→ can be seen i normal power microscope

INTRA CYTOPLASMIC INCLUSION BODIES [Eosinophilic]

① Negri bodies → Rabies
② Guarnieri bodies → Vaccinia
③ Paschun bodies → variola
④ Bollinger bodies → Fowl pox
⑤ HP bodies / [Henderson Peterson] Molluscum bodies → Molluscum contagiosum

INTRA NUCLEAR INCLUSION BODIES

① Cowdry type (A) inclusions seen in

H → Herpes virus [Lipschvltz bodies also seen]
(A) →
Y → Yellow fever virus [Torres bodies also seen]

② Cowdry type (B) inclusions seen in

(B)
A → Adeno virus
P → Polio virus

→ INTRA NUCLER + INTRA CYTOPLASMIC INCLUSION BODIES SEEN IN MEASLES
[aka WARTHIN FINKELDEY GIANT CELLS]

→ Intra nuclear + intra cytoplasmic inclusion bodies also seen in CMV
[Owl Eye Appearance of infected cells]

HERPES

FAMILIES

α VIRINAE	β VIRINAE	γ VIRINAE
→ includes HSV 1 HSV 2 HHV 3	→ includes HHV 5 HHV 6 HHV 7	→ includes HHV 4 HHV 8
→ affects Ganglionic cells	→ affects Salivary glands	→ affects B cells [dlt CD21 / CR2 Receptors]

→ HHV – 3 → Varicella zoster [chicken pox virus]
HHV – 4 → EBV
HHV – 5 → Cytomegalo virus
HHV – 8 → Kaposi sarcoma causing virus
HSV – 1 → Herpes simplex virus 1
HSV – 2 → Herpes simplex virus 2

HSV

HSV 1 → causes infection above the waist
HSV 2 below the waist
} not a rule

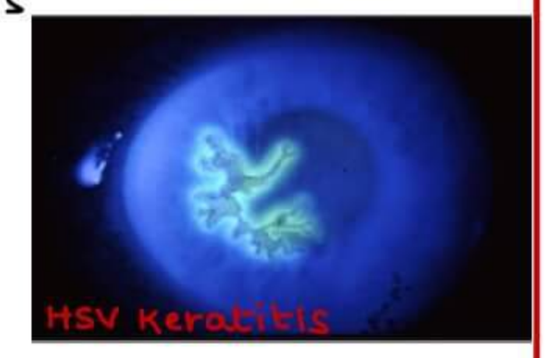

Herpes labialis

MUCOSAL INVOLVEMENT

→ mc site → Buccal mucosa
→ causes → Acute gingivo stomatitis
→ mc recurrent herpes lesion → herpes labialis

CUTANEOUS INVOLVEMENT

→ mc site → face
→ HERPETIC WHITLOW → occurs in nurses, doctors
GLADIATORUM → occurs in wreslers



CNS

→ Temporal lobe encephalitis
→ MOLLARET'S MENINGITIS [Recurrent lymphocytic meningitis]

OPHTHA INVOLVEMENT

→ cause acute dendritic ulcers
steroids are contraindicated
Topical Acyclovir indicated


Eczema herpeticum by HSV1/2

GENITAL INVOLVEMENT

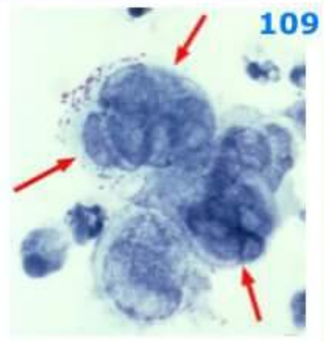
→ causes B/L vesicular, pustular, erythematous lesions
→ Prevent^{n} OF HSV 2 infection to baby → cesarean section done

LAB DIAGNOSIS

① PCR
② ELISA } for HSV 1 & 2

③ TZANCK SMEAR

Tissue biopsy + toluidine blue → TZANCK CELLS
[mutinucleated giant cells]


TZANCK CELLS

VARICELLA ZOSTER / CHICKEN POX VIRUS

→ Portal of entry → Respiratory tract | conjunctiva
→ SAR (secondary attack Rate) → >90%
→ infectivity period → 2 days before ← RASH → 5 days after
[until the scab falls]
→ centripetal Rash tnt , DEW DROP APPEARANCE & Pleomorphism seen
→ IP → 14 - 16 days
→ mc complication → secondary bacterial infection
mc extra cutaneous involvement → Brain
most serious complication → Varicella Pneumonia

→ CONGENITAL VARICELLA SYNDROME
→ chorio retinitis
→ limb hypoplasia
→ microcephaly
→ cicatrising lesions

→ Vaccine strain → OKA STRAIN

LAB Dx
① PCR/ELISA for HHV-3
② TZANCK SMEAR

REACTIVATION → Herpes zoster [shingles]
→ occurs in stress
→ Types
H Z oticus / RAMSAY HUNT SYNDROME
→ Bell's palsy
Vesicles over EAC, TM & cheeks
→ Geniculate ganglion involved

H Z Ophthalmicus
→ single sided lesion tnt on face
→ dermatomal involvement [T_3-L_3]
→ Ophthalmic branch of trigeminal nerve involved

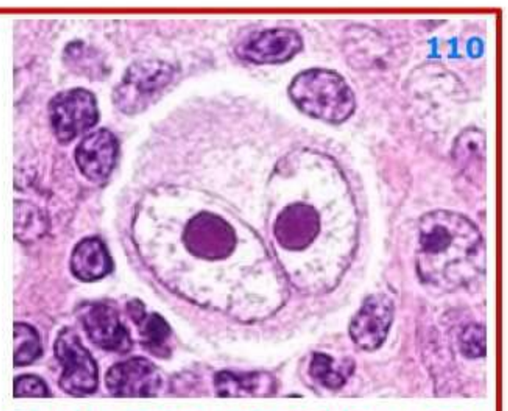
→ LAB DIAGNOSIS → PCR/ELISA available


zoster Ophthalmicus

CMV

→ Largest herpes virus

→ OWL EYE APPEARANCE OF infected cell seen



OWL EYE APPEARANCE

congenital cmv SYNDROME

FEATURES

→ hepatosplenomegaly [mc]

→ petechial lesions

→ chorio retinitis

→ calcifications

→ convulsions

→ microcephaly

→ mental Retardation

MONONUCLEOSIS LIKE SYNDROME

FEATURES

→ Atypical lymphocytes [CD_8 T cells mainly]

→ PAUL BUNNEL TEST → -ive

→ causes CMV Pneumonia in post Kidney transplant
post bone marrow transplant
[post transplant (1 - 4 months) more vulnerable]

CMV RETINITIS

→ Risk Factor → CD_4 count → <50

→ DOC → GANCICLOVIR

LAB DIAGNOSIS

① CULTURE on Human fibroblast cell line [WI-38]

② PCR for CMV

③ ELISA for CMV

EBV

→ affects B cells → Polyclonal proliferat^n of B cells

→ DISEASES

① NASO PHARYNGEAL CA [NPC]

② BURKITT's LYMPHOMA [starry sky appearance]

③ HODGKIN & NON HODGKIN'S LYMPHOMA

④ ORAL HAIRY LEUKOPLAKIA [OHL]

⑤ DUNCAN SYNDROME → x linked lympho proliferative syndrome

⑥ TONSILLAR CARCINOMA

⑦ INFECTIOUS MONONUCLEOSIS / KISSING DISEASE

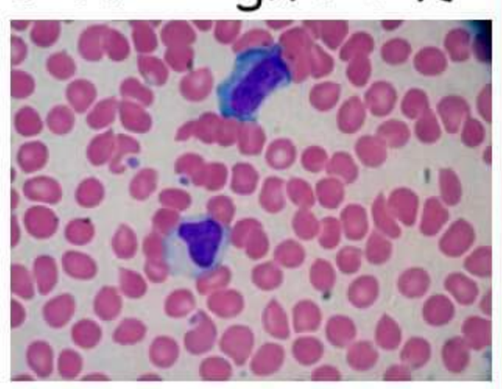
→ fever, rash, lymphadenopathy

→ $CD_4 : CD_8$ ratio reversed for some times

→ atypical lymphocyte cells +nt

→ paul Bunnel test is positive

BURKITT'S LYMPHOMA [Tumor of Jaw]



Atypical lymphocytes

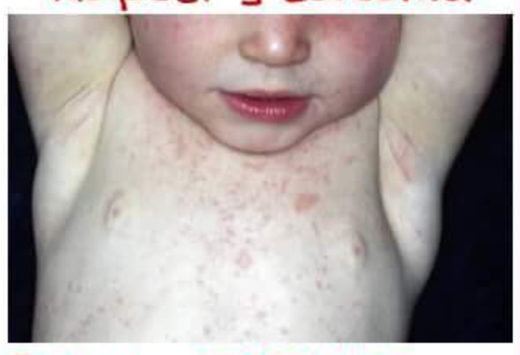
LAB DIAGNOSIS

① PCR for EBNA [Epstein Barr Nucleic acid]
② PCR for VCA [viral capsid antigen]
③ ELISA for EBV

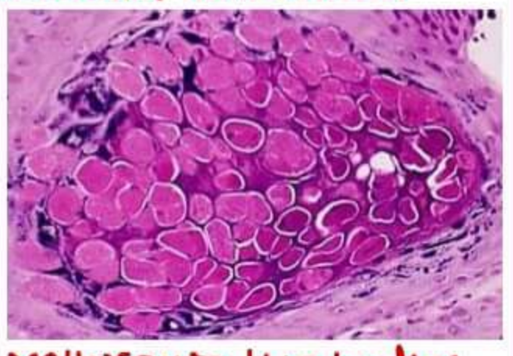

Kaposi's sarcoma

HHV -8 [KAPOSI SARCOMA CAUSING VIRUS]

→ common among HIV +ive
→ vascular tumor
→ also alw Primary effusion lymphoma
Castle man's disease


Roseola infantum

HHV 6 & 7 → causes Exanthem Subitum / Roseola infantum


Molluscum / HP bodies

POX VIRUS

MOLLUSCUM CONTAGIOSUM

→ pearly white umblicated nodule over the skin / genitals +nt
→ caused by MCV 1, 2, 3, 4 [mcv1 → mc]
→ On biopsy, HENDERSON PETERSON [HP] BODIES seen [intra cytoplasmic]
→ Rx → PODOPHYLLIN RESIN + CRYOSURGERY


Molluscum contagiosum

PARVO VIRUS

→ SS DNA VIRUS
→ PARVO B 19 → important
→ affects erythroid precursor cells ⟶ APLASTIC CRISIS in Sickle cell anemic Patients

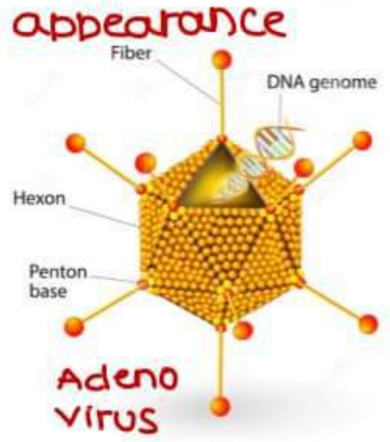
→ ASSOCIATED WITH
NIHF [Non Immune Hydrops fetalis]
Polyarthralgia syndrome
- pain is more in upper limb joints than Lower limb joints

Erythema infectiosum / 5th Disease
- SLAPPED CHEEK APPEARANCE +nt


Slapped cheek appearance

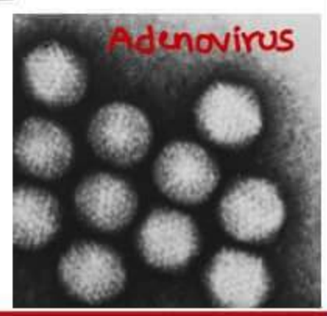
ADENO VIRUS

→ ds DNA virus
→ non enveloped virus
→ Space vehicle look like
→ Types 3, 4, 7, 14 → causes Pharyngo conjunctival fever or Swimming pool conjunctivitis



Adeno virus

→ Types 8, 19, 37 → causes Epidemic Kerato conjunctivitis
→ Type 37 → causes STD
→ Type 40, 41 → causes Infantile diarrhoea
→ Type 11, 21 → causes Hemorrhagic cystitis



AAV & VACCINIA [AAV → Adeno Associated Virus]

→ Used for making recombinant vaccines using genetic engineering

POLYOMA & PAPILLOMA VIRUS

POLYOMAVIRIDAE

→ mostly oncogenic in Lab animals
→ non - enveloped
→ Icosahedral
→ 45 nm in size
→ DS DNA Genome
  ↳ Early region → interacts ī P53 & RB genes
  ↳ Late region → capsid

JC VIRUS

→ cultured in human fetal glial cells
→ infection → Ubiquitous
→ Risk factor → HIV +ive, transplant, Hodgkin's Lymphoma
→

cross Blood Brain Barrier
↓
Infects oligodendrocytes & astrocytes
↓
causes subacute demyelinating disease
↓
PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

→ LAB DIAGNOSIS
  → PCR on brain biopsy tissue for JC virus

→ NO treatment & fatal

BK VIRUS

→ Post kidney transplant infection [Borrowed Kidney]
→ cultured in human diploid cell lines
→ Latency in Kidney & reactivated by Kidney transplants [BK NEPHROPATHY]
→ DECOY CELLS are seen in BK nephropathy
→ DIAGNOSIS
  → Urine → Culture
  → PCR

→ TREATMENT → immunosuppression to be corrected

SV - 40 [SIMIAN VACOULATING-40]

→ causes Progressive multifocal leukoencephalopathy in Rhesus monkeys
→ cytopathic effect → cytoplasmic vacoulation

merkel cell carcinoma virus
KI virus [Karolinska institute]
WU virus [washington university virus]

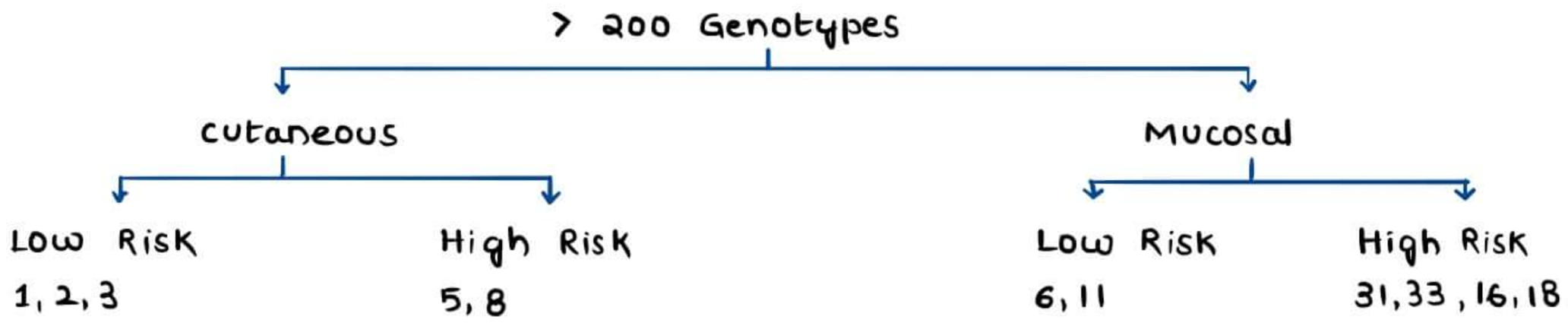
GENERAL PROPERTIES OF PAPILLOMAVIRIDAE

1. All are oncogenic in lab animals
2. Non - enveloped
3. Icosahedral
4. DS DNA Genome [circular]
   - → regulatory region
   - → Late region → L1 L2 → capsid
   - → Early region → E1, E2, E3, E4, E5, E6, E7
     - • E6 → inactivate products of P53 gene
     - • E7 → inactivate products of RB gene

INFECTIONS CAUSED BY PAPILLOMA VIRUS

→ cause infections in the breaks of skin & mucous membrane, exposing the basement membrane

TYPES OF PAPILLOMA VIRUS ON NUCLEOTIDE SEQUENCING



DISEASES CAUSED BY PAPILLOMA VIRUS

1. SKIN WARTS
   - → most common types → 1 - 8
   - → Epidermoplasia verruciformis / TREE MAN SYNDROME caused by HPV 5, 8

2. GENITAL WARTS
   - → condyloma acuminata, Laryngeal warts } caused by HPV 6, 11

3. CARCINOMA
   - → FEMALES → cervical carcinoma, vulval, vaginal, Anal carcinoma
   - → MALES → Anogenital carcinoma [Penile, Anal]

   - → VACCINES
     - a. CERVARIX [Bivalent vaccine] → HPV 16, 18
     - b. GARDASIL [Quadrivalent vaccine] → HPV 6, 11, 16, 18
     - c. GARDASIL - 9 → HPV 6, 11, 16, 18, 31, 33, 45, 52, 58

DIAGNOSIS

HISTOPATHOLOGY

- → Koilocytosis
- → cytoplasmic vacuolation & nuclear enlargement

PCR for HPV

condyloma acuminata

RNA VIRUSES

PICORNAVIRIDAE

Includes small RNA viruses

① Polio virus
② coxsackie virus
③ Entero virus
④ ECHO [Entero cyto Pathogenic Human Orphan virus] → Aseptic meningitis
⑤ Rhino virus → common cold virus

POLIO VIRUS

→ Type 1 → most common wild type
Type 2 → most antigenic
Type 3 → VAP [vaccine associated Paralysis]

SWITCH OVER

→ New vaccines are Bivalent from old vaccines [Trivalent]
→ NO Type 2 Strain

POLIO

→ Route → Faeco oral contamination
→ >95% → inapparent infections
<1% → paralysis (Anterior horn cells are damaged)

→ Tonsillectomy should be avoided during a polio epidemic
→ more physical activity should be avoided during polio epidemic

→ CULTURE → on Monkey kidney cell lines
ELISA | PCR for Polio Virus

→ ANTIGENS
C antigen [coreless antigen] } Type specific
D antigen [Dense antigen] }

COXSACKIE VIRUS

COXSACKIE VIRUS A

→ Causes
Flaccid Paralysis in mice
Herpangina [Acute vesicular pharyngitis]
Acute haemorrhagic conjunctivitis [cox A 24 + Enterovirus -70]
HFMD [Hand foot Mouth Disease] → cox A16
Aseptic meningitis

COXSACKIE VIRUS B

→ Causes

- Bornholm's disease [Acute pleurodynia or Devil's grip]
- DM [B_4]
- HFMD
- Aseptic meningitis

→ inoculation into mice cause Spastic paralysis

→ CULTURE on Newborn Suckling mice [<48 hrs]
Arboviruses also cultured

ENTEROVIRUS

→ includes

- Enterovirus 70 → causes Acute Haemorrhagic conjunctivitis
- Enterovirus 71 → causes HFMD
- Enterovirus 72 → Hepatitis A

MYXOVIRUSES

ORTHOMYXOVIRIDAE

includes

- Influenza A, B, C

PARAMYXOVIRIDAE

includes

- Parainfluenza viruses
- mumps
- Measles
- Rubella [Togaviridal family, Arbovirus
- RSV
- metapneumo virus
- Nipah virus [Henipa virus]

ORTHOMYXOVIRIDAE

INFLUENZA A, B, C

→ SS RNA

→ negative sense
↓ RNA Polymerase
tive sense

SEGMENTED GENOME		
B	→	Bunya virus
I	→	Influenza virus
R	→	Reo virus
A	→	Arena virus

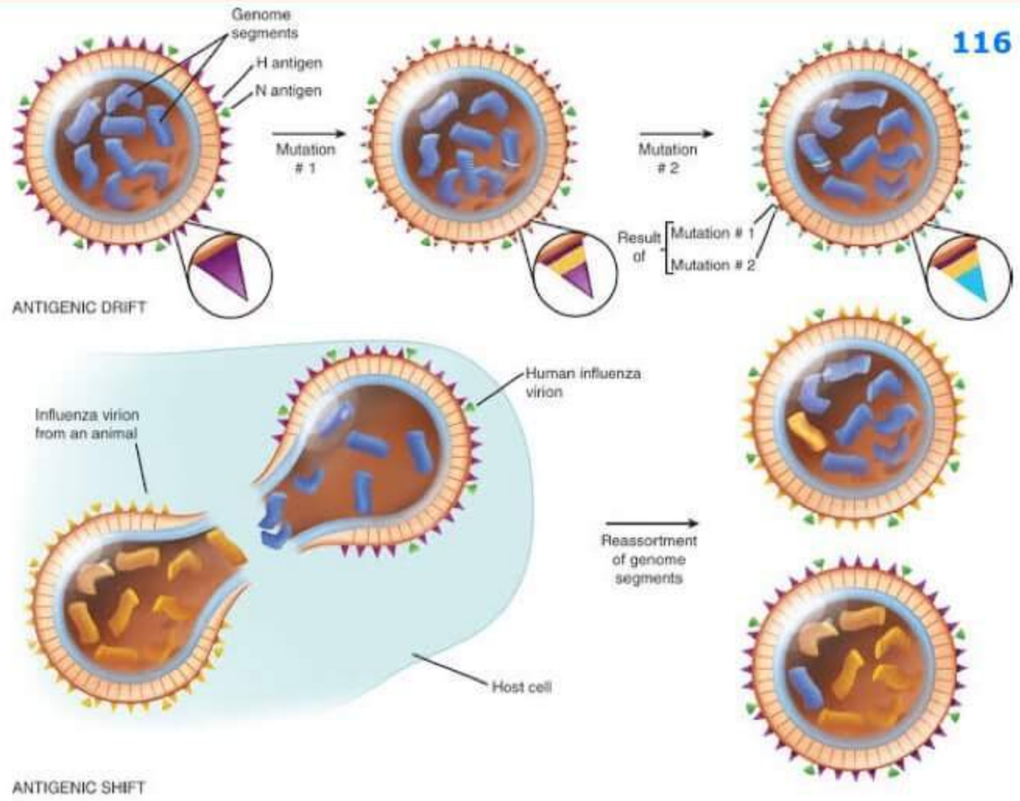
→ have segmented genome

- A → 8 Segments
- B → 8 Segments
- C → 7 segments

→ Glycoprotein H [Hemagglutinin] → adheres to cell
Glycoprotein N [neuraminidase] → Recepor Destroying Enzyme
– process is known as ELUTION

ANTIGENIC SHIFT

→ abrupt, drastic & discontinuous process
→ dlt Reassortments
→ causes Pandemic
→ $H_3N_2 \longrightarrow H_1N_1$



ANTIGENIC DRIFT

→ Single point mutation & continuous in nature
→ $H_3N_2 \longrightarrow H_{3a}N_{2b}$
→ Antigenic shift & drift mostly happens in Influenza A
Sometimes in Influenza B

H_5N_1 [BIRD FLU]

→ infected birds transmit to humans
→ human to human transmission not possible
→ $PB1F_2$ gene → targets the mitochondria of cell & damages it

H_1N_1

→ combination of Human influenza strain, swine strain, avian flu strain
→ LAB DIAGNOSIS
1 NASO PHARYNGEAL SWAB
→ taken ĩ proper precautions
→ Extract RNA [ĩ RNA Extraction Kits]
↓
Real time PCR [< 2 hrs]
RNA → cDNA → amplify

→ chemoprophylaxis → TAMIFLU [OSELTAMIVIR], given in initial stages of disease / During an epidemic
→ Killed & live vaccines are available → Temporary immunity

PARAMYXO VIRIDAE

PARA INFLUENZA

→ para influenza type 1 & 2 causes CROUP [Acute laryngotracheo bronchitis]
para influenza type 3 causes LRTI [Lower respiratory tract infection]

MUMPS

→ mc presentation of mumps → B/L Parotitis
→ COMPLICATIONS
U/L orchitis [in young males]
Oophritis [in females]
Aseptic meningitis
Pancreatitis


MUMPS

→ SAR → > 85%.
→ IP → 2-3 WKS
→ LAB DIAGNOSIS → PCR/ ELISA for Mumps virus
→ vaccine strain → JERYL LYNN STRAIN
→ some times Meningo encephalitis preceeds Parotitis

MEASLES

→ most characteristic feature → KOPLIK SPOT [opp. to lower 2nd molar]
→ SAR → > 90%.
→ IP → 10 - 14 days
→ Infectivity period → 4 days Before ← RASH → 5 Days after
→ INCLUSION BODIES → WARTHIN FINKELDAY GIANT CELLS

→ CLINICAL FEATURES
- Diarrhoea
- Pneumonia
- Sinusitis
- Otitis media

→ Chronic / Late (after years) complicatⁿ → SSPE [Subsclerosing Pan encephalitis]
→ vaccine strain → EDMONSTON JAGREB / MORATEN / SCHWARTZ

vaccine is given by Subcutaneous route [BCG → Intra dermal]
constituted by Distilled water [BCG → normal Saline]

→ LAB D_X → PCR/ ELISA for measles virus

RUBELLA / GERMAN MEASLES

→ Togaviridae family & Arbo virus
→ Infectivity period → 1 Week before ← RASH → 1 week after
→ IP → 2-3 weeks
→ FORCHHEIMER SPOTS are seen
→ BLUE BERRY MUFFIN RASH seen over uvula

BLUE BERRY MUFFIN RASH
IUGR
Thrombocytopenia
} BLUE BERRY MUFFIN SYNDROME


Forchheimer spots

→ CONGENITAL RUBELLA SYNDROME
→ classical triad
C → Cataract
H → Heart Disease [PDA]
D → Deafness [sensorineural]


Congenital Rubella Cataract

→ LAB DIAGNOSIS → PCR/ ELISA for measles virus

RSV Respiratory Synctial Virus

→ causes Acute Bronchiolitis
→ DOC → RIBAVIRIN
→ age group → 1 month to 6 months
→ FUSION PROTEINS + nt → Hf multinucleate giant cells
→ LAB DIAGNOSIS → PCR / ELISA For RSV

METAPNEUMO VIRUS

→ Age group → 6 months to 12 months
→ causes Acute Bronchiolitis
→ DOC → RIBAVIRIN
→ LAB DIAGNOSIS → PCR for Metapneumo Virus

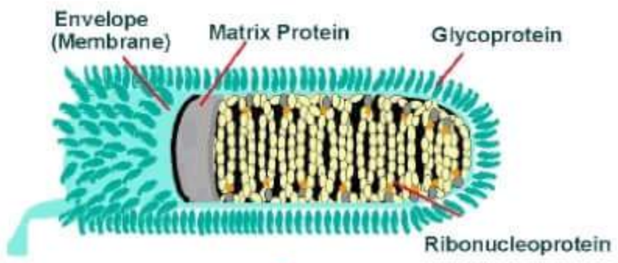
NIPAH VIRUS [HENIPA VIRUS]

→ NIPAH → malaysian village
→ Reservoir → fruit Bats
→ Amplifiers → Pigs
→ MOT → contact ± the secretions of infected person
eating & drinking contaminated food & water
ingestion of contaminated coconut palm syrup

→ mortality → > 70%.
→ LAB DIAGNOSIS → PCR for NIPAH Virus

RABIES VIRUS

→ Family → Rhabdoviridae
Sub family → Lyssa viridae



Rabies virus

→ SS RNA Virus
Negative sense $\xrightarrow[\text{Polymerase}]{\text{RNA}}$ Positive sense

→ Speed of Rabies virus → 3 mm/hr
→ ↓ ACh at various sites & induce neural cell apoptosis

NATURAL / STREET VIRUS	FIXED / DUMB VIRUS
→ causes Natural / furious rabies	→ causes Dumb rabies → used in vaccine production
→ Hydrophobia, Negri bodies } Present	→ Absent

TYPES OF BITES

CLASS I BITES → Licks over intact skin → Nothing

CLASS II BITES → Licks over broken skin → PEP
slight grazing +nt
no oozing of blood

CLASS III BITES → Facial Bites → PEP + Igs
constant oozing of blood +nt
Untraceble animal after bite
Wild animal bites

Post Exposure Prophylaxis [PEP] → 0, 3, 7, 14, 28
→ Im
→ watch out for 10 days

PRE EXPOSURE PROPHYLAXIS → 0, 7, 21 or 28
→ indicated for
Veternarians, Researchers

VACCINES → PCEC [Purified Chick embryo cell vaccine]
PVC [Purified vero cell vaccine]
HDC [Human Diploid cell vaccine]

ANTE MORTEM DIAGNOSIS

→ done ī corneal smear
Nape of neck hair follicle

→ Rabies antigen demonstration by DFAT
DFAT → Direct flourescent Antigen testing

→ by Animal inoculation
→ Now a days PCR is done

POST MORTEM DIAGNOSIS

→ Demonstration of Negri bodies in hippocampus

Brain Biopsy Tissue + Basic Fuchsin → SELLER'S TECHNIQUE
↓
Negri bodies can be demonstrated

→ by Animal inoculation

NEGRI BODIES → Intra cytoplasmic eosinophilic inclusion bodies

DOSE OF RABIES IMMUNOGLOBULIN → Human Rabies Ig [20 units/kg]

HEPATITIS VIRUS

HEP A	Hep B	Hep C	Hep D	Hep E
Picorna viridae	Hepadnaviridae only DNA virus	Flaviviridae	Dependent over B	calciviridae
Faeco oral transmission	Parenteral Sexual & vertical transmission	Parenteral		Faeco oral transmission
IP → 15-50D	IP → 50-150D	IP → 15-150D	IP → 50-150D	IP → 15-50D
Fulminancy 0.1% [Adults]	0.1 - 1%.	0.1%.	5-20%.	1-2%. → 20%. [pregnancy]
chronicity	1 - 10%.	>85%.		
carrier	0.1 - 30%.	1.5 - 3.2%.		

HEPATITIS A

→ only hepatitis virus that can be cultured → has good vaccine

→ LAB DIAGNOSIS → ELISA

1 IgM anti HAV demonstration

a stool examination

b serum examination [more significant]

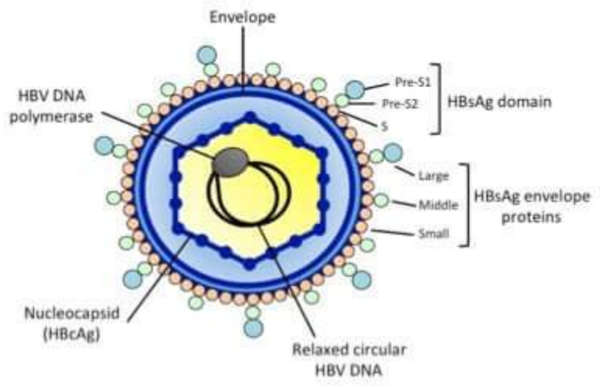
HEPATITIS B

→ FORMS

Spherical → most common

Tubular

Dane particle → complete virus genome [42 nm]



→ ACUTE HEP B

→ HBs Ag + IgM anti HBC → markers for Acute Hepatitis B

IgM anti HBc [c-core Ag] → better marker

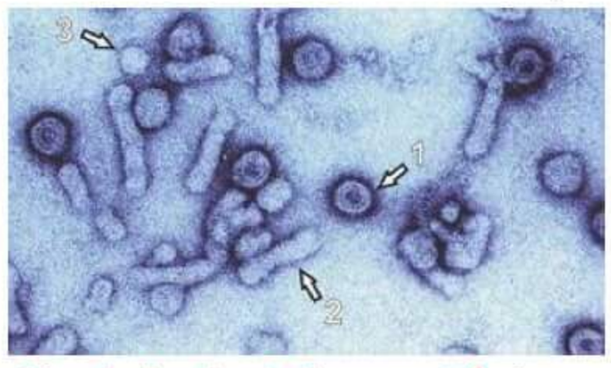
→ HBe Ag +ive → Active Replicating phase
maximum liver damage occurs

→ Anti HBe +ve → Active replicative phase → ↓ing

→ CHRONIC HEP B → >6months HBs Ag +ive

chronicity → 1 - 10%.

carrier Rate → 0.1 - 30%



Morphological forms of HBV

→ if vaccinated,

→ Anti HBs should be > 10 IU/ml

→ Schedule → 0, 1, 6 months

→ Hep B vaccine

- recombinant vaccine
- prepared in yeast

→ GENES

p → polymerase [largest gene]
c → precore
core
s → surface Antigen
x → carcinogenicity

→ pre core mutants → lack HBe Ag & carry poor prognosis
→ Escape mutants → no HBs Ag

LAB DIAGNOSIS

→ PCR for HBV DNA [series of PCR done]
→ Hepa card Test for HBs Ag

HEPATITIS C

→ >85% chronicity → maximum
→ a/w more ↑
Cirrhosis
Hepatocellular carcinoma

→ No Good vaccine available
→ d/t QUASI SPECIES [Antigenic variations]

→ LAB DIAGNOSIS
→ Series of PCR for HCV
→ ELISA
→ HCV Tridot Testing

HEPATITIS D

① Co infection → B+D [reduce each other's virulence]
② Super infection → $\textcircled{B}^{D}$ [more dangerous; 5-20% fulminant]

→ LAB DIAGNOSIS → ELISA for IgM anti HDV

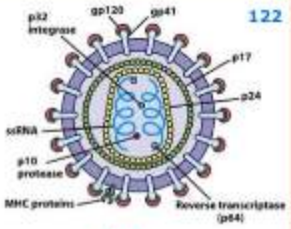
HEPATITIS E

→ fulminancy → 1-2%
→ 20% [in pregnancy]
→ LAB DIAGNOSIS → ELISA for IgM anti HEV [Serum > stool]

NON-ENVELOPED RNA

P → Picornaviridae → Hepatitis A
A → Astroviridae
R → Reoviridae
C → Calciviridae → Hepatitis E

HIV



HIV

→ Family → Retroviridae
Sub family → Lentivirinae

→ Prevalence in India
→ HIV 1 >>> HIV 2
→ in HIV 1 → group 'M' → subtype 'C'

→ STRUCTURAL GENES OF HIV1
gag → codes for capsid, matrix protein, p24, p17, 18
env → codes for Gp120 / GP 41
pol → codes for p31, 51, 61
polymerase / reverse transcriptase

→ LAB DIAGNOSIS
→ most sensitive test → ELISA
most specific test → HIV RNA PCR > Western Blot

→ MODES OF TRANSMISSION [MOT]
① Sexual → 0.1 – 1%. [mc mot in India]
② Blood Transfusion → > 90%.
③ Needle stick injury → 0.5 – 1%. [0.3 – 3%.]
④ Mother to child Transmission → 30%.

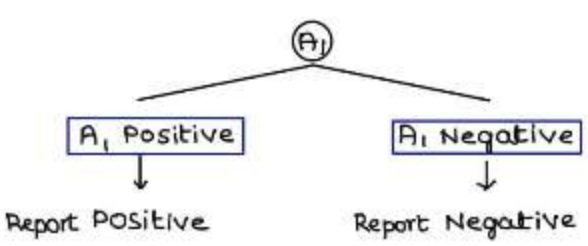
→ can ↓transmission from Mother to child
to 15% → in developing countries
to 0-5% → in developed countries
by even avoiding breast feed

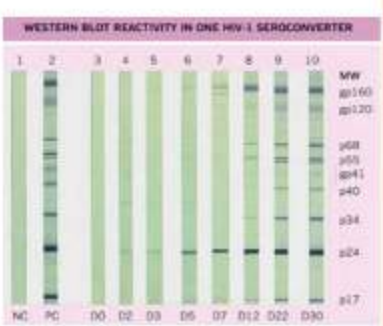
NACO STRATEGIES

STRATEGY I → done in blood banks
STRATEGY II A → done by PSM [for HIV surveillance]
II B → done in Symptomatic HIV persons
STRATEGY III → done in asymptomatic persons

STRATEGY 1 / ALOGRITHM 1

→ done for transfusion & transplantation safety
→ one test kit is required

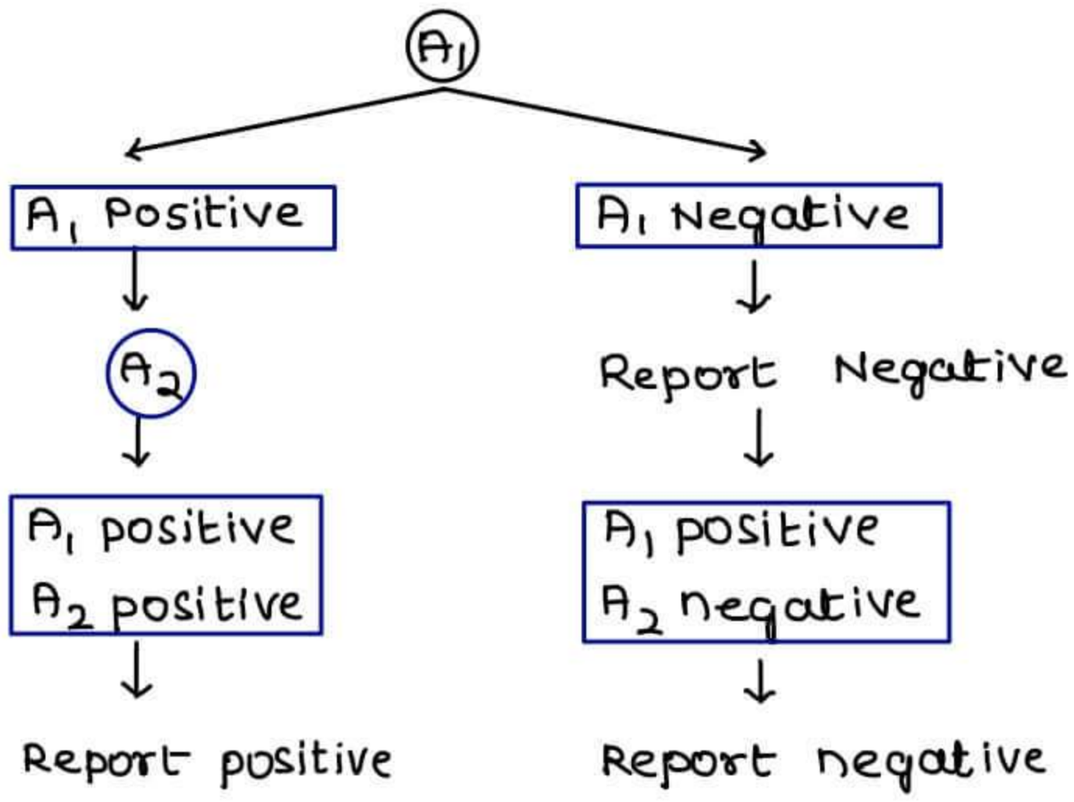




STRATEGY / ALOGRITHM II

→ done for surveillance by PSM department

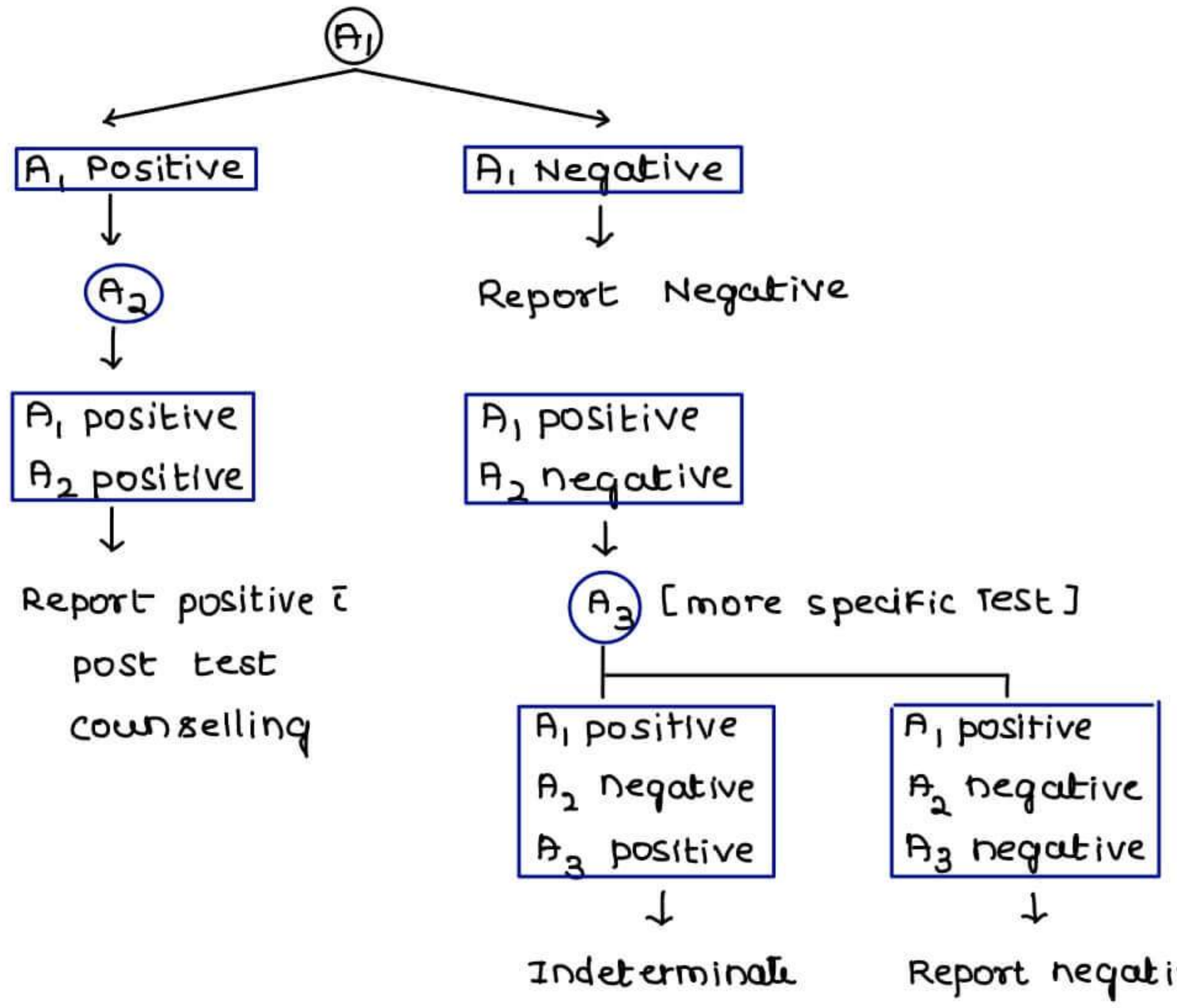
→ 2 test kits are required



STRATEGY / ALOGRITHM II B

→ done in symptomatic individuals

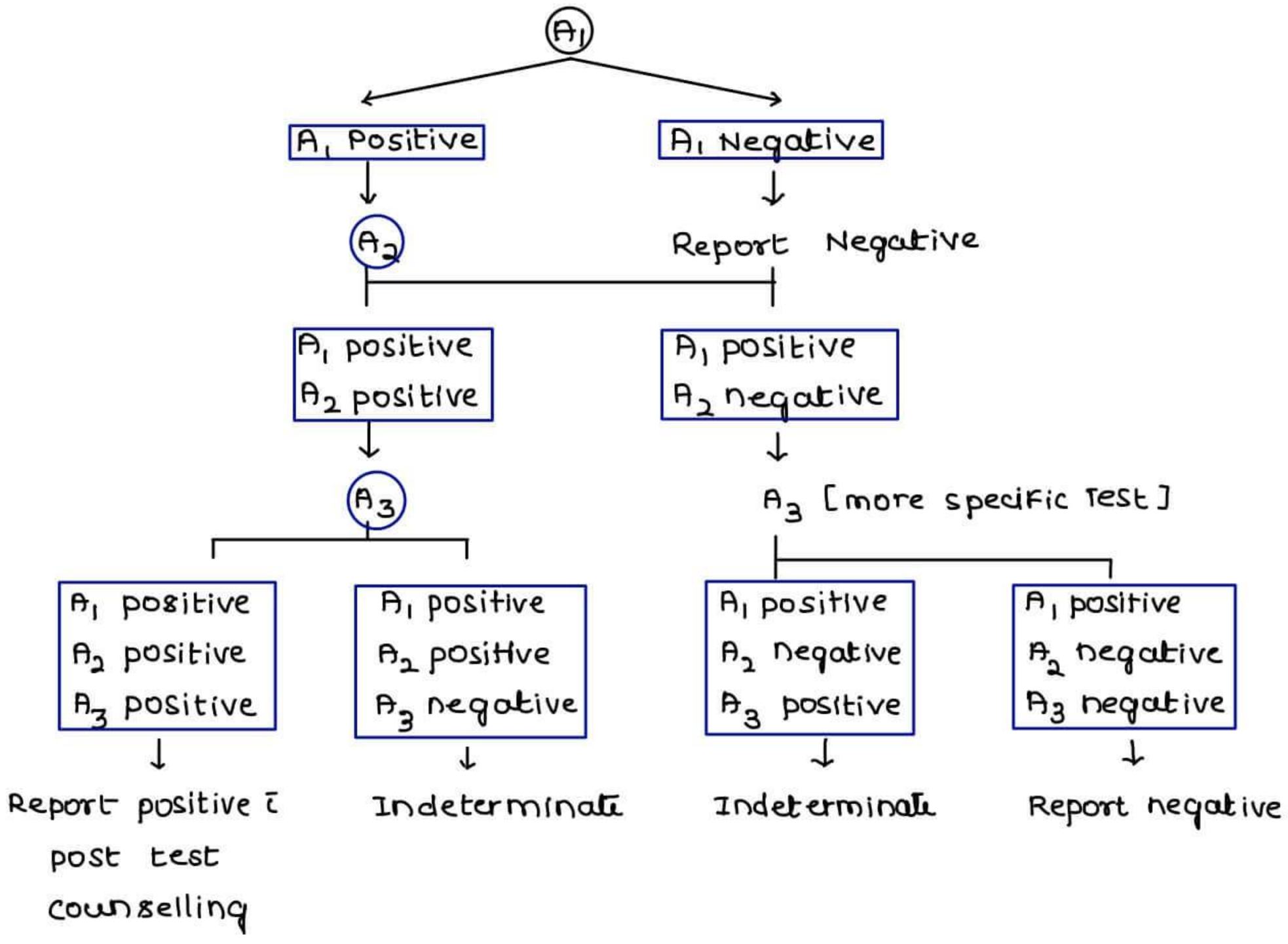
→ 3 test kits required



STRATEGY / ALOGRITHM III

→ done to detect HIV infection in asymptomatic individuals

→ 3 test kits required



CLINICAL FEATURES

① ACUTE HIV SYNDROME

→ occurs 3-6 wks after primary infection

→ burst of plasma viremia +nt

→ resembles acute infectious mononucleosis [glandular fever]

→ most cases follow by prolonged clinical latency

② ASYMPTOMATIC STAGE [CLINICAL LATENCY]

→ median time of latency → 10 yrs

→ Active viral replicatn is ongoing & progressive

→ Lymphoid tissues are major sites of replication [not in blood]

→ It is clinical latency NOT MICROBIOLOGICAL LATENCY

  → low level of viremia +nt

  → Pt. is infective

  → steady progressive decline [not rapid] of CD_4 T cell count

    Average T cell decline → 50/ml per year

  → Rate of disease progression ∝ HIV RNA levels

→ Persistent generalised lymphadenopathy → >1cm in diameter for >3 months in 2 or more non-conigous extra inguinal sites

→ AIDS Related complex

- constitutional symptoms or minor opportunistic infections

③ SYMPTOMATIC DISEASE

→ occurs when CD4 count is very low

→ opportunistic infection & neoplastic diseases present

CD_4 T cell count	Opportunistic infections
400 - 200	Mycobacterium TB Candida (Oral Thrush) Herpes zoster koposi sarcoma Hairy leukoplakia Herpes Simplex
Below 200	P → Pneumocystis carinii C → Cryptococcus neoformans Coccidioidomycosis Cryposporidiosis T → Toxoplasma gondii
Below 50	MAC [mycobacterium Avium Intracellulare] cmv retinitis CNS lymphoma

ARBO VIRUSES

FAMILY	GENUS	Imp. SPECIES
TOGAVIRIDAE	ALPHA VIRIDAE	Chickengunya Eastren Equine Encephalitis virus Westren Equine Encephalitis virus
FLAVIVIRIDAE	FLAVI VIRUS	Japanese encephalitis B, Dengue West nile fever Yellow fever Kyasanur forest Disease virus Zika virus, Hepc
BUNYA VIRIDAE	BUNYA VIRUS	California encephalitis
	PHLEBO	Sandfly fever virus Rift valley fever virus
	NAIRO	Crimen cango Hemorrhagic virus Ganjam virus
	HANTA	Hantaan, Seoul, Puumala
REOVIRIDAE	ORBIVIRUS	Colorado Tick fever
RHABDO VIRIDAE	VESICULO VIRUS	Chandipura virus Vesicular Stomatitis virus

→ Hantaan → causes Hantaan Renal syndrome, Huntaan pulmonary Syndrome
→ a/w Rodent Faeces

INCUBATION PERIODS

Dengue	→	5 - 6 Days
Chikungunya	→	5 - 6 Days
Japanese encephalitis	→	5 - 15 Days
Yellow fever	→	3 - 6 Days
Kyasanaur forest Disease	→	4 - 8 Days

JAPANESE ENCEPHALITIS

→ Reservoir → Ardeid birds [white cranes]
Transmitters → culex tritaeniorhyncus
Culex vishnui
→ Amplifiers → Pigs
→ mortality → 30%.

DENGUE

→ Serotypes → 1, 2, 3, 4, 5
→ transmitted by → Aedes aegypticus
→ ADE [Antibody Dependent Enhancement]

Serotype 1 infected 1st time & recovered
↓
Serotype 2 infected 2nd time
↓ Leads to
DHF [Dengue Haemorrhagic fever]
DSS [Dengue Shock Syndrome]

→ instead of protection in 2nd time infection by antibodies, there Will be promotion to more severe form of Disease
→ ANTIBODY DEPENDENT ENHANCEMENT

→ DHF
→ Fever - Acute, high & Continuous
→ TOURNIQET TEST Positive → > 20 petechial lesions | Sq. inch seen
→ Thromocytopenia → < 100000
→ Haemo concentration → Haematocrit raises by ↑ 20%.

→ DSS
→ DHS Plus Weak & rapid pulse
narrow pulse pressure

→ Break Bone fever occurs in Dengue

→ LAB DIAGNOSIS

① NS 1 ANTIGEN TESTING

→ done in the initial 2 days
→ NS → Non structural or Non Specific protein
→ done by ELISA
→ Upto 5th day → IgM capture ELISA can be done

② PCR for Dengue virus & serotypes

YELLOW FEVER

→ 1st viral disease to be discovered
→ Not present in India
→ Prevented by yellow fever vaccination
→ vaccination certificate is valid for life time
→ 17D vaccine is used
→ trasmitted by Aedes aegypticus

KFD

→ prevalent in Karnataka
→ mild haemorrhagic fever
→ Reservoirs → Squirrels / Rats
→ Transmitter → Ticks
→ Amplifier → monkeys

CHIKUNGUNYA

→ means Bending upon
→ immense joint pain present
→ never complicates

TRANSMISSION

AEDES	CULEX
Y → Yellow Fever	B → Bancroftian filariasis
D → Dengue	E → Encephalitis Japanese
R → Rift valley fever	W → West nile fever
C → Chikungunya	A → Arthritis like Features

CULTURE → on New born suckling mice [<48hrs]

FILOVIRIDAE

IMPORTANT SPECIES

- → MARBURG
  EBOLA VIRUS

- → Filamentous in Shape
- → MOT → contact ī the secretion of infected patients
- → IP → 3 – 15 Days
- → BSL 4 [Bio safety level 4] is maintained
- → IOC → PCR For Ebola virus

ARENA VIRIDAE

- → Segmented genome is present
- → Sandy appearance on electron microscopy
- → Important viruses
  - → Lymphocytic chorio meningitis virus
  - → Lassa virus
  - → Junin virus
  - → Machupo virus

REOVIRIDAE

- → DS RNA virus
- → Important viruses
  - → Orth O reo virus
  - → Orbi virus
  - → Rota Virus
  - → Colti virus

ROTA VIRUS

- → On Electron Microscopy → Wheel with spoke appearance
- → DS RNA virus
- → Segmented genome tnt
- → mc cause of diarrhoea in children
- → IOC → Rota virus antigen detection in stool by ELISA
- → VACCINES → Rotateq / Rotavarix etc are available
- → NSP – 4 → responsible for secretory diarrhoea

VIRUS a/w GASTROENTERITIS

- → Enteric Adenovirus Type 40, 41 → infantile diarrhoea
- → Norwalk virus
- → Calci virus
- → Astro virus

ONCOGENECITY

DNA VIRUSES

→ Papova viridae
→ Herpes viridae

Papova viridae

HPV → Genital warts

Herpes viridae

EBV [HHV-4] → nasopharyngeal CA
Burkitt lymphoma
Hodgkin | Non Hodgkin lymphoma

HHV 8 → Kaposi sarcoma
Primary Effusion lymphoma
castlemann disease

Hep B → Hepato cellular carcinoma

RNA VIRUSES

Retro viridae → HTLV 1 [Adult T cell leukaemia]
Flavi viridae → Hepato cellular carcinoma
Hep C

ZIKA VIRUS

→ Family → Flaviviridae
→ Transmitted by → Aedes
Sexual Transmission
Mother to child transmission
→ if pregnant mother infected
↓
New born might have
microcephaly
GBS [Guillian Barre Syndrome]

PRIONS

→ proteinaceous infectious particles
→ most resistant forms
→ Mechanism of virulence → abnormal folding [PrP → PrPSc]
→ HUMAN DISEASES
K → Kuru
F → familial Fatal insomnia
C → Creutzfeld Jacob disease
gaye → Gerstman Straussler Schienker syndrome

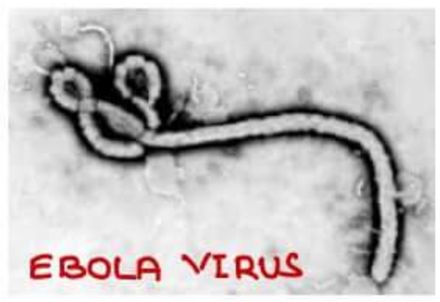
ANIMAL DISEASES

- → Scrapie of goat
- → Bovine spongiform encephalopathy
- → Mink & feline encephalopathy
- → Wasting disease of deer

MUST KNOW

EBOLA VIRUS

- → discovered in 1976
- → Family → Filoriviridae
- → elongated filamentous molecules
- → VIRION contains ss, non segmented, negative sense viral genomic RNA
- → 5 SUB TYPES
  - ZAIRE EBOLA VIRUS [ZEBOV] → most virulent
  - SUDAN EBOLA VIRUS [SEBOV]
  - IVORY COAST EBOLA VIRUS [ICEBOV] → least virulent
  - EBOLA RESTON [REBOV]
  - BUNDI BUGYO EBOLA VIRUS [BEBOV]


EBOLA VIRUS

VIRAL HAEMORRHAGIC FEVER CAUSES BY

① Family ARENAVIRIDAE
- → Lassa Fever [Lassa virus]
- → Lujo virus
- → Argentine [Junin virus]
- → Bolivian [Machupo virus]
- → Brazilian [Sabia virus]
- → Chapare haemorrhagic fever [chapare virus]
- → venezuelan [Guanarito virus]
- → White Water Arroyo virus

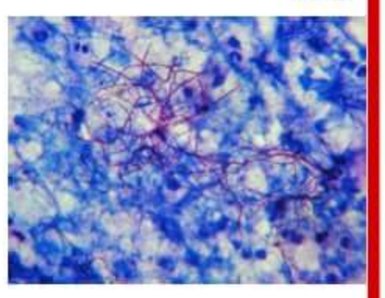
② Family BUNYAVIRIDAE
- → Haemorrhagic fever i renal syndrome [Hanta virus]
- → Crimean - Congo hemorrhagic fever virus from genus Nairovirus, reported from Gujrat
- → Garissa virus and Ilesha virus from Orthobunya virus
- → Rift valley fever virus from genus Phlebovirus

③ Family FILOVIRIDAE
- → Ebola virus
- → Marburg virus

④ Family FLAVIVIRIDAE
- → bengue & yellow fever and
- → Tick borne encephalitis group → Omsk Hemorrhagic fever virus
  Kyasanur forest Disease Virus

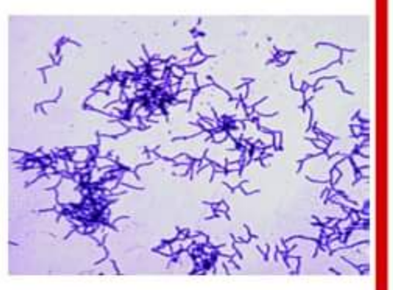
⑤ Family RHABDOVIRIDAE

NOCARDIA



→ 1% Acid Fast
→ associates ī actinomycetoma
→ Pulmonary involvement is +nt mainly
→ cultured by Paraffin bait Technique

ACTINOMYCETES



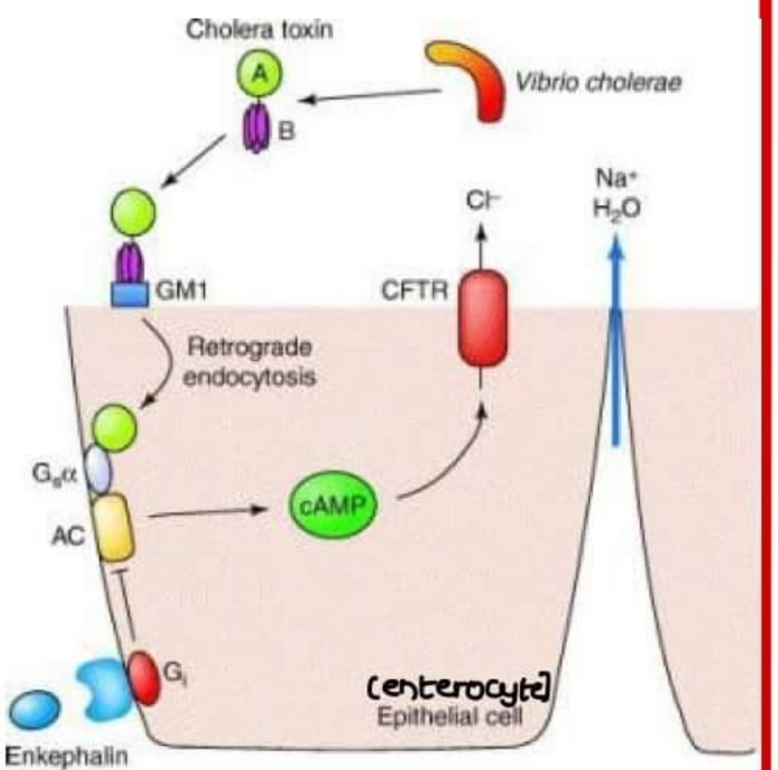
→ Gram positive bacilli ī 'ANTLER-LIKE BRANCHING'
[Filamentous branching]
→ modified acid fast negative
→ NON acid fast mostly
→ mostly non cultured
→ leads to Actinomycetoma
→ causes Oral Actinomycosis [mc Location - Cervicofacial]
→ Sulphur granules are seen
→ Sun Ray appearance

CHOLERA TOXIN

→ Cholera toxin binds to enterocytes [intestinal cells]
ī interact^{n} of Pentameric B Sub unit of toxin
ī GM_1 ganglioside receptor on enterocyte

↓

Endocytosis of toxin



→ A/B Cholera toxin undergo clevage of A_1 domain from A_2 domain & A_1 becomes active enzyme

→ In the cytoplasm, active A_1 activates the G protein $G_{s\alpha}$ through an ADP ribosylation reaction that locks G protein in its GTP-bound form & continoually stimulate to produce cAMP

→

High cAMP levels

↓

Activates CFTR

[cystic Fibrosis Transmembrane conductance Regulator]

↓

Dramatic efflux of ions & water from infected enterocytes

↓

WATERY DIARRHOEA

→ ENKEPHALINS IN R_1 OF CHOLERA
→ by direct action on enterocytes
→ MOA

binds to Opioid receptors on enterocytes

↓

inhibit stimulat^{n} of cAMP through G proteins

GAMMA DELTA T CELLS [γδ T cells]

→ have distinctive T cell Receptor
  → most T cells composed of α & β glycoprotein chains
  → γδ T cells composed of one γ & one δ glycoprotein chains

→ Less common the αβ T cells
  But highest in the gut mucosa, ∴ in INTRA EPITHELIAL LYMPHOCYTES

→ do not require antigen processing & MHC presentat^{n} of epitopes

GAS PLASMA STERILIZATION

→ GAS PLASMA
  → Fourth state of matter [after solid, liquid, gas]
  → generated in an enclosed chamber under deep vaccum using radio Frequency or microwave energy to produce charged particles from gas
  → many of them are FREE RADICALS [atom ∴ unpaired electron, highly reactive]
  → Gas used is H_2O_2

→ Effectiveness depends on
  1 Type of seed gas used
  2 depth of vaccum

CORONA VIRUS

→ Family → corona viridae
  Subfamily → corona virinae

→ causes SARS [Severe Acute Respiratory Syndrome]
  → causes both Upper & Lower respiratory tract infections
  → presents ∴ muscle pain, headache & fever followed in 2-14 days by onset of respiratory symptoms

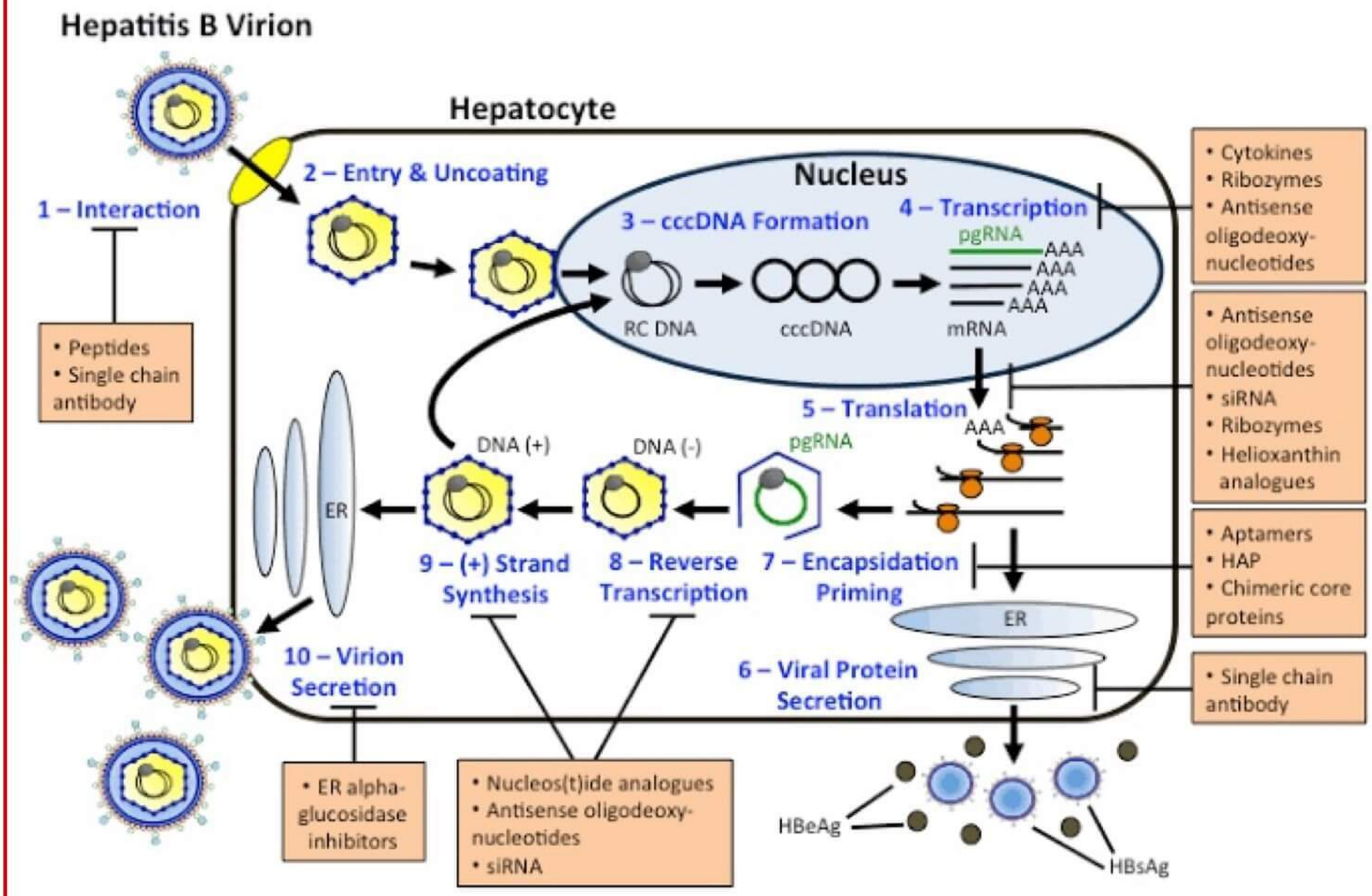
→ 6th new type identified in 2012
  Initial name → NOVEL CORONA VIRUS
  New name → MIDDLE EAST RESPIRATORY SYNDROME CORONA VIRUS [MERS - CoV]

CRYOGLOBULINEMIA

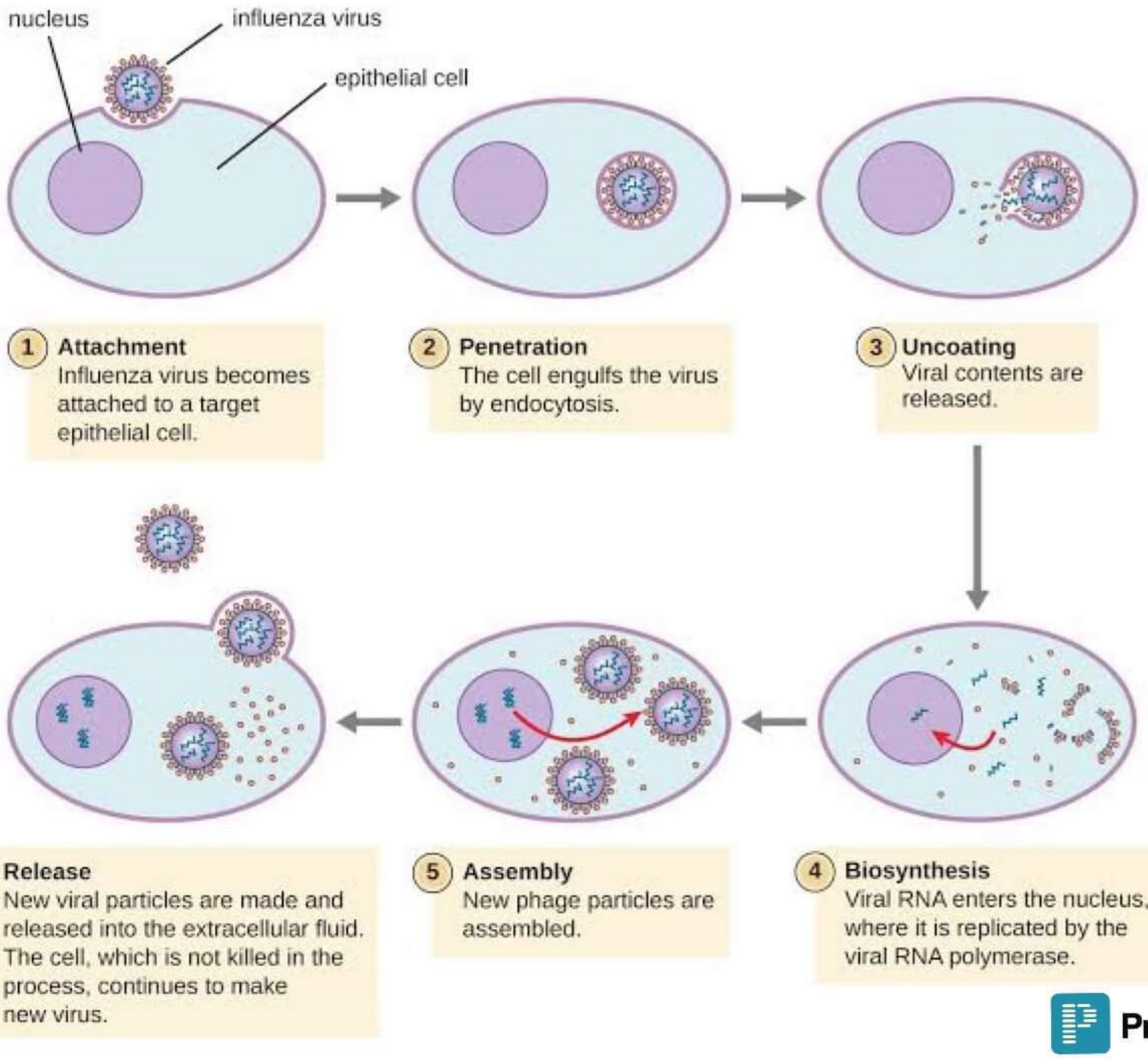
→ characterized by clonal expansion of rheumatoid factor expressing B cells in liver, LN & peripheral blood, resulting in presence of cryoglobulins in circulat^{n}.

→ CRYOGLOBULINS
  → cold insoluble immune complexes
    contains Rheumatoid factor, polyclonal Ig G, HCV RNA
  → they precipitate & deposit on vascular endothelium, causing VASCULITIS in skin, kidneys & peripheral nerves

HEPATITIS B VIRION



INFLUENZA VIRUS PATHOGENESIS



HIV

1 Fusion of HIV to the host cell surface.

2 HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.

gp120

CD4

Co-receptor (CCR5 or CXCR4)

Preintegration complex

Host Cell

3 Viral DNA is formed by reverse transcription.

Viral RNA

Reverse transcriptase

4 Viral DNA is transported across the nucleus and integrates into the host DNA.

Integrase

Viral DNA

Host DNA

New viral RNA

Mature Virion

5 New viral RNA is used as genomic RNA and to make viral proteins.

6 New viral RNA and proteins move to the cell surface, and a new, immature HIV forms.

7 Virus is released. Viral protease cleaves new polyproteins to create mature infectious virus.

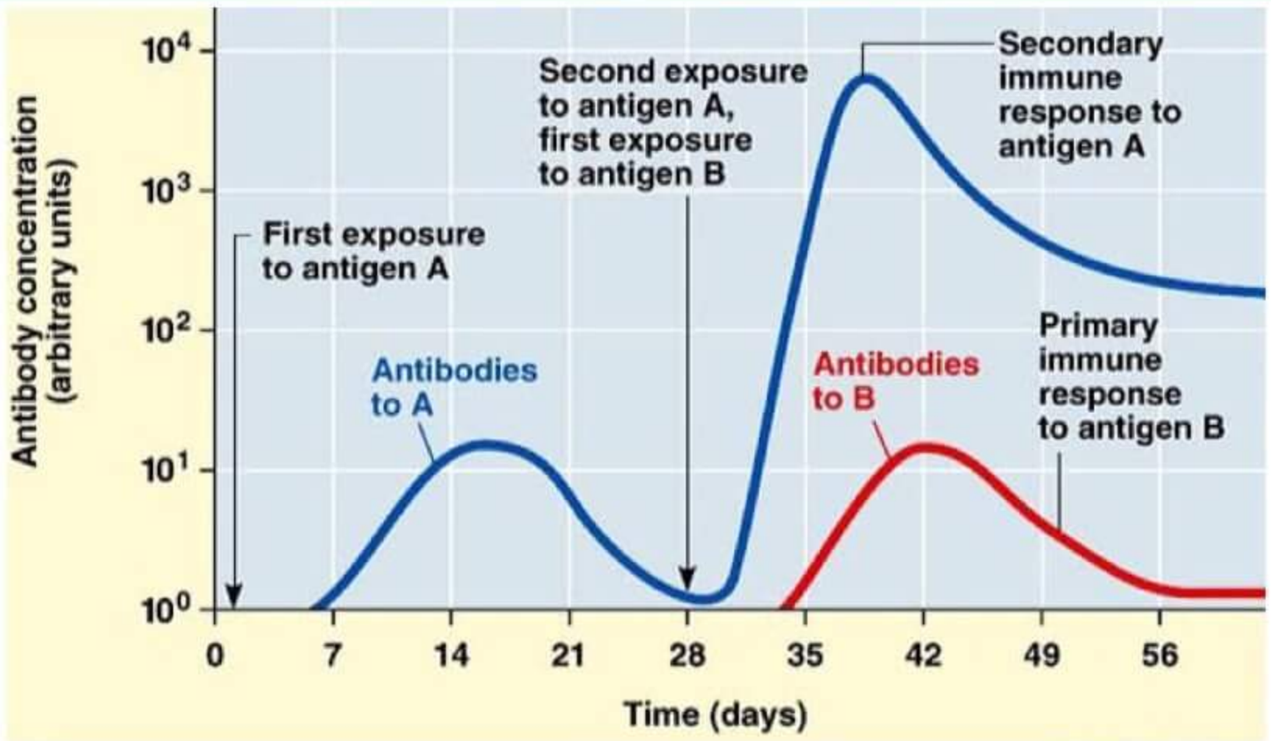
IMMUNOLOGY

IMMUNITY → Resistance offered by the host against offenders

INNATE IMMUNITY	ACQUIRED IMMUNITY
Resistance to the infection from birth by its genetic or constitutional make up	Resistance to infection acquires during life time
Depends on S Species specific I Individual specific R Race specific	Different forms Natural Active → infections Natural Passive → mother to child Artificial Active → vaccines Artificial Passive → Immunoglobulins
COMPONENTS Phagocytes [monocytes, macrophages, neutrophils] NK (natural Killer) cells Alternate complement pathway Acute Phase Proteins [CRP, MBP, Serum amyloid protein] Normal resident Flora Inflammation, fever Skin & mucosal barrier	COMPONENTS B cell T cell classical complement Pathway Antigen Presenting cells [APC]
occurs in minute Diversity Limited non specific no memory	occurs in days against wide range of infections specific memory present

MBP → mannose Binding Protein

PIR [Primary Immune Response]	SIR [Secondary Immune Response]
→ Slow to start Sluggish Short Lived	→ Prompt Powerful Prolonged
→ IgM produced mostly	→ IgM → IgG ISOTYPE/CLASS SWITCHING – occurs d/t change in the constant portion of heavy chain



ANTIGEN

→ Any foriegn substance which induces antibody formation under optimum conditions

→ HAPTEN → incomplete antigen
→ requires carrier protein to be immunogenic

→ FACTORS AFFECTING IMMUNOGENECITY

① Nature of the substance
② More Molecular weight & size → ↑ Antigenic
③ Tissue enzyme response
④ Foreigness ∝ immunogenecity
∝ Hypersensitivity

NATURE OF SUBSTANCE

Proteins > carbohydrates > Lipids > Nucleic Acids

SUPER ANTIGENS

→ non MHC restricted

→ attached to TCR on Lateral side on Vβ (variable β) domain
↓
induces > 20% T lymphocytes activatn
↓
Leads to massive lymphokine release
↓
SHOCK

→ Examples

① STAPHYLOCOCCAL TOXINS
Enterotoxin A → Food Poisoning
Exfoliate Toxin A,B → SSSS [Staphylococcal Skin Scalded syndrome]
Entero Toxin F → Toxic shock syndrome Toxin

② Streptococcus → Scarlet fever / Erythrogenic toxin A & C
③ Mycoplasma arthritis
④ Malasezzia furfur
⑤ Yersinia enterocolitica
⑥ EBV
⑦ CMV
⑧ HIV
⑨ Rabies virus

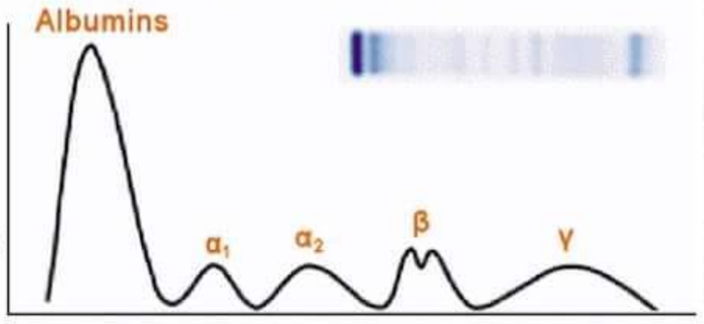
T INDEPENDENT ANTIGEN	T DEPENDENT ANTIGEN
→ Simple → LPS, capsule, Flagella	→ Complex
→ Dose dependent	→ immunogenic over wide range of dose
→ IgM & IgG3	→ All Ab rise
→ NO memory	→ Memory + nt
→ NO macrophage processing	→ macrophage processing needed
→ Rapidly metabolised	→ rapidly metabolized
→ Directly acts on B cells & causes polyclonal B cell activat^{n}	

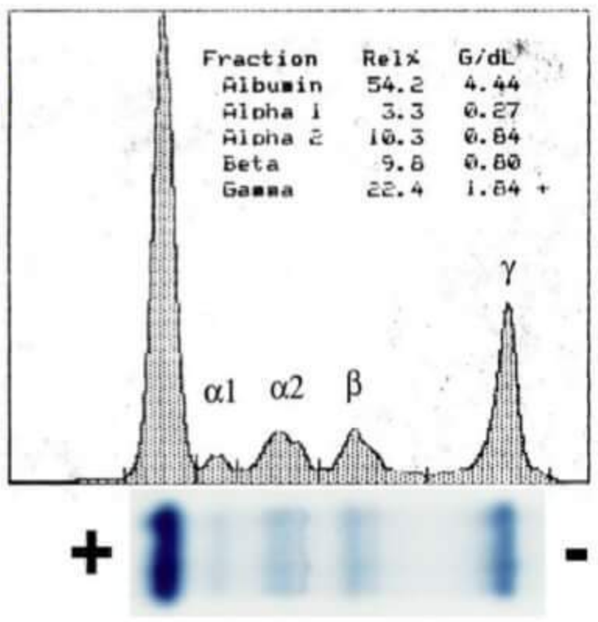
ANTIBODIES

→ Antibodies are immunoglobulins [γ globulins]

→ KABAT & TISELIUS found them by doing electrophoresis

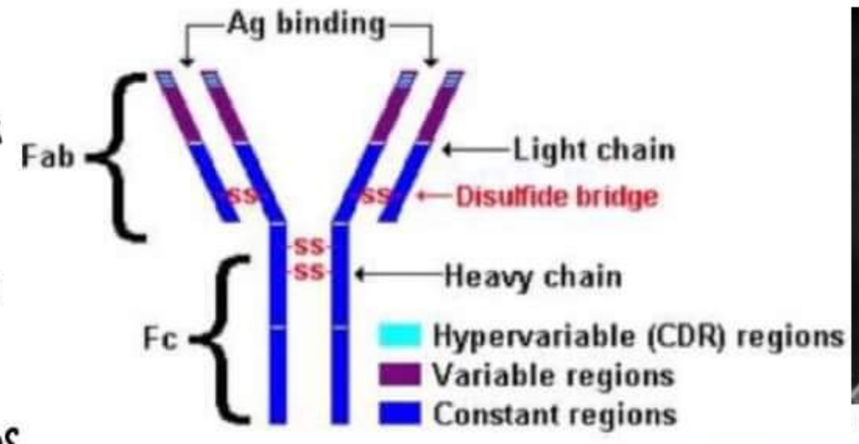
Figure 1: Normal electrophoretic graph and Blood Proteins





ANTIBODY STRUCTURE

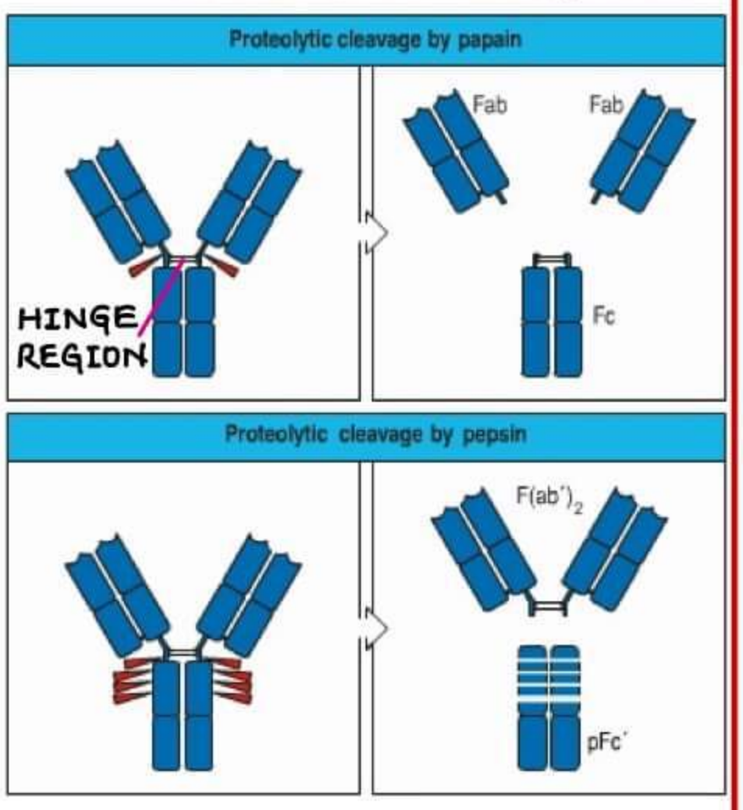
Each antibody consists of
2 Light chains & 2 Heavy chains
Each light chain have
1 variable & 1 constant region
Each heavy chain have
1 variable & 3/4 constant regions




Gerald M. Edelman

Rodney R. Porter

Upper portion → Fab [ab = antigen binding]
Lower portion → Fc [c = constant portion]



HYPER VARIABLE REGION / PARATOPE / HOTSPOTS

→ Actual Antigen binding takes place here
- Epitope of antigen binds here

→ present in both Light & heavy chains

Light & Heavy chains binds ī each other by Disulfide bond
Two heavy chains binds ī each other by Disulfide bond

pApain cleaves Above the hinge region → 3 fragments [2Fab + 1Fc] released
Pepsin cleaves Below the hinge region → 2 fragments [$F(ab')_2$ + Fc'] released

PROPERTIES OF ANTIBODIES

Ig G

- → maximum in concentratⁿ
- → maximum $t_{1/2}$ → 23 Days
- → Sub Groups → Ig G_1, G_2, G_3, G_4
  G_1 → mc
- → Fc portion of Ig G involves in Opsonisation [enhancement of phagocytosis]
- → only Ig which pass through placenta
  Exceptⁿ → Ig G_2 doesn't cross placenta
- → Ig G_4 → doesn't takes place in complement pathway
  Ig G_3 → doesn't involve in coagglutinatⁿ

- → Role of Ig G is more in precipitation [Precipitatⁿ > Agglutinatⁿ]

Ig A

- → Ig A_1 → present in serum
  Ig A_2 → present in mucosal surface & body secretions

- → Dimer form bounded by joining chains
- → mc selective Ig deficiency → Ig A

Ig M

- → maximum molecular weight
- → aka MILLIONARE MOLECULE
- → has maximum sedimentatⁿ co-efficient
- → valency → 2×5 = 10
  Pentamer

- → 1st Ig to be produced in any immune response
- → MA → Both have joining chains
- → also play a role in Opsonisation

Ig M & D → present over Naive B cells

Ig E

→ minimum in concentratⁿ

- → shortest $t_{1/2}$ → < 2 Days
- → plays a role in HOMOCYTOTROPISM (movement towards mast cells)
  - Occurs in Type 1 Hypersensitivity

- → only heat labile antibody
- → aka REAGINIC ANTIBODY

LIGHT CHAINS

- → Kappa : λ → 2:1

ANTIGEN ANTIBODY INTERACTIONS

POST ZONE [Antigen Excess]

→ ZONE OF EQUIVALENCE [Antigen = Antibody → LATTICE]

PROZONE [Antibody Excess]

→ most reactions happen in zone of Equivalence

SEROLOGICAL REACTIONS ≅ Ag Ab Interactions

AFFINITY → INTENSITY OF ATTRACTION between the antigen & antibody

AVIDITY → STRENGTH OF BOND after the formatⁿ OF antigen & antibody complex

COMPARITIVE EFFICIENCY OF IG IN DIFFERENT SEROLOGICAL REACTIONS

REACTION	IgG	IgM	IgA
Precipitation	Strong	weak	variable
Agglutination	weak	Strong	moderate
complement fixation	Strong	weak	negative
Lysis	Weak	Strong	negative

PRECIPITATION

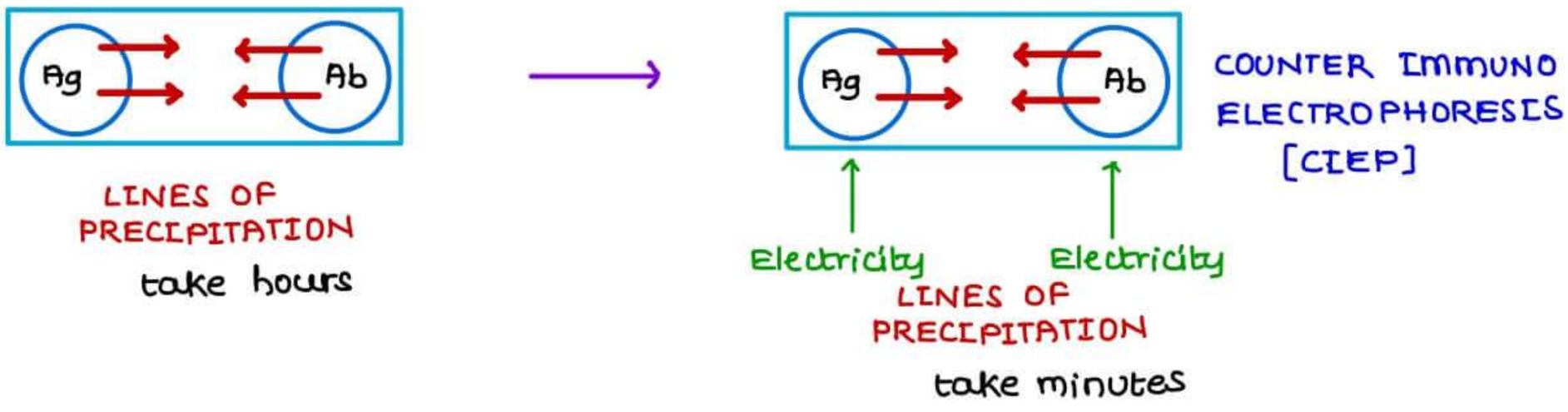
→ Soluble antigen + Antibody → Insoluble precipitate

Eg → ASCOLIS THERMO PPT. TEST → Done for Anthrax antigen

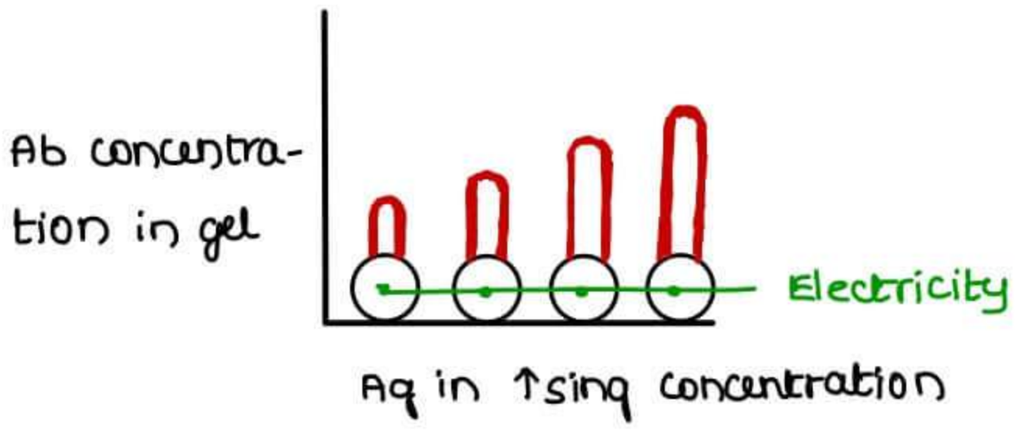
→ FLOCCULATION → modificatⁿ of precipitatⁿ

Eg → VDRL (Best test to assess prognosis)

→



→ ROCKET ELECTROPHORESIS



IMMUNO DIFFUSIONS

① SINGLE DIFFUSION IN ONE DIRECTION [OUDIN PROCEDURE]

Antibody incorporated in agargel in a test tube
↓
Antigen solutⁿ layered over it
↓
Antigen diffuses downward
↓
Form a line of precipitation
↓
Number of bands indicates
the number of different antigens present

② DOUBLE DIFFUSION IN ONE DIMENSION [OAKLAY – FULTHORPE PROCEDURE]

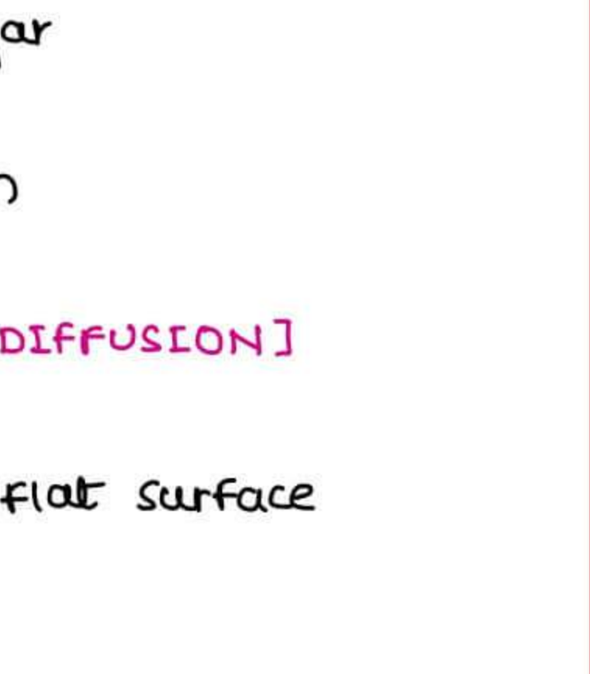
Antibody incorporated in gel
↓
A column of Plain agar placed above it
↓
Antigen layered on top of agar
↓
Antigen Antibody move towards each other
through the intervening column of plain agar
↓
Band of precipitate at Optimum proportion

③ SINGLE DIFFUSION IN TWO DIMENSIONS [RADIAL IMMUNODIFFUSION]

Antiserum incorporated in agar gel poured on a flat surface
↓
Antigen is added to the wells
↓
Ag diffuses radially & forms rings shaped bands of precipitatⁿ → HALO
Diameter of halo → concentratⁿ of antigen

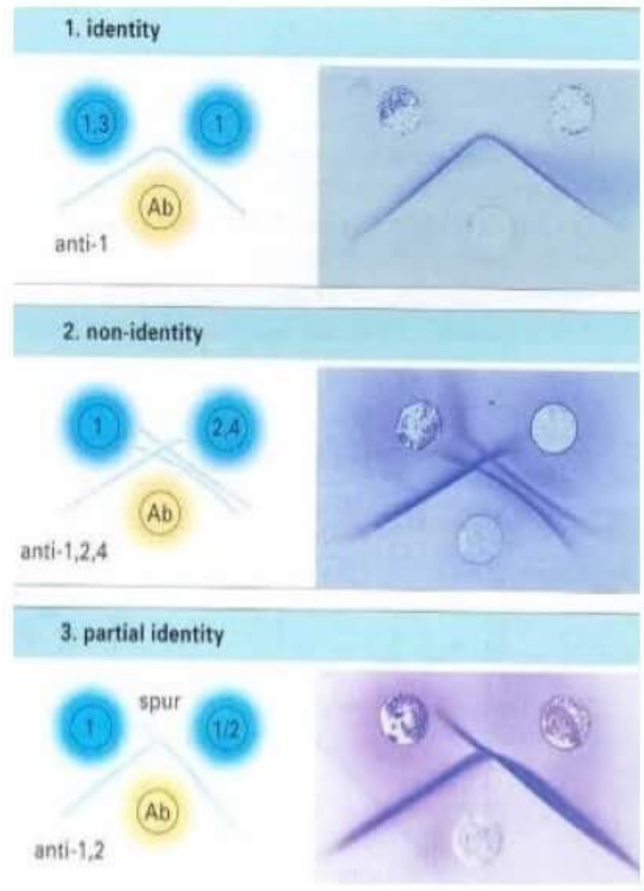
USES

① Estimatⁿ of Ig in sera
② Screening of antibodies to influenza viruses



RADIAL IMMUNO DIFFUSION

④ DOUBLE DIFFUSION IN TWO DIMENSIONS [OUCHTERLONY PROCEDURE]



Agar gel poured on a slide
↓
Wells are cut
↓
Antiserum placed in the central well
Antigens in the surrounding wells
↓
if two adjacent antigens are
identical → Line of precipitate fuse

if unrelated → Lines cross each other

partial identity → Spur formation

AGGLUTINATION

→ Insoluble antigen + Antibody → CLUMPS
[Particulate antigen]

→ AGGLUTINATION >>> PRECIPITATION

→ EXAMPLES

① WIDAL TEST → Antibodies against 'O' & 'H' are measured
② CAT → COLD AGGLUTINATION TEST → for Mycoplasma
③ SAT → STANDARD AGGLUTINATION TEST → for Brucellosis
④ MAT → MICROSCOPIC AGGLUTINATION TEST → for Leptospirosis
⑤ COOMB'S TEST

DIRECT COOMB TEST → done for fetal Ab [In vivo test]
INDIRECT COOMB TEST → done for Maternal Ab [In Vitro test]

coomb's sera → Antiglobulin
Incomplete IgG Antibodies

Rh^+ father + Rh^- mother → Rh^+ child
Do incomplete IgG Antibodies testing
if Free incomplete IgG Antibodies in maternal serum
+
O Rh^+ RBCs [For tagging incomplete IgG Ab] [in vitro]
+
COOMB SERA
↓
AGGLUTINATION

PASSIVE LATEX AGGLUTINATION TEST

→ Precipitation → Agglutination

Soluble antigen + Latex particle → insoluble antigen

→ Examples

ASO

CRP

Rheumatoid factor Test

NEUTRALISATION REACTIONS

→ Toxin + Antitoxin

→ Examples

① SCHICK TEST → done for Diphtheria

② NAGLER'S REACTION → done for clostridium perfringens [α lecithinase]

COMPLEMENT FIXATION TEST

① WASSERMAN REACTION → done for syphilis

② SABINFELDMAN DYE TEST → done for Toxoplasmosis

→ Antigen + Antibody + RBC system + complement

↓

if Ab present

↓

Ag + Ab complex

↓

activate complement &

complement attaches to complex

↓

NO HEMOLYSIS [Ab present]

CARD TESTS

→ based Immuno chromatographic principle

→ sample runs on nitrocellulose paper

→ Eg. HEPA CARD → Test becomes positive if HBS Ag present

→ if control is negative, then card test is not valid

IMMUNO FLUORESCENCE

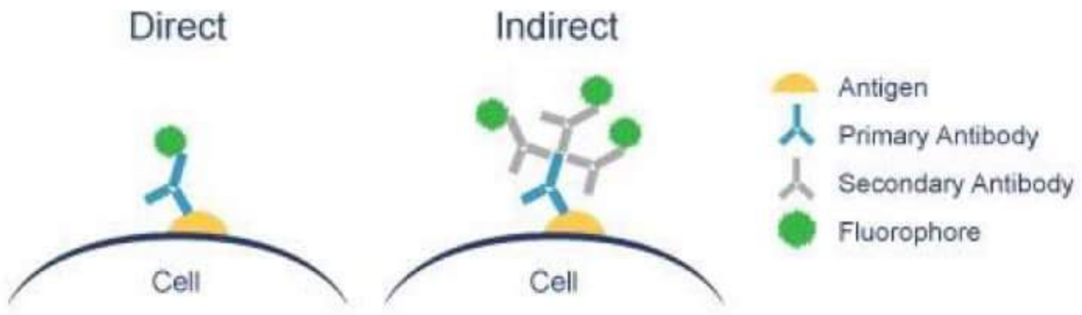
→ TYPES

Direct

Indirect

→ Fluorescent dyes

Fluorescein isothiocyanate
Lissamine rhodamine
Rhodamine - Auramine etc



→ DIRECT IMMUNOFLUORESCENCE

- Detect antigens
- Principle → Antibodies tagged ī fluorescent dyes
  ↓
  Detect unknown antigen

- Uses
  Detectⁿ of bacteria, virus, other antigens in blood, CSF, urine, Faeces, tissues
  Diagnosis of Rabies [DFAT - Direct fluorescent Antigen Testing]

- Disadvantage → Specific Fluorescent labelled Ab required

→ INDIRECT IMMUNOFLUORESCENCE → Detection of Antibody

Known antigen
↓
unknown antibody [serum]
↓
Ab presents - binds ī antigen
↓
Fluorescene tagged Ab to human globulin is added
↓
Fluorescence occurs

→ Advantage → single antihuman globulin fluorescent Ab to any Ag

ELISA [ENZYME LINKED IMMUNO SORBENT ASSAY]

TYPES

① Direct ELISA
② Indirect ELISA
③ Compititive ELISA
④ Sandwich ELISA
⑤ capture ELISA

COMPONENTS

① ENZYME CONJUGATE
- Enzyme conjugated Ab
- Used as the indicator Ab

→ commonly used enzymes
  ⓐ Horse Radish Peroxidase [HRP] – more widely used
  ⓑ Alkaline Phosphotase [AP]

→ There is no significant difference b/w test sensitivities

② SUBSTRATE & CHROMOGEN

→ used as indicator in detecting enzyme - substrate reaction

→ commonly used substrates
  ⓐ p - Nitrophenyl Phosphate [pNPP]
  ⓑ Hydrogen peroxide – 0.003 - 0.015%

→ Chromogen – are light sensitive so it is necessary to incubate in dark

They are
TMB → Tetra Methyl Benzidine
ABTS → Azino Benthiazoline Sulphonic Acid
OPD → O - phenylene Diamine

→ Absorbance (wavelength) commonly used is 450 nm
Other wavelength used are 620 nm
490 nm
405 nm etc
They depend upon supplied kits

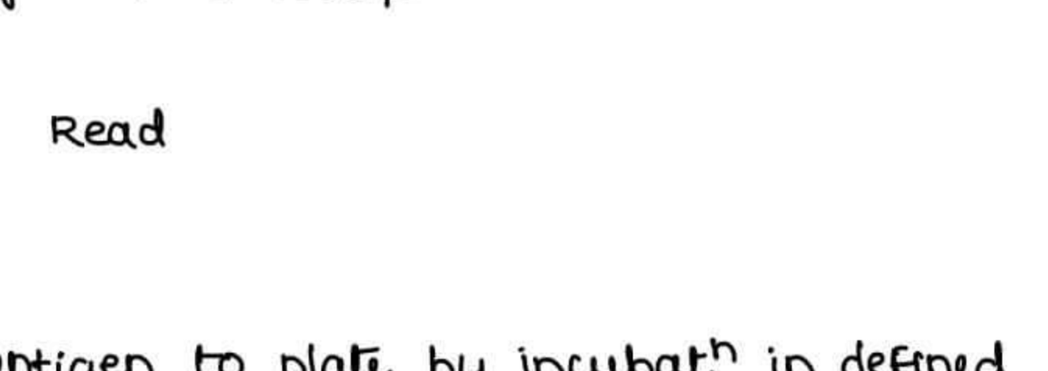
DIRECT ELISA → Detects particular Antigen in a sample

→ $\underset{W}{IAg} + \underset{W}{Ab^{*}E} + S \rightarrow$ Read

→ Stage (i) → passive absorptⁿ of antigen to plate by incubatʰ in defined buffer
Stage (ii) → Wash
Stage (iii) → Addition & incubation of enzyme labeled antibody
Stage (iv) → Wash
Stage (v) → addition & incubation of color development system

INDIRECT ELISA

→ A well tagged ī HIV Ag taken
→ Patient serum ī Ab_1 added to it
→ $Ab_1 \xrightarrow{\text{injected to}}$ Goat $\xrightarrow{\text{produces}} Ab_2$
Ab_2 + Enzyme + Substrate added
→ IF COLOUR appears → +ive
→ can measure OD values [Optical Density values]

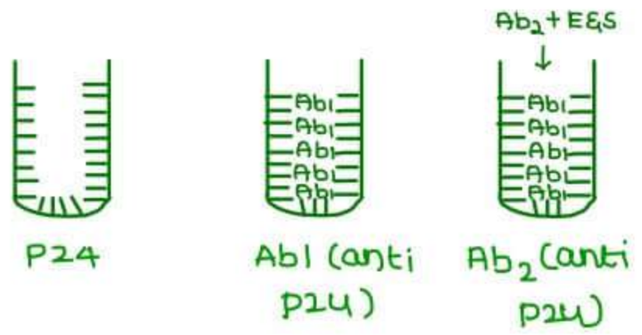


→ COLOR [+ve]

COMPETITIVE ELISA

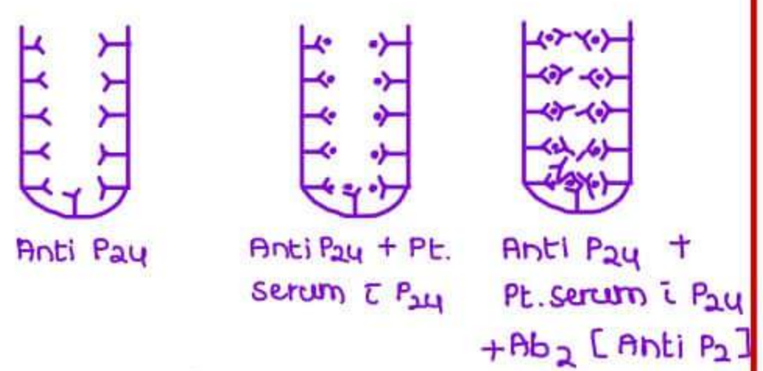
→ A well tagged ī P_{24} Ag taken
→ Patient serum ī Ab_1 (anti P_{24}) added to it
→ P_{24} —injected to→ Goat —produces→ Ab_2 [anti P_{24}]

Ab_2 + Enzyme + substrate added
NO COLOUR → POSITIVE



SANDWICH ELISA

→ A well tagged ī anti P_{24} Ab taken
→ Patient serum ī P_{24} added to it
→ Ab_2 [anti P_{24}] + Enzyme + substrated added
→ IF colour appears → +ive
→ can measure OD (optical density ī Optical densitometer]



HYPERSENSITIVITY

→ An altered state of reactivity to an ANTIGEN / HAPTEN in a sensitized host resulting in an unexpected, exaggerated reaction which is injurious to the host leading to tissue damage / disease / death

→ A → Anaphylactic hypersensitivity → TYPE I
C → Cytotoxic hypersensitivity → TYPE II
I → Immune complex mediated hypersensitivity → TYPE III
D → Delayed hypersensitivity → TYPE IV

TYPE I

→ Occurs in 2 phases
1 Sensitization Phase
2 Effector phase

① Sensitization Phase

→ Allergen → Processed by APC &
Peptides of it presented to CD_4 T cells
↓
Activates TH_2 cells
↓
releases IL_4
↓
Activates B cells
↓
IgE Production
↓
HOMOCYTOTROPISM [movement towards the mast cells]

② EFFECTOR PHASE

Shocking Dose

↓

Mast cell Degranulation

↓

PRIMARY MEDIATORS

- P → Protease
- E → Eosinophil chemotactic factor
- N → Neutrophil chemotactic factor
- Hai → Histamine, Serotonin } causes Vasodilation & Bronchoconstriction

SECONDARY MEDIATORS

- B → Bradykinins
- P → Prostaglandins
- L → Leukotrienes
- P → PAF [Platelet Activating factor]
- C → Cytokines [IL_1, TNF α]

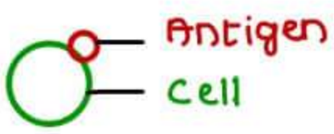
LOCALIZED ANAPHYLAXIS / ATOPY

- → run in families
- → Eg. Bronchial asthma
  Food allergies
  Allergic rhinitis
  Atopic dermatitis

EXAMPLES

- → P - K Reaction
- → Theobald Smith Phenomenon
- → Casoni Test

TYPE II HYPERSENSITIVITY

→ 

+ ANTIBODY → complement Activated

- → Extracellular matrix antigens
- → Exogenous antigens absorbed on host cells
  Eg. Drugs can coat up RBC membrane

- → Antigen is bound to cell

- → complement dependent cytolysis, complement dependent inflammation, complement mediated Phagocytosis } occurs

COMPLEMENT MEDIATED CYTOLYSIS → involves MAC (C_5-C_9)

COMPLEMENT DEPENDENT INFLAMMATION → involves C_{3a}, C_{5a} (chemo attractants)

COMPLEMENT MEDIATED PHAGOCYTOSIS → involves C_{3b} / C_{4b} (opsonins)

Best opsonin → C_{3b}

EXAMPLES

B
Positive → Pernicious anemia
Blood → Blood Transfusion Reaction
Group → Good Pasture syndrome
Graves Disease

IS → ITP
Insulin resistant DM

Mine → Myasthenia gravis

D → Drug induced Hemolytic anemia
A → Auto Immune Hemolytic anemia
T → Transfusion Reactions
E → Erythroblastosis Fetalis
Palm → Pemphigus vulgaris

ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY [ADCC]

→ Ig G Ab attach to target cell antigens through Fab region
Fc portion of Ig G inturn binds to Fc receptors of NK cells

→ Leads to destruction of the target cell

→ used in killing & destruction of
parasites
Tumor cells
Graft tissue

ANTIBODY DEPENDENT CELLULAR DYSFUNCTION [ADCD]

→ Auto Ab mediated
→ TYPES
① STIMULATORY ADCD
Eg. Grave's Disease
→ Activatⁿ of receptors takes place
→ Auto Ab [Long Acting Thyroid stimulators LATS] produced
→ Stimulate Thyroid to produce hormones

② INHIBITORY ADCD

Eg. Myasthenia gravis

→ inhibition of receptor takes place

→ Anti Ach receptor Ab are produced

→ Blocking of Ach receptors happens & leads to Profound muscular weakness

TYPE III

→ Immune complex mediated

→ Antigen is not bound to cells

→ Antigens can be exogenous [bacteria & virus] or endogenous [DNA etc]

Ab Excess

↓

Immune complexes are large & insoluble & tend to localise near the site of antigen administration

↓

Localised Reaction [ARTHUS REACTION]

Localised area of neurosis dlt vasculitis

Ag Excess [monovalent Antigens]

↓

small solubles complexes tend to travel through blood to various sites & get deposited

↓

Generalised Reactn [SERUM SICKNESS]

↓

occurs following serum therapy [mostly ∵ Horse antitetanus serum]

manifests after 7-8 Days

Edema, Erythema, Glomerulonephritis can occur

→ EXAMPLES

H → Hypersensitivity Pneumonitis, HSP

A → Arthus Reaction

R → Reactive arthritis

P → PAN, PSGN

E → Epstein Barr Infections

R → Rheumatoid Arthritis

S → Serum sickness, SLE

TYPE II Lepra Reaction

TYPE IV HYPERSENSITIVITY

→ Delayed Hypersensitivity

→ SENSITIZATION PHASE

APC present peptide fragments ī MHC_2 to TH_1 cells

↓

TH_2 cells differentiate to form T Delayed type Hypersensitivity [T_{DTH}] cells

Other T cells are also involved → also act as DTH cells

CD_8 T cells

CD_4 TH_{17} cells

→ EFFECTOR PHASE

T_{DTH} cells on subsequent contact ī antigen

↓

release cytokines

↓

attract & recruit inflammatory cells at the sites

CYTOKINES

I → Interferon γ [most important]

I → IL2

M → MCAF [Monocyte chemo Attractant factor]

to → TNFβ [Tumor Necrosis factor β]

Medical → MIF [macrophage Inhibition factor]

Institute → IL3

Gujarat → GmCSF [Granulocyte monocyte colony stimulating factor]

IFN γ → activates resting Macrophages

IL 2 → acts in autocrine manner & helps in proliferat^n of T_{DTH} cells

PATHOLOGY

→ continuous T_{DTH} reaction for killing intracellular microbes

↓

Formation of GRANULOMA [TB, Leprosy etc]

T helper cell infiltrate is replaced by macrophages in 2-3 weeks

Macrophage transforms into

① Large, flat ; eosinophilic cell [Epithelioid cell]

② Epithelioid cells fuse [ī the help of INFγ] to form multinucleated gaint cells

EXAMPLES

TUBERCULIN TEST → Delayed type hypersensitivity

CONTACT DERMATITIS

nickel, oak poison act as Haptens
↓
Haptens + Skin proteins
↓
Internalized by Skin APC [Langerhan cells]
↓
Presented to T_{DTH} cells
↓
Activates macrophages
↓
release lytic enzymes
↓
causes skin redness & Postule formation

HYBRIDOMA TECHNOLOGY

→ done for mass production of monoclonal antibodies

→ Principle → Bcells fused up ĩ myeloma cells on HAT media
[Hypoxanthine Aminopterine Thymine media]

- mouse is injected ĩ desired Antigen

- B cells [HGPRT +ive]
- B cells + myeloma cells [HGPRT -ive] on HAT media

→ unfused B cells → Do not survive
unfused myeloma cells → Do not survive
Fused cells [Hybrid cells] → Survive

→ Aminopterin
blocks denovo pathway for Purine Synthesis
Now every cell will go through salvage pathway to synthesize purine

Hybrid cells → Mice
↓
Desired monodonal antibodies

COMPLEMENT SYSTEM

- → Non specific proteins
- → constitute 5% of serum proteins
- → Heat labile (56°C × 30 min)
- → can bind to F_c portⁿ of Ig

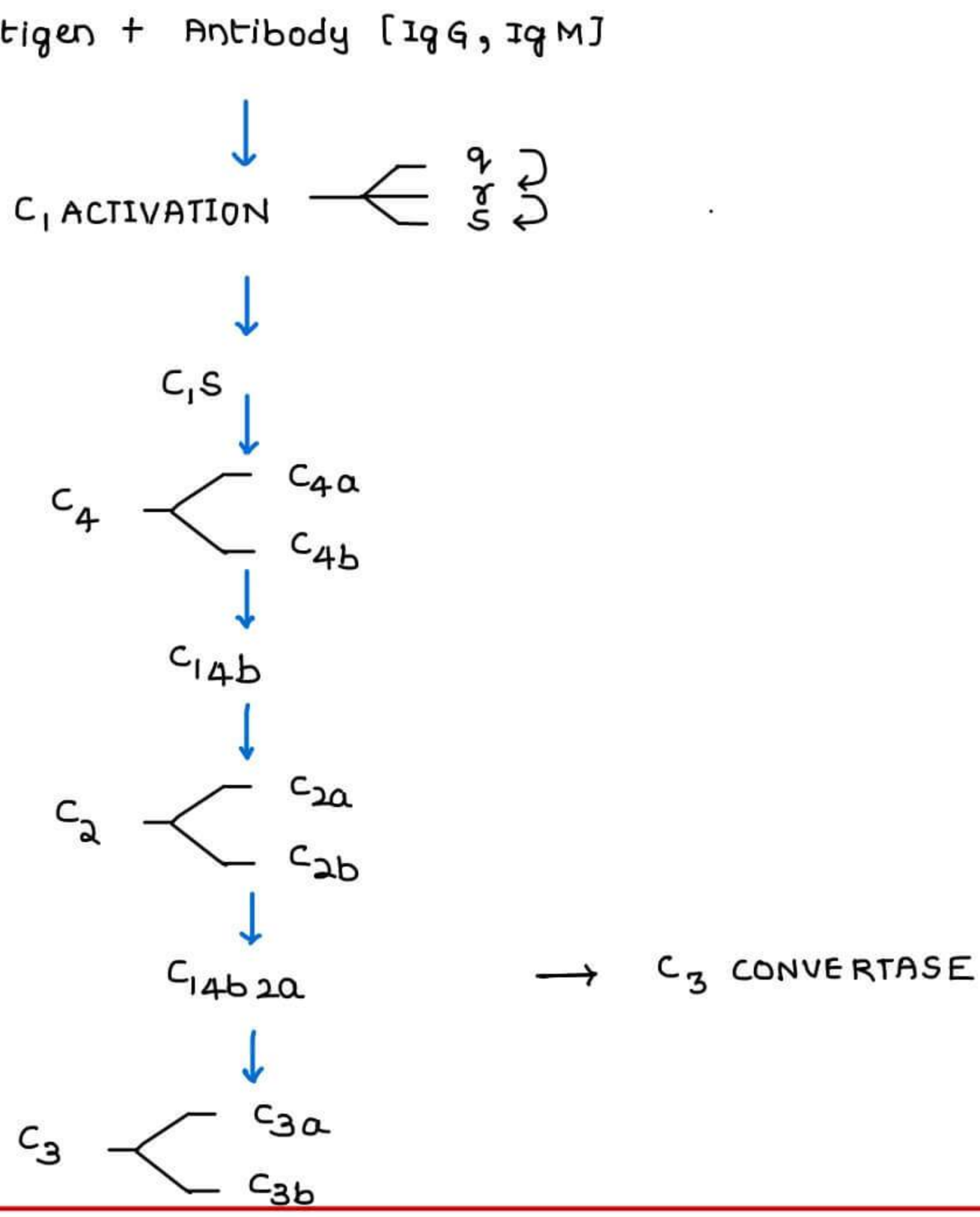
SITES OF SYNTHESIS

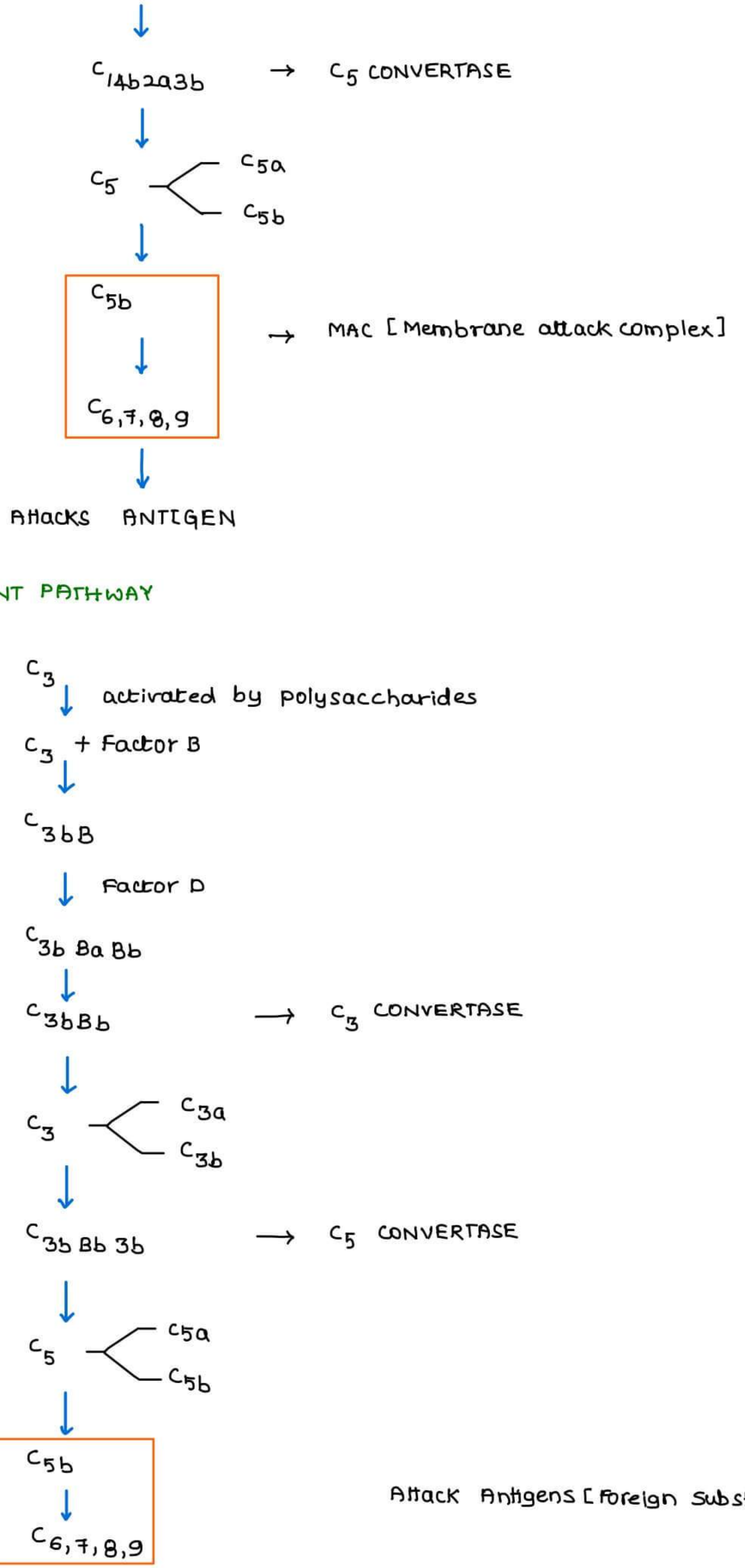
→ GIT	→	C_1
Macrophage	→	C_2, C_4
Spleen	→	C_5, C_8

TYPES

	CLASSICAL	ALTERNATIVE	LECTIN
ACTIVATORS	Ag + Ab complex	Zymogen Endotoxin Nephritic factor Properidin	mannose binding lectin
1st ACTIVATED	C_1	C_3	C_4
PART OF	Acquired immunity	innate immunity	innate immunity

CLASSICAL COMPLEMENT PATHWAY





ALTERNATE COMPLEMENT PATHWAY

→ $C_{1,4,2}$ → will not consumed in alternate complement pathway

LECTIN PATHWAY

→ similar to complement pathway, but starts ī C_4

→ C_1 is not consumed

BIOLOGICAL ROLES

① Lysis of target cells

② Chemotactic → C_{3a}, C_{5a}

Anaphylactic → C_{3b} → best opsonin

③ mediate Type 2 & Type 3 Hyper sensitivity Reactions

④ Auto immune diseases

⑤ Endotoxic shock

DEFICIENCY / Absence

MAC Deficiency → Leads to Recurrent Neisserial infections

D & I absence → Leads to Pyogenic infections

H absence → leads to Hemolytic uremic syndrome

C_1 esterase inhibitor deficiency → Heriditory angioneurotic edema

→ reason for ACE Inhibitor side effect

INTERFERONS

→ induced by virus

→ can confer protection against same virus or unrelated virus

→ not virus specific

but Species specific

→ MOA → inhibit viral protein synthesis

→ Secreted by

L → Leucocytes → IFN α

F → Fibroblasts → IFN β

T → T- lymphocytes → IFN γ

→ USES

1 Rx of Hepatitis B & C

2 Rx of multiple sclerosis

STRUCTURE OF IMMUNE SYSTEM

MYELOID SYSTEM

- RBC
- WBC
- Platelets
- Granulocytes

LYMPHOID

- Lymphoid cells
- Lymphoid organs
  - Thymus
  - Bone marrow

LYMPHOID ORGANS

- Central → Thymus, Bone marrow
- Peripheral → Spleen, Lymph nodes, MALT [Mucosa Associated Lymphoid Tissue], GALT [Gastric], Liver

LYMPHOID CELLS

- T cells
- B cells
- NK cells

THYMUS

- → develops from 3rd & 4th pharyngeal pouches
- → maturation of T cells happens

BONE MARROW

- → B & T cells originates from BM
- → B cells mature in BM

PERIPHERAL LYMPHOID ORGANS

- → T dependent area in a LN → Para cortical area
- → B dependent area in a LN → Cortical follicle

SPLEEN

- → T dependent area in a LN → around periarteriolar lymphoid sheath
- → B dependent area in a LN → Germinal follicle [marginal zone]

CD MARKERS

- T cells → CD_1 to CD_8
- B cells → CD_{10}, CD_{19} to CD_{23}

B LYMPHOCYTES

→ constitute 10-20% of total lymphocytes

→ also acts as APC [Antigen Present cells] [↑ surface Ig m]

→ CD_{21} → EBV receptor for B cells
CD_2 → Measles receptor for B cells

→ on Electron Microscopy → shows micro villi on surface

→ BLAST TRANSFORMERS of B CELLS → [Endotoxins], EBV Ag
BLAST TRANSFORMERS of T CELLS → concavalin
Phyto Haemagglutinin

→ ROSETTES TYPES ON RBC
B cells → EAC
T cells → E

T CELLS

→ constitute 60-70% of total lymphocytes

TYPES

① CD 4 T CELLS

TH_1 → Secretes IL-2
IFN r

TH_2 → secretes IL-4, 5, 6, 10

→ The earliest identifiable T cell precursor in bone marrow are CD 34 + Pro T cells

② CD 8 T CELLS [CYTOTOXIC T CELLS] → causes lysis of target cells

NK [NATURAL KILLER] CELLS

→ constitutes 5-10% of all lymphocytes

→ kills virus infected cells & Tumor cells

→

APCs [Antigen Pregenting cells]
↓
Secretes IL1
↓
Stimulates TH1 cells
↓
Secretes IL2
↓
Stimulates NK cells → LAK CELLS
[Lymphokine Activated Killer cells]

MHC [Major Histocompatibility complex]

→ present on chromosome 6p

CLASS I MHC GENES

→ present in all nucleated cells
→ provides peptide fragment of cells to CD8 cells
→ helps in processing of virus infected cells & Tumor cells

CLASS II MHC GENES

→ DP / DQ / DR
→ Glyco protein pregent on APC
→ provides exogenous peptide fragments to CD4 T cells.
→ play central role in transplantatn antigens immune response

CLASS III MHC GENES

→ a/w complements → C_2, C_4, properidin factors, C_3 convertage
TNF, HSP (Heat shock protein)

MHC RESTRICTION

Exogenous antigen	Endogenous antigens
↓	↓
Peptide fragments ī MHC II	Peptide fragments ī MHC I
↓	↓
CD4 T cells	CD 8 T cell

→ only above process will occurs

MACROPHAGES

→ play a role in Phagocytosis
→ acts as APCs

→ MODIFIED MACROPHAGES

Peripheral Blood	→	Monocytes
Liver	→	Kuffer cells
Brain	→	microglia
kidney	→	mesangial cells
Bone	→	osteoclasts
Lungs	→	alveolar macrophages
skin	→	Langerhan cells

→ acts / kills by Phagocytosis
Phago lysosomal fusion
lysosomal degranulatn
O_2 free radical killing

DENDRITIC CELLS

→ APCs

→ derived from Bm
transported to lymph nodes

INTERLEUKINS

	SECRETED BY	FUNCTION
IL 1	monocytes macrophages Dendritic cells	target vasculature induces inflammatⁿ, fever
IL 2	TH 1 cells	LAK cells from NK cells
IL 3 [GMCSF]	TH cells	Granulocyte Monocyte colony Stimulating factor
IL 4	TH 2 cells	class switching to Ig G
IL 5	TH 2 cells	class switching to Ig A Eosinophil attractant
IL 6,10,13	same function as 4	
IL 8	Macrophages	Neutrophil chemo attractant
IL12	Macrophages	promotes TH1 induction & inhibits TH 2

CHEMOKINES

CXC CHEMOKINES

→ act on neutrophils

→ IL8, IL1, TNF

CC CHEMOKINES

→ monocyte chemo attractant protein 1

→ Eotoxin; RANTES

C CHEMOKINES

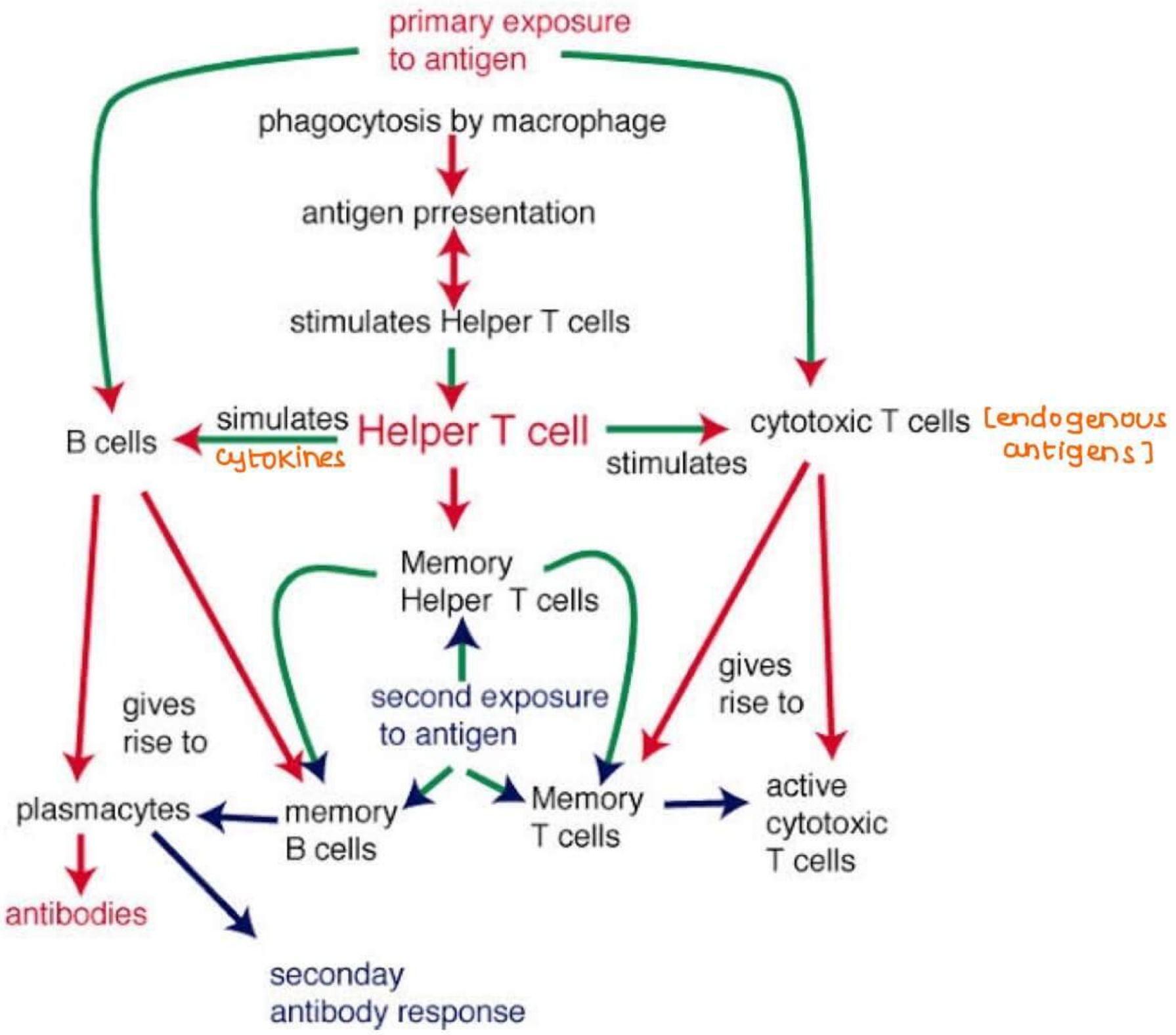
→ specific for lymphocytes & lymphotactin

CX_3C CHEMOKINES [FRACTALKINE]

FUNCTIONS

T cells → Cell mediated Immunity

B cells → Humoral Immunity



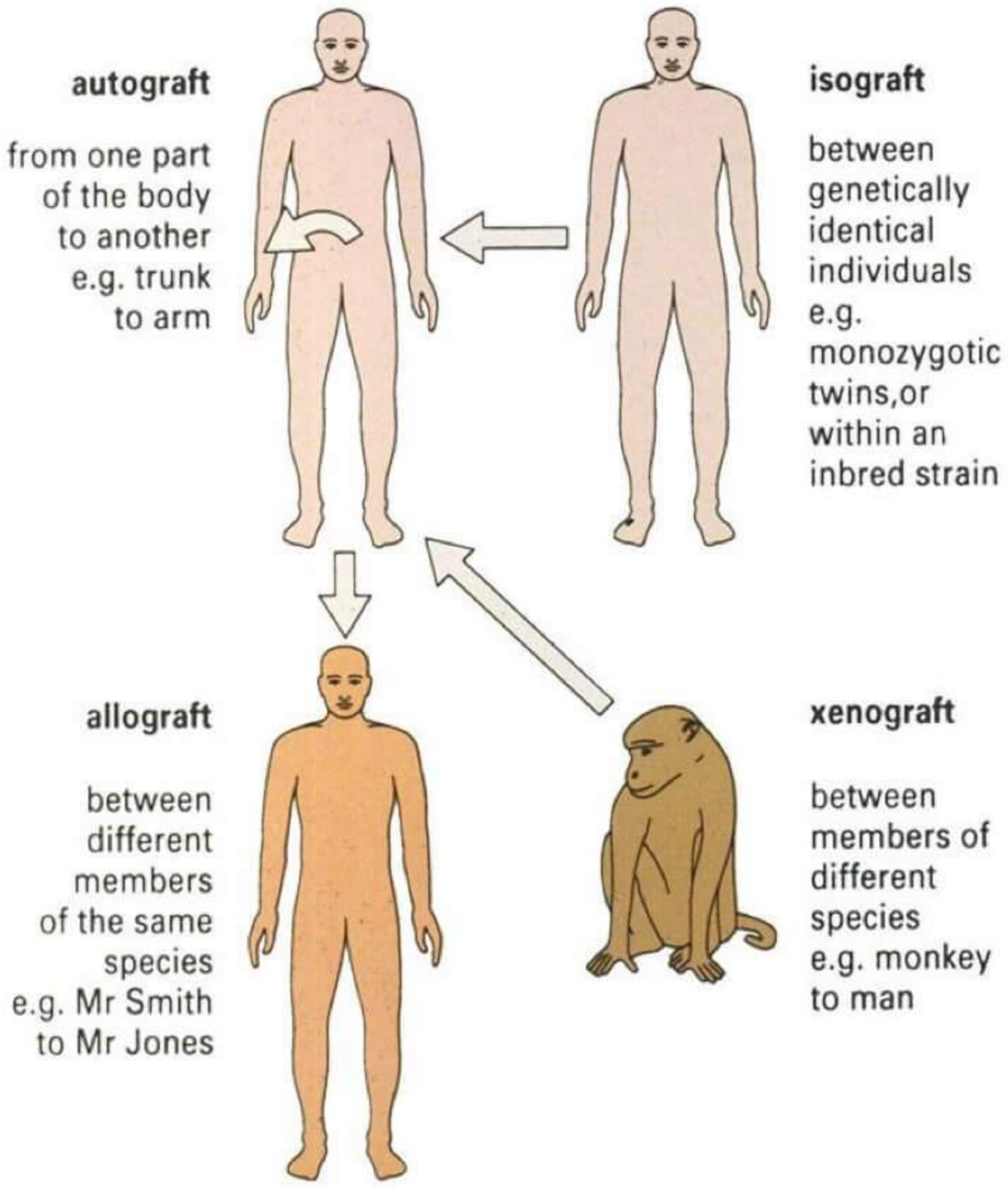
CELL MEDIATED IMMUNITY ROLES

① Immunity against intracellular organisms [TB, Leprosy]
② Immunological surveillance & immunity against cancer
③ Transplantation immunity
④ Delayed hypersensitivity
⑤ Autoimmune diseases

ADJUVANT

→ ↑ses the immunogenicity of Ag or vaccine
→ Examples
  $Al(OH)_3$
  $AlPO_4$
  LPS of B. pertussis [in DPT vaccine]

GRAFT



XENOGRAFT / ALLOGRAFT REACTION

SERIES OF EVENTS

- → Graft is vascularised, healthy in first 3 days
- → From 4th day
  - → There is inflammation ↑ lymphocytes, macrophages
  - → ↓ blood vessels, occluded by thrombi
  - → vascularity diminishes & graft undergoes ischemic necrosis by 10th day
  - → Necrosis & scab like appearance → Sloughs OFF [1ST SET RESPONSE]
- → if another graft from the same donar → rejected in an accelarated fashion
  - → vascularisation commenses
  - → soon interrupted by inflammatory response
  - → Necrosis sets early
    Graft sloughs OFF by 6th day

GRAFT REJECTION

TYPE	TIME TAKEN	MECHANISM
HYPER ACUTE	→ minutes to hours	→ Preformed Ab
ACUTE	→ days to week	→ CD4T cell activation
CHRONIC	→ months to years	→ fibrosis & Wide spread arteriopathy [T cell]

GRAFT VS HOST REACTION

BILLING HAM'S CRITERIA

① Graft contains immuno competent cells
② Host is immunosuppressed
③ MHC incompatible [MHC Typing not done properly]

→ Skin, Liver & GIT are the main targets
→ GVH in animal model → RUNT DISEASE
- cachexia occurs
- animal dies

TYPING METHODS

HLA TYPING

① SEROLOGICAL METHODS
→ Micro cyto toxicity
→ Mixed leucocyte Reactions

② DNA PCR TYPING [Best]

AUTO IMMUNITY

→ Immune response to its own antigens leads to various pathological conditions / diseases

MECHANISMS

1 Exposure of sequestrated antigen
2 formation of neo antigens
3 immunological damage by cross reactivity
4 Breakdown of immunological homeostasis
5 Exposure of forbidden clones

SEQUESTRATED ANTIGENS

→ Lens protein, Sperm protein } considered as foreign

FORMATN OF NEO ANTIGENS

→ Biological exposure, Chemical exposure } Neo antigens

IMMUNOLOGIC REACTION BY CROSS REACTIVITY

→ Carbohydrate 'C' cross reacts ī cardiac valves
Carbohydrate 'M' cross reacts ī myocardium

BREAK DOWN OF IMMUNOLOGICAL HOMEOSTASIS

→ NO coordination blw cell mediated & humoral immunity

FORBIDDEN CLONES are later exposed

EXAMPLES

① AIHA [Auto Immune Hemolytic Anaemia]

② Myasthenia Gravis

③ SLE [COD is due to Kidney lesions mainly]

④ Rheumatoid Arthritis

→ Ig M against fc portion of Ig G
Ig m + Ig G Complex deposited in Synovium of joints & activate complement

⑤ Hashimoto's Thyroiditis

→ age group → middle aged women
→ infiltratn of gland by sensitised lymphocytes leads to progressive destruction of thyroid follicles
→ presents ⁻ goitre

MECHANISM OF AUTO IMMUNITY IN A NUTSHELL

① Exposure of sequestrated antigens
② formation of neo antigens
③ cross reacting antigens
④ Polyclonal activation of B cell
⑤ Inappropriate expression of MHC class □ molecules

IMMUNO DEFICIENCY OF DISORDERS

DISORDERS OF PHAGOCYTOSIS

① CGD [Chronic Granulomatous Disease]

→ deficiency of NADPH Oxidase
→ leads to recurrent catalase positive infections [staph & coliforms]
→ NITRO BLUE TETRAZOLIUM TEST → -ive

② LAD SYNDROME

→ absence of CD18
problem in common β chains of integrins
→ leads to recurrent infection

③ CHEDIAK HIGASHI SYNDROME

→ Defective phagolysosomal fusion
→ ↓↓↓ in melanocytes

④ JOB SYNDROME / HYPER Ig E SYNDROME

→ deficiency of TH 17
→ Leads to recurrent staph. cold abscess
→ ↑ Ig E

⑤ MPO DEFICIENCY

→ Myeloperoxidase deficiency

→ mild or none presentation

B CELL DISORDERS

① BRUTON'S AGAMMA GLOBULINEMIA / X LINKED AGAMMA GLOBULINEMIA

→ defect in B tyrosine kinase gene

→ PRO B CELLS —✕→ PRE B CELLS
BTK gene defect

→ upto 6 months of neonatal life, nothing happens [d/t maternal Abs]
after that severe infections occurs

② X LINKED HYPER IgM SYNDROME

→ Deficiency of CD 40L on activated T cells

→ ↑ serum IgM

→ T cells are normal

③ SELECTIVE IgA DEFICIENCY

→ Leads to repeated sino pulmonary infections

④ COMMON VARIABLE IMMUNO DEFICIENCY

→ Unknown defect

→ B CELLS —✕→ Plasma cells

→ ↓↓ immunoglobulins

→ Late onset

→ Commonly a/w Giardiasis

⑤ TRANSIENT HYPO GAMMA GLOBULINEMIA

→ delayed onset of normal IgG synthesis

→ can resolve itself upto 2-6 yrs

COMBINED B & T CELL DISORDERS

① WISKOTT ALDRICH SYNDROME

→ WASP gene defect

→ R → Recurrent infections
E → Eczema
T → Thrombocytopenia

→ IgM → ↓ed
IgG & IgA → Normal
IgE → ↑ed

② ATAXIA TELANGIECTASIA

→ defect in Kinase involved in cell cycle
→ deficiency of Ig A & Ig E
→ leads to
  cerebral ataxia
  telangiectasia
  ovarian dysgenesis

③ SEVERE COMBINED IMMUNO DEFICIENCY [SCID]

ETIOLOGY

→ defect in common gamma chain of IL-2 receptor
  defect in receptors of IL 4, 7, 9
→ 2nd most common defect → Adenosine deaminase deficiency
→ Rag 1 & Rag 2 genes defect → Total absence of T & B cells
→ Deficiency of PNP [purine nucleoside phosphorylase]

TREATMENT → Bone marrow transplantation

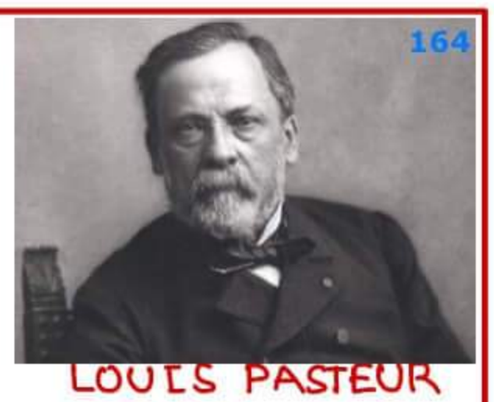
T CELL DEFECTS

① DI GEORGE SYNDROME

→ 22q 11 defect
→ absence thymus & para thyroid glands
→ presents c
  hypocalcemic tetany
  congenital heart defects

② CHRONIC MUCOCUTANEOUS CANDIDIASIS

→ also a T cell Disorder
→ causes recurrent candidiasis



LOUIS PASTEUR

LOUIS PASTEUR

- → Father of microbiology
- → proposed fermentation principle
- → Dis approved abiogenesis theory
- → proposed Germ Theory
- → proposed Autoclave & Hot air oven
- → proposed Pasteurisation of milk
- → vaccines
  - C → Cholera
  - A → Anthrax
  - R → Rabies → 1st rabies vaccine given to JOSEPH MEISTER

ROBERT KOCH

ROBERT KOCH

- → Father OF modern Microbiology
- → Proposed KOCH POSTULATES
- → Discovered TB, cholera bacilli
- → proposed Hanging Drop method
- → proposed Aniline dye staining
- → proposed Solid media concept

- → KOCH POSTULATES
  - MTB [example]
  - constant association ~ disease must be present ~ causative organism
  - isolation in a pure culture media should be possible
  - culture growth inoculated in animal shold cause same lesion
  - Reisolation should be possible from experimental animal
  - Antibodies should be produced from antigens

BACTERIA Does not follow KOCH POSTULATES

Mycobacterium leprae

Treponema pallidum

PAUL EHRLICH

- → proposed Acid fast staining
- → Standardization of toxin & anti toxin done by him

JOSEPH LISTER

- → Father of antiseptic surgery

ANTONY VON LEEUWENHOEK

- → Father OF microscopy
- → coined the term "Little Animalcules" for micro organisms

→ Father of vaccination → EDWARD JENNER
→ Father of PCR → KARY B MULLIS

→ KLEB LOFFLER'S BACILLI → Corynebacterium diphtheriae
→ PREISS NOCARD BACILLI → Corynebacterium pseudo TB
→ KOCH WEEK BACILLI → Haemophilus aegypticus
→ WHITMORE BACILLI → Burkholderia pseudomallei
→ PFEIFFER'S BACILLI → Haemophilus influenzae

	VIRUSES	BACTERIA	FUNGI	PROTOZOA / HELMINTHS
Cells	NO	yes	yes	yes
Diameter [μm]	0.2 - 2	1 - 5	3 - 10 (yeast)	15 - 25 [trophozoite]
Nucleic Acid	DNA or RNA	DNA & RNA	DNA & RNA	DNA & RNA
Type of Nucleus	none	prokaryotic	Eukaryotic	Eukaryotic
Ribosomes	Absent	70s [50s + 30s]	80 s [60s + 40s]	80 s [60s + 40s]
Mitochondria	Absent	Absent	present	present
Outer surface	capsid	Rigid wall peptidoglycan	Rigid wall Chitin	Flexible membrane
Motility	none	some	none	most
Replication		Binary fission	Budding mitosis	mitosis

MICROSCOPY

→ Depends on
- Magnification
- Resolution → ability to distinguish two points as seperate
  - Human eye → 0.2 mm
  - Light microscope → 0.2 μ
  - Electron microscope → 0.2 nm

contrast can ↑ed by staining

LIGHT MICROSCOPE

→ White light is used as soure
→ used For
- motility [hanging drop method] demonstratⁿ
- Gram staining
- Acid fast staining

→ internal details can not be demonstrated
→ Highest magnificatⁿ achieved by Oil immersion lens [100x × 10x = 1000x]

PHASE CONTRAST MICROSCOPY

→ light emerges in different phases
↓
Difference in intensity
↓
Reveal internal structures of living cells

→ can be used for motility demonstratⁿ

DARK FIELD MICROSCOPY

→ Background is dark by Dark field condensers
→ Reflected light is used
→ visualise Spirochaetes

FLUORESCENT MICROSCOPY

→ When UV rays falls on fluorescent substance, wave length of invisible light increases & becomes Luminous
→ STAINS

Auramine / Rhodamine for	→	MTB
calcoflour white for	→	Fungus
Acridine orange for	→	Malarial parasite

ELECTRON MICROSCOPE

→ beam of electrons used instead of Light
→ beam focussed by circular magnets instead of lens
→ object is held in path of beam
→ Scatters the electrons by object
→ image is formed on fluorescent viewing screen
→ maximum resolution can obtained [0.2nm - 0.5nm]
(100 times better than light microscope)
→ Vacuum is used

	PROKARYOTES	EUKARYOTES
Nuclear membrane	absent	present
Nucleolus	absent	present
Ribonucleo protein	absent	present
cell division	Binary fission	mitosis/meiosis
chromosom	one, circular	many, linear
cell wall	NAM, NAG	Sterols [mycoplasma also]
membrane bound organelles	absent	present
Respiratⁿ	Mesosomes	Mitochondria
cytoskeleton	absent	present
Ribosomes	70S	80S
mRNA	no introns	Introns & Exons +nt

SHAPES

- cocci in clusters → staphylococcus
- cocci in chains → strepto coccus
- cocci in pair [diplococci] → Pneumo coccus
- Kidney shaped → Gonococci
- lens shaped → Meningococci
- cocci in Tetrads → Micrococci
- cocci in octate → Sarcina
- Spectacle like appearance → Entero coccus

BACILLI

- Bamboo Stick appearance → Bacillus anthracis
- Diplo bacilli → Klebsiella pneumoniae
- Branching → Actinomycetes
- curved GNB → Campylo bacter → Gull wing shape
  Helico bacter
- Spiral → spirillum
  Spirochaetes
- Pleomorphic → Haemophilus
  Brucella

All cocci are Gram positive **Except**

- meningo cocci
- Gono cocci
- Veilonella
- Moraxella

All Bacilli are Gram negative **Except**

- M → Mycobacterium Species
- A → Anthracis Bacillus
- C → Clostridium Species
- D → Diphtheriae Corynebacterium
- O
- N → Nocardia
- A → Actinomycetes
- L → Listeria
- D → Diphtheroids

	GRAM POSITIVE	GRAM NEGATIVE
THICKNESS	100 layer thick	1-2 layer thickness
LIPID CONTENT	2-5%	15-20%
TEICHOIC ACID	Present	Absent
VARIETY OF AA	Few	Several
LPS [Endotoxin]	absent	present
NAG - NAM attached ī a tetrapeptide side chain cross linked by	penta peptide crossbridge	Tetra peptide are directly linked to each other

NAG → N acetyl Glucosamine
N acetyl Muramic Acid

AA → Amino Acids

CELL WALL DEMONSTRATION

R → Reaction ī specific antibody

M → micro dissection , Mechanical Rupture of cell

P → Plasmolysis

Doctor → Differential staining

INCLUSIONS

STORAGE GRANULES

→ corynebacterium diphtheriae
- Polymetaphosphati
- Volutin granules
- meta chromatic granules
- Babes ernest granules

→ energy deposits of bacteria

→ Granules are stained by

P → PONDER'S STAIN

A → ALBERT STAIN

N → NEISSER STAIN

CAPSULE

→ Polysaccharide mostly
Polypeptide in Bacillus anthracis

→ Virulence factor
prevents phagocytosis

→ Demonstrated by

Negative staining for cryptococcus

Quellung Reaction For Pneumococcus

Mc Fadyean's Reaction

FLAGELLA

→ Used for motility

→ 0.02 μ thick

3-20 μ long

→ Antigenic

→ PARTS

Filament

Hook

Basal Body → attached to cytoplasmic membrane by rings

→ T → Tumbling → Listeria

D → Darting → Vibrio

S → Swarming → Proteus

Falling leaf like → Giardia lamblia [dlt 4 pairs of Flagella]

→ WIDAL TEST → measures antibodies against 'O' & 'H' [more immunogenic]

FIMBRIA

→ Fine hair like structure

→ 0.5 μ long

→ helps in adhesion

conjugation [sex pili]

→ Antigenic

SPORES

→ Highly resistant resting forms

→ Spore forming Bacteria

B → Bacillus anthracis

S → Sporo sarcina

C → clostridium

→ Drum stick appearance seen in Cl. tetani

→ most have sub terminal spores [most of clostridia]

→ central spore seen in Cl. bifermentans

→ Bacillus cereus also produces central spores

PROTOPLAST → seen in Gram positive

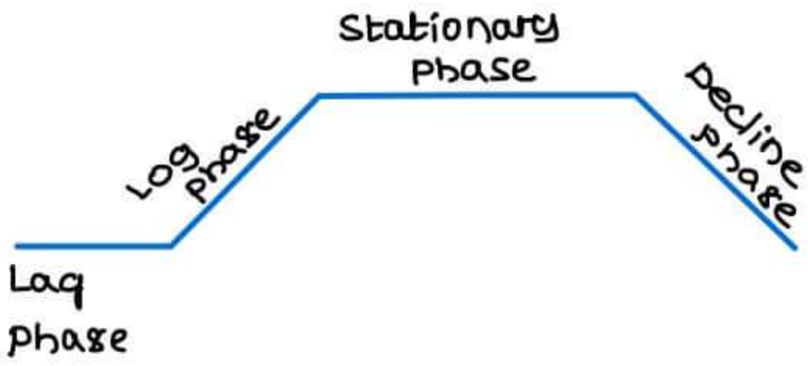
SPHEROPLAST → seen in Gram negative [Still some cell wall is present]

[out cell wall Forms [looses cell wall in Hypertonic Solution]

L FORMS

- cell wall free forms
- KLINBERGER named them
- seen in Streptobacillus moniliformis
- unstable L forms seen in Mycoplasma

GROWTH CURVE



→ **Lag Phase**
- Build up enzymes & metabolites
- adapts to the conditions → ↑ in size

→ **Log Phase**
- exponential division occurs
- maximally active
- uniformly stained
- $(2)^n$ exponential division

→ **Stationary Phase**
- Live = Dead
- Sporulation happens
  Exotoxin production happens
  Antibiotic production [some bacteria] happens

→ **Decline Phase**
- complete accumulat^n of toxic metabolites
- involution forms formed

STERILIZATION & DISINFECTION

STERILLIZATION

→ Making an article free of pathogens, non pathogens including spores

DISINFECTION

→ Removal of pathogens & non pathogens but not spores

ASEPSIS

→ Process in which the chemical agents [antiseptic] applied to body surfaces will Kill / inhibit pathogenic micro organisms

DECONTAMINATION or SANITIZATION

→ Reduction of pathogenic microbial population to a level in which items are considered safe to handle

NATURE OF ORGANISMS [RESISTANCE IN DECREASING ORDER]

→ Prions > cryptosporidium oocysts > Bacterial spores > mycobacteria > Other parasite cysts [eg. Giardia] > Small non-enveloped virus > Trophozoites > Gram negative bacteria > fungui > large non-enveloped viruses > Gram positive bacteria > Enveloped viruses

CLASSIFICATION OF STERILIZATION METHODS

1. SUN LIGHT
2. DRYING
3. HEAT
   - a. DRY HEAT
     - Flaming
     - incineration
     - Hot air oven
   - b. MOIST HEAT
     - < 100°C
       - Pasteurization
       - Inspissation
       - water bath
     - = 100°C
       - Tyndallisation
       - Boiling
     - > 100°C
       - Auto clave
4. FILTRATION
   - Depth filters
   - membrane filters
5. RADIATION
   - a. Ionising — Gamma rays & X Rays
   - b. Non ionising — UV Rays & infrared rays
6. ULTRASONIC VIBRATION
7. CHEMICALS — Alcohols, Aldehydes, phenolic compounds Biguanides, Halogens, Oxidising agent, Heavy metal salts, Surface active agents, dyes
8. GAS STERILIZATION — ETO, BPL, Plasma sterilization

METHOD	PRINCIPLE
1. SUN LIGHT	Active microbicidal activity of Light UV LIGHT
2. DRYING	70 - 80% weight of bacterial cell is water
3. DRY HEAT	C → Charring O → Oxidative damage D → denaturation E → Elevated electrolytes
4. MOIST HEAT	Denaturation & Coagulation of Proteins
5. FLAMING	Flame of BUNSEN BURNER
6. INCINERATION	Inoculating wires, loops 870°c to 1200°C burns the anatomical & microbiological waste

HOT AIR OVEN

→ temperature → 160°C for 2 hrs

→ Electrically heated chamber ī a fan to ensure adequate & even distribution of hot air

→ materials sterilized
- → Glassware → syringes, petridishes, flasks, pipettes
- → surgical instruments → scalpels & scissors
- → chemicals like → Dusting powder, grease, paraffin, oils

→ PRECAUTIONS TO BE TAKEN
- → overloading should not be done
- → Equal & free air circulation should be maintained
- → material should be properly dried before putting
- → Paper wrapping of items should be done
- → oven should be allowed to cool for 2 hrs before opening the door

→ CONTROLS
- ① Spores of Bacillus subtilis
- ② Spores of clostridium tetani [non pathogenic]

MOIST HEAT STERILLIZATION

→ coagulatⁿ & denaturatⁿ of proteins

→ < 100°C	→	pasteurization, inspissation
at 100°C	→	Boiling, Tyndallisation
> 100°C	→	Autoclave

PASTEURLZATION OF MILK

Flash method	→	72°C for 15 - 20 sec	→ bring down to 13°C
	→	Kills coxiella burnetti spores also	
	→	Better method than Holder's	
Holder's method	→	60°C for 30 min	

WATERBATH

→ Disinfection of serum, BodyFluids & vaccines can be sterilized
→ Bacterial vaccines → disinfected at 60°c x 1 hr
Serum or heat labile body fluids → disinfected at 56°c x 1 hr

INSPISSATION

→ 80 - 85°c for 20 - 30 minutes for 3 days
→ First exposure → vegetative forms killed
2nd & 3rd exposure → spores germinating also get killed

→ Done for
↳ LJ [LOWENSTEIN JENSEN] MEDIA | DORSET EGG MEDIA
↳ LSS [LOEFFLER'S SERUM SLOPE for Diphtheriae]

AT 100°C

BOILING

→ 100°c for 15 minutes
→ most of vegetative forms killed
→ but SPORES are NOT KILLED

KOCH'S ARNOLD STEAM STERILIZER

→ used for those medias which decompose at higher temperature of autoclave
→ Articles exposed to steam [100°c] at atmospheric pressure for 90 min.

TYNDALLIZATION

→ 100°c x 20-30min x 3 Days
→ Done for Sugar & Gelatin containing Substances

AT > 100°C

AUTOCLAVE

→ WATER BOILS WHEN ITS VAPOUR PRESSURE EQUALS SURROUNDING ATMOSPHERIC PRESSURE
→ As atmospheric pressure increases, Boiling temperature also increases
→ At normal pressure, boiling point of water is 100°c.

3 PARTS OF AUTOCLAVE

→ pressure chamber
→ Lid
→ Electrical heater

TEMPERATURES OF AUTOCLAVE

→ 121°c x 15 min x 15 psi [Pounds square inch]
→ 126°c x 10 min x 20 psi
→ 133°c x 3 min x 30 psi

Done For

→ Surgical instruments
→ All culture medias except LJ & LLS | Dorset egg
→ Autoclavable plastic containers

→ Plastic tubes & pipette tips
→ Biohazard waste
→ Surgical Sutures except catgut

CONTROLS → Spores of GeoBacillus stearothermophilus
→ Killed at 121°C x 12 min

CHEMICAL INDICATORS
→ Class I → External pack control [Autoclave tape]
→ Class II → Equipment control [Bowie dick Test]
→ Class IV/V → Internal pack control

PHYSICAL CONTROL
→ Digital displays on equipment temperature, pressure & time

FILTRATION
→ mainly done for Heat sensitive substances
↳ vaccines
↳ antibiotics
↳ Serum
↳ toxin
↳ Sugar solution

2 TYPES OF FILTERS
1. DEPTH FILTERS - POROUS FILTERS [metal polymer ceramic random mats]
→ Eg → candle filters
→ unglazed porcelain filter
→ Seitz filter
→ Sintered glass filter

2. MEMBRANE FILTERS
→ most widely used filters for bacterial filtration
→ made up of cellulose acetate, cellulose nitrate, polycarbonate
→ Pore size → 0.22 μ → mc used
→ 0.45 μ → retains coliforms
→ 0.8 μ → retains airborne micro organisms

→ USE → to sterilize sugar, sera & antibiotic solutions

AIR FILTERS
1. HEPA [High efficiency particulate air] FILTER
→ Removes 99.97% of particles having a size 0.3 μ or more

2. ULPA [Ultra Low Particulate/ Penetration]
→ Removes 99.999% of dust, pollen, molds

CONTROL → Brevundimonas diminuta & Serratia marcesens

RADIATION

IONIZING RADIATION / COLD STERILIZATION

→ Gamma rays / x Rays
→ breaks upon DNA ī out temperature rise → COLD STERILIZATION
→ used to sterilize Plastic syringes & catgut sutures
→ CONTROL → Spores of Bacillus pumilus

NON IONIZING RADIATION / HOT STERILIZATION

→ UV rays / Infra red rays
→ temperature of surface increases
→ used for OTs, Biosafety cabinets

HIGH FREQUENCY ULTRASONIC WAVES

→ disrupt the bacterial cells
→ not reliable, not done these days

CHEMICAL METHODS OF STERILIZATION

VARIOUS GROUPS OF DISINFECTANTS

ALCOHOLS

→ most widely used disinfectants & antiseptics

ETHANOL & ISOPROPONOL [70-80%]

→ MOA → Bactericidal & fungicidal but not Sporicidal
→ some enveloped virus like HIV are also destroyed

ETHYL ALCOHOL → used as surgical spirit [70%]
→ used in antirubs as antiseptics

ISOPROPYL ALCOHOL → used for stethoscope & thermometers x 10 - 15 min

ALDEHYDES

→ MOA → combine ī nucleic acids, proteins & inactivate them by cross-linking & alkylating the molecules
→ also sporicidal [used as chemical sterilants]

FORMALDEHYDE

→ FORMS
1. GAS → used for OT fumigation
2. LIQUID [FORMALIN] → used to preserve anatomical & patho specimens

GLUTARALDEHYDE

→ Less toxic, less irritant
→ 2% Gluteraldehyde → CIDEX
- used for BronchoSCOPES, CystoSCOPES, endoscopes

→ disinfects objects within 20 min
→ available in inactive form & activated by alkalinisation before use
→ remains active only for 14 days

ORTHOPHTHALDEHYDE

→ 0.55 % solution can also be used for sterilizing cytoscopes & Endoscopes

Advantages

→ does not require activation
→ low vapour property
→ Better odour
→ more stable
→ ↑ mycobactericidal activity

PHENOLIC COMPOUNDS

PHENOL [CARBOLIC ACID]

→ 1 st widely used antiseptic
→ discovered by JOSEPH LISTER in 1867
→ derived from coal tar
→ MECHANISM → denature proteins & disrupts cell membranes
→ Advantage → high mycobactericidal activity & can act upon in presence of organic waste

→ CRESOLS, XYLENOL, LYSOL are used as disinfectants in Labs & Hospitals
→ retain activity in the presence of organic waste

CHLORHEXIDINE → components of SAVLON [chorhexidine + cetrimide]
CHLOROXYLENOL → component of dettol

HALOGENS

→ Iodine & chlorine have antimicrobial activity
→ Exist in free State & form salt like compounds ī sodium & most other metals

IODINE

→ skin antiseptic
→ oxidising cell constituents & iodinizing cell proteins
→ at higher concentration, it may kill spores

TINCTURE of IODINE [2% IODINE]

→ in water Ethanol Solution of Potassium Iodide

IODOPHOR

→ complexing iodine ī Organic carrier → BETADINE

CHLORINE

COMMON ACTIONS

1. Bleaching in laundry & homes by oxidation
2. Disinfection in hospitals by coagulation
3. Odour control in toilets by Precipitation
4. Chlorination of drinking water in water tanks by Septication
5. Elimination of slime & algae in Swimming pool by pH adjustment

PREPARATIONS

1. CHLORINE GAS
2. SODIUM HYPOCHLORITE
3. CALCIUM HYPOCHLORITE

} Produce HClo [Hypochlorous Acid]

↓

oxidation of cellular materials & destruction of vegetative bacteria & fungi

→ Organic matter interferes ī its action

OXIDIZING AGENTS

H_2O_2
Peracetic Acid
Plasma sterilization

H_2O_2

→ strong oxidizer [high level disinfectant] Liberates toxic free hydroxy radicals which are active ingredients
attack membrane, DNA, lipid & other cellular components

→ most effective concentration → 3-6%.
→ Spores might require → 10%. [higher concentration]

PERACETIC ACID

→ powerful oxidizing agent
→ more active than H_2O_2
→ concentration → < 1%. is sporicidal

At low temperature

→ high level disinfectant
→ used to sterilize endoscopes
→ may corrode steel, iron, copper

PLASMA STERILIZATION

→ Plasma Refers to gaseous state consisting of ions & free electrons
→ Chemical sterilants H_2O_2 & Peracetic acid used to induce plasma state
→ Plasma sterilizers [STERRAD] → maintains uniform vaccum inside the chamber
→ Active agents → UV photons & radicals used
→ maintain low temperature
→ used in sterilization of surgical instruments
→ CONTROL → Bacillus stearothermophilus spores

HEAVY METAL SALTS

MECHANISM OF ACTION

→ combine ī bacterial cell proteins, often ī their sulfhydryl groups & inactivate them & precipitate cell proteins

→ most heavy metals are bacteriostatic
- ↳ SILVER SULFADIAZINE [used for Burns]
- ↳ SILVER NITRATE [1%]
- ↳ COPPER SULPHATE [fungicidal]
- ↳ MERCURY SALTS
  - → MERCUROCHROME → known antiseptics
  - → THIOMERSAL → preservatives in vaccine & sera

SURFACE ACTIVE AGENTS or SURFACTANTS

→ Lower surface tension blw 2 liquids or blw liquid & solid

→ CLASSIFIED INTO
- → Anionic
- → cationic
- → Non ionic
- → Amphoteric

CATIONIC SURFACTANTS

→ Quaternary Ammonium compounds are most popular cationic disinfectants

→ have positively charged quaternary nitrogen & a long hydrophobic aliphatic chain

→ they disrupt the microbial membranes & denature the proteins

→ Gram positive bacteria are better killed than gram negative bacteria but not MTB or Spores

→ cationic detergents → disinfectant for food utensils & small instruments

→ Eg → Alkyl trimethyl ammonium salts
Acetyl trimethyl ammonium bromide [cetrimide]

ANIONIC SURFACTANTS [common soaps]

→ have strong detergent action

→ have Weak antimicrobial action

→ most active at acidic pH

AMPHOTERIC [Tego compounds]

→ have detergent property of anionic compounds

→ have Antimicrobial property of cationic compounds

→ act upon compounds at a wide range of pH but activity reduced in presence of Organic waste

→ Eg. TEGO Compounds → Active compound is DDAG [Dodecyl Diamino Ethyl Glycine]

→ used as a antiseptic in dental practices

DYES

→ ANILINE & ACRIDINE are 2 groups of dyes
→ used as skin & wound antiseptics

ANILINE DYES

1. CRYSTAL VIOLET
2. GENTIAN VIOLET
3. MALACHITE GREEN

→ more active against Gram positive than Gram negative
→ But no activity against MTB
→ reduced activity in presence of Organic waste
→ interferes ī synthesis of peptidoglycan in cell walls

ACRIDINE DYES

ACRIFLAVINE
EUFLAVINE
PROFLAVINE
AMINACRINE

} affected very little by Organic waste

→ more active against Gram positive than Gram negative
→ interfers in synthesis of Nucleic Acid & proteins in bacterial cells

GASEOUS STERILIZATION

→ Ethylene oxide [ETO]
→ Low temperature Steam formaldehyde
→ β propiolactone

ETO

→ most widely used gaseous chemical sterilant
→ Both microbicidal & sporicidal
→ acts by combining ī cell proteins
→ high penetrating power
→ used for prepacked syringes
→ highly inflammable, irritant & explosive
→ mixed ī CO_2 or dichlorodifluoromethane

3 factors affect ETO disinfectant power

→ ETO concentration
→ Humidity
→ Temperature

At ETO concentration of 700 mg/l & 40-50% relative humidity at 38°c sterilizatⁿ is achieved [5-8 hrs]

CONTROL → Bacillus globigi spores used

USES

- to sterilize
  - disposable plastic petri dishes
  - syringies
  - heart lung machines
  - Respirators
  - Sutures
  - catheters
  - dental equipments

LOW TEMPERATURE STEAM FORMALDEHYDE

- used for fumigation of OTs
- not used now

BPL [Beta Propiolactone]

- 0.2% is active against all micro-organisms including spores
- used for inactivation of vaccines
- found to be carcinogenic, so not used for fumigation

TESTING OF DISINFECTANTS

PHENOL COEFFICIENT TEST / RIDEAL WAEKER TEST

- determined by dilution of disinfectant
- used to sterilize suspension of salmonella typhi in given time divided by dilution of phenol which sterilizes suspension in same time
- if phenol coefficient > 1 → disinfectant is better than phenol

- PHENOL COEFFICIENT TEST =

$$\frac{\text{Highest dilution of test disinfectant that kills S. typhi in given time}}{\text{Highest dilution of phenol that kills S. typhi in given time}}$$

- it doesn't assess in presence of organic waste

CHICK MARTIN TEST

- modificatn of RIDEAL WALKER CO EFFICIENT TEST
- checks whether Disinfectant works in presence of organic waste [faeces, yeast] to simulate natural condition

CAPACITY TEST [KELSEY SYKES TEST]

- capacity of a disinfectant to retain its activity when repeatedly used microbiologically in increasing load

IN USE TEST [KELSEY & MAURER TEST]

- disinfectant is microbiologically contaminated
- routinely performed in hospitals once in 3 months

SPORICIDAL AGENTS

E → Ethylene oxide
F → Formaldehyde
G → Glutaraldehyde [2%]
H → Hydrogen Peroxide
→ Sodium Hypochlorite [blood spills]

O - phthalic Acid
Peracitic acid
Autoclave
Hot air oven
plasma sterilization

CHEMICAL DISINFECTANTS - CLASSIFICATION

LEVELS OF DISINFECTANTS	BACTERIAL SPORES	TB BACILLI	NON ENVELOP. VIRUS	FUNGI	ENVELOPED VIRUS	VEGETATIVE BACTERIA
LOW LEVEL	NO	NO	NO	+/-	YES	YES
INTERMEDIATE LEVEL	NO	YES	YES	YES	YES	YES
HIGH LEVEL	may be	YES	YES	YES	YES	YES
CHEMICAL STERILANT	YES	YES	YES	YES	YES	YES

SPAULDING CLASSIFICATION

SEMICRITICAL DEVICES

→ includes those comes in contact ī mucous membranes like respiratory equipments [laryngoscope]
→ High level disinfection needed

CRITICAL DEVICES

→ includes those Enters sterile sites like Surgical instruments, catheters
→ High level disinfection needed

NON - CRITICAL DEVICES

→ includes those come in contact ī skin like BP cuff, ECG Electrodes
→ Low / intermediate level disinfection needed

MEDICAL EQUIPMENTS

→ includes Examination tables, computers
→ Low level disinfection needed

BACTERIAL GENETICS

DNA

→ made of

- Deoxy ribose
- Phosphate groups
- 4 nitrogenous bases
  - 2 purines → Adenine & Guanine
  - 2 pyrimidines → Thymin & cytosine

- Strands held together by hydrogen bonds

RNA

→ made up of

- Ribose sugar
- Uracil replaces the Thymine

→ Types

- mRNA [messenger RNA]
- tRNA [Transfer RNA]
- rRNA [Ribosomal RNA]

GENE → Segment of DNA that specifies for a particular polypeptide

CODON → Genetic information stored in DNA

ANTICODON → Binds to codon

NON SENSE CODON → UAA, UAG, UGA
→ Do not code for any amino acid
→ terminate the synthesis of polypeptide
→ aka STOP CODONS

EXTRA CHROMOSOMAL GENETIC MATERIALS

PLASMIDS

→ Free in cytoplasm of bacteria
→ When they integrate host cell → EPISOME
→ Not essential for the survival of bacteria
→ Plasmid Encode genetic information for
① Resistance to antibiotics
② Bacteriocins
③ Entero toxin production
④ Enhanced pathogenicity
⑤ Degrading complex organic molecules

BACTERIAL VARIATIONS

PHENOTYPIC	GENOTYPIC
physical expression in environment	Sum total of gene make up ↓ Genetic Apparatus of the cell
Examples ① TYPHOID BACILLI – normally flagellated – no Flagella grow in Phenol agar ② LACTOSE FERMENTATION by E.coli – by β galactosidase	Examples ① Mutations ② Transfer of genes by – conjugation – Transformation – Transduction

MUTATION

→ Random, undirected, heritable variation

→ Alterat^{n} of nucleotide sequences happens
by addition
deletion
Substitution [one or more bases]

→ Bacteria undergo mutations → 10^{-2} to 10^{-10} per bacterium per division

→ MUTAGENIC AGENTS
UV Rays
Alkylating agents
Acridine dyes

→ All genes are susceptible for mutations
but all mutations are not expressed

→ LETHAL MUTATION → Harmful, Destroys the vital function

→ CONDITIONAL LETHAL MUTANTS
Example → Temperature sensitive mutants
- can lives at 35°C
- but dies at 39°C [Restrictive temperature]

→ TYPES

① Point mutation
Base pair substitution
frame shift mutat^{n}

② Multisite Mutations
Gain, Loss or Duplicat^{n} can occur

POINT MUTATION

TRANSITION	TRANSVERSION
mc type purine is replaced by purine pyramidine is replaced by pyramidine	purine can be replaced by pyramidine & VICE VERSA

FRAME SHIFT STUFF

(Serine)

Normal → AAT AGT GCC

Deletion → AAT GTG CCA

(valine)

(Serine)

Normal → AAT AGT GCC

Insertion → AAT CAGT GCC

(Glutamine)

MULTI SITE MUTATIONS

TYPES

① Addition / Gain
② Deletion / Loss
③ Duplication
④ Inversion

MISSENSE MUTATIONS

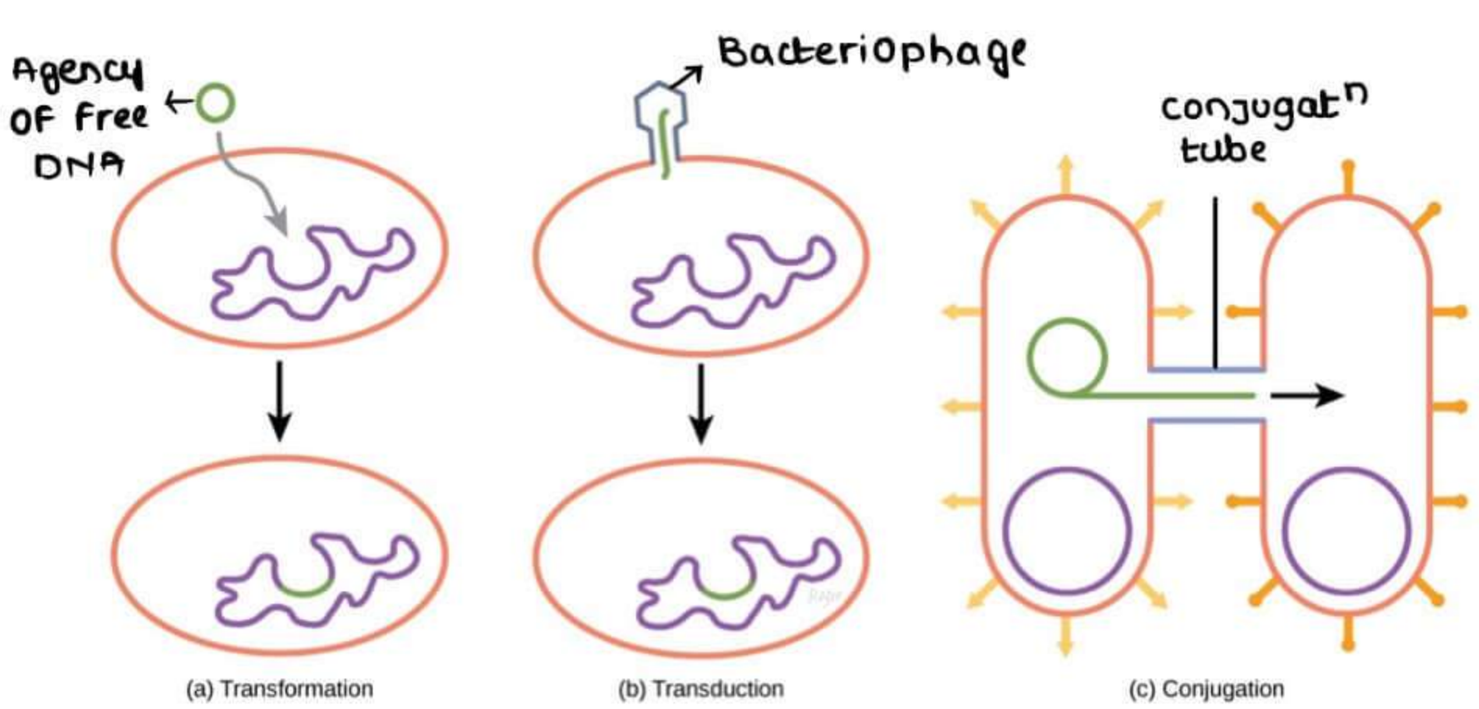
→ Triplet code is altered, different amino acid produced from that normally located at that particular position

NONSENSE MUTATION

→ premature polypeptide chain terminat^{n} by stop codon

GENE TRANSFERS [sexual process]

① TRANSFORMAT^{N} → naked DNA taken up from the environment by bacterial cells
② TRANDUCTION → Use of bacteriophages [bacterial virus] to transfer DNA b/w cells
③ CONJUGATION → direct transfer OF DNA from one bacterial cell to another



(a) Transformation (b) Transduction (c) Conjugation

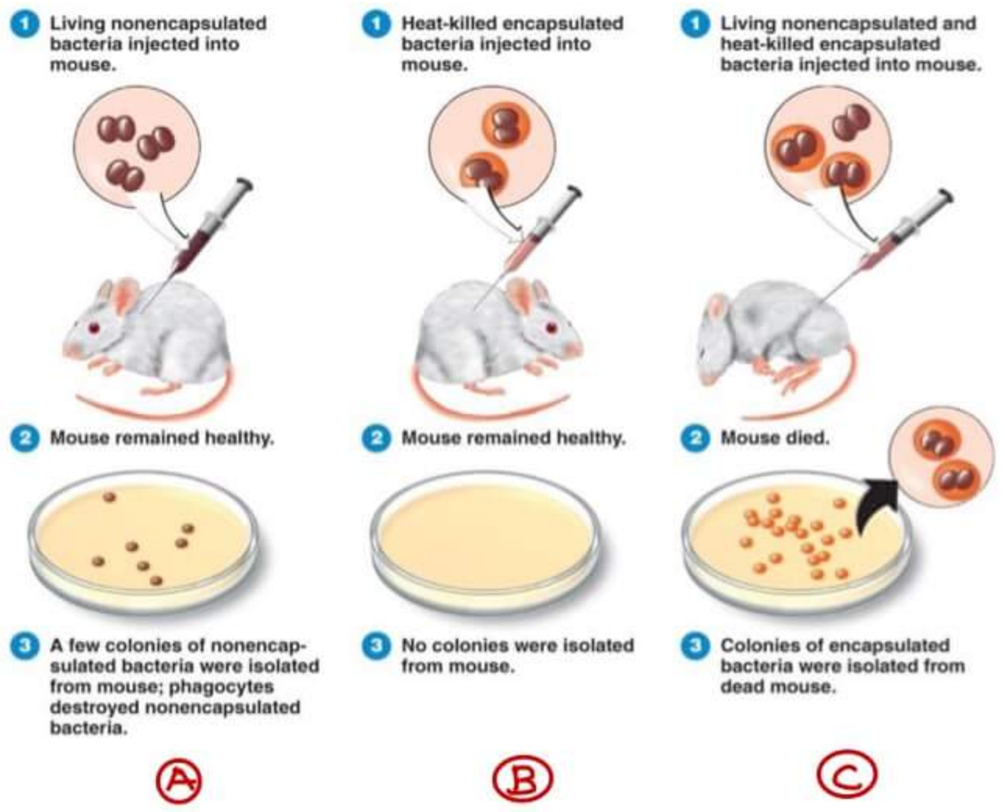
TRANSFORMATION

→ Discovered by GRIFFITH
Rediscovered by AVERY, MACLEOD & MCCARTY [1994]

→ CASE A
Living non-encapsulated bacteria [Rough strains of Pneumococcus when injected into mouse → Mouse dies

→ CASE B
Heat killed encapsulated pneumococcus when injected into mouse → No effect

→ case C
Heat killed smooth strain of pneumonia + Live rough strains OF Pneumonia
When injected into mouse → Mouse dies
colonies of living encapsulated pneumococci are isolated from dead mouse [Through agency of free DNA]



Demonstratⁿ OF TRANSFORMATION by Avery, MacLeod & McCarty

TRANSDUCTION

→ transmission of portion of DNA from one bacterium to another by a bacteriophage
→ MECHANISM
↳ part of host DNA may accidentally incorporate into bacteriophage
↳ gets transferred to recipient bacterium so that it leads to acquisition of new characters by the recipient bacterium from donar DNA

TYPES

LYTIC or VIRULENT CYCLE

→ Bacteriophage multiplies in host cytoplasm & produce large no. of daughter phages
→ Subsequently gets released, causing death & lysis of host cycle

LYSOGENIC OR TEMPERATE CYCLE

→ HOST bacterium unharmed
→ phage DNA integrate ī bacterial chromosome as prophage that multiplies Synchronously ī bacterial DNA
→ When phage DNA tries to come out it is disintegrated from host chromosome, comes out in cytoplasm & behaves like a lytic phage;
→ it replicates to produce daughter phages which subsequently released by host cell lysis

TYPES OF TRANSDUCTION

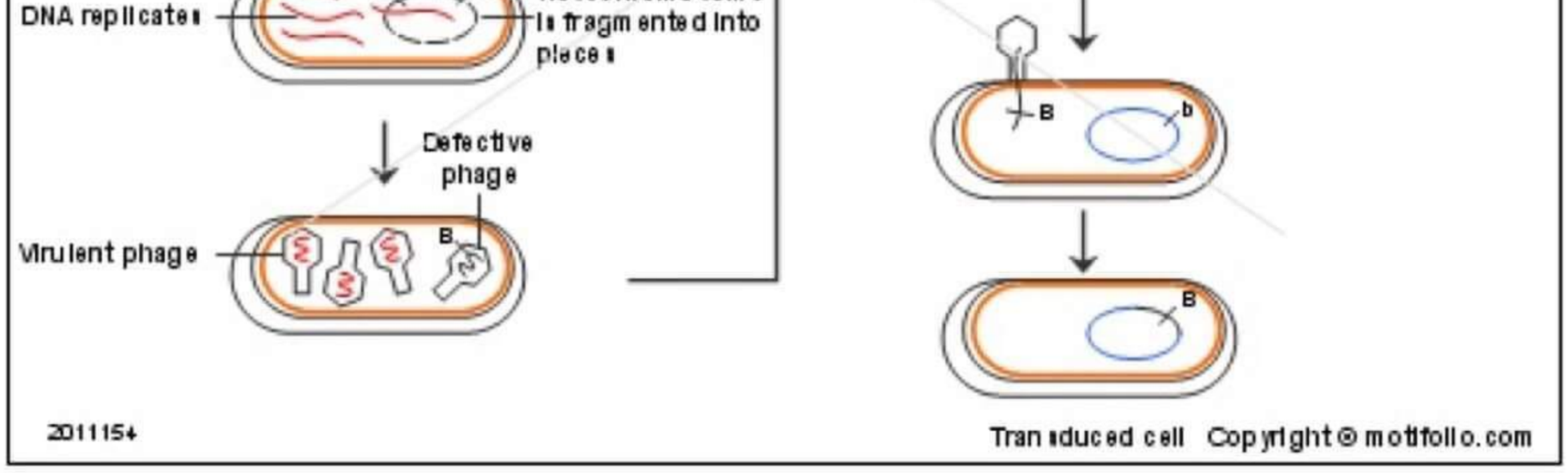
GENERALISED TRANSDUCTION

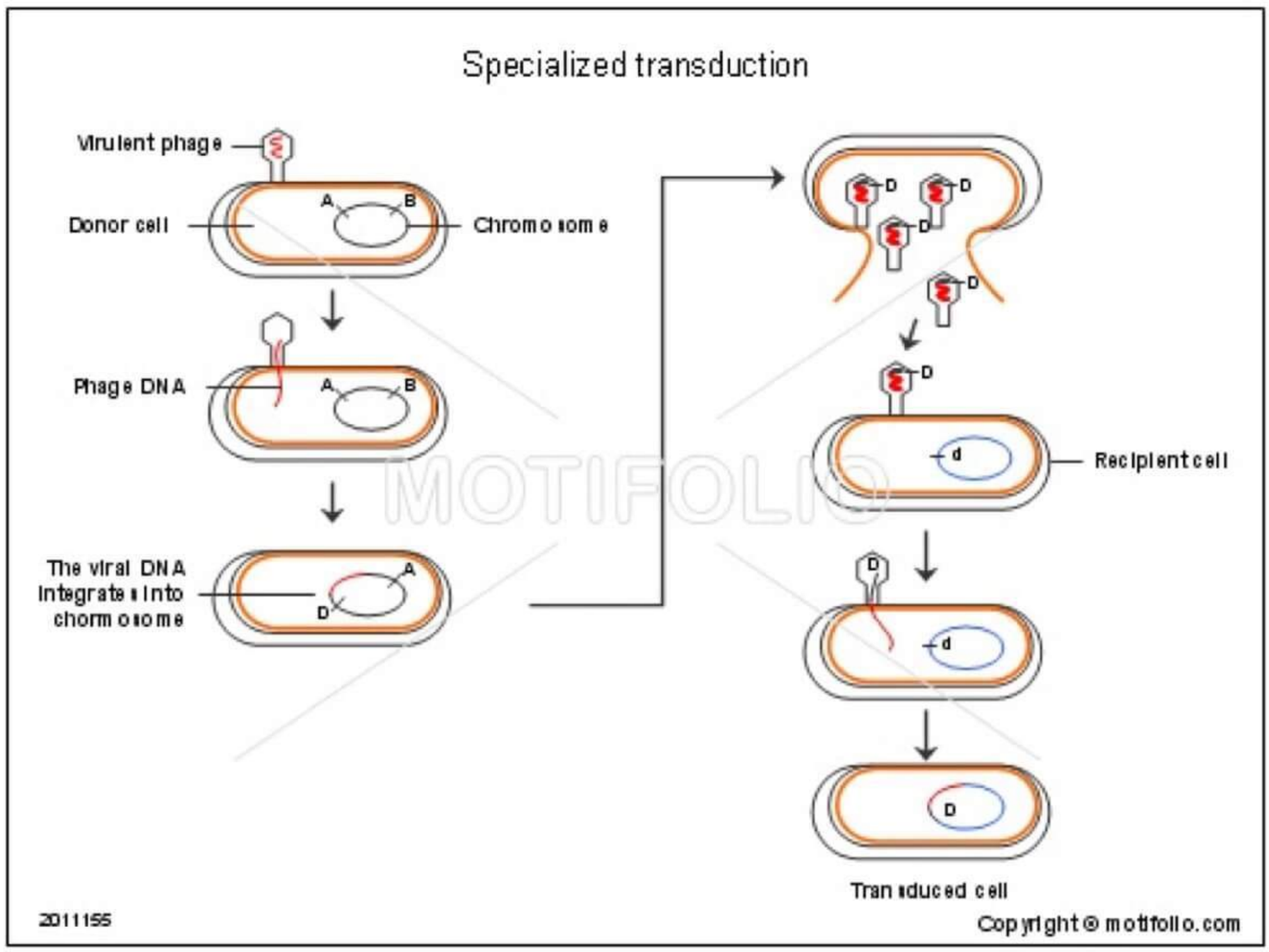
→ involves transfer of any part of donor bacterial genome to recipient bacteria

→ occurs as a result of defective assembly during the lytic/virulent cycle & Sometimes temperate phages

SPECIALIZED / RESTRICTED TRANSDUCTION

→ part adjacent to integration of phage DNA ī host cell DNA





LYSOGENIC CONVERSION

→ occurs mostly in temperate / lysogenic life cycle

→ Phage DNA incorporates in Bacterial DNA & gives it a new property → LYSOGENIC CONVERSION

BACTERIAL TOXINS THAT ARE CODED BY LYSOGENIC PHAGES

A → A & C pyrogenic toxins of Strepto. pyogenes

B → Botulinum toxin C & D

C → Cholera toxin

D → Diphtheria toxin

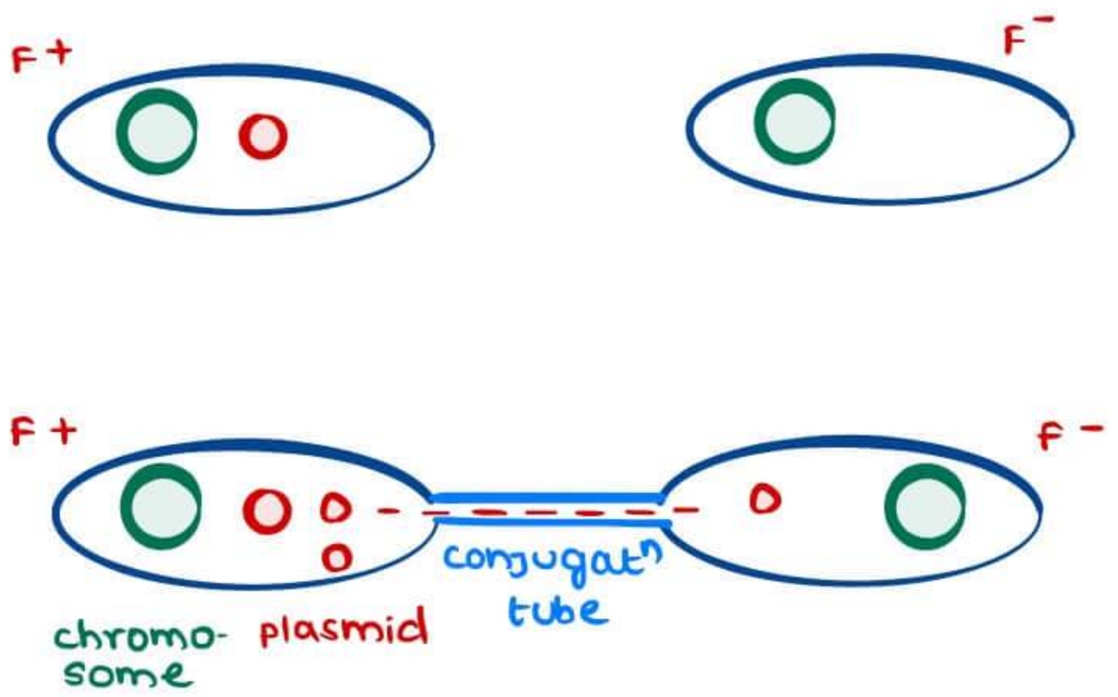
E → EHEC [Entero Haemorrhagic E.coli] [aka VeroToxin]

Panton valentine toxin of Staph. aureus

CONJUGATION

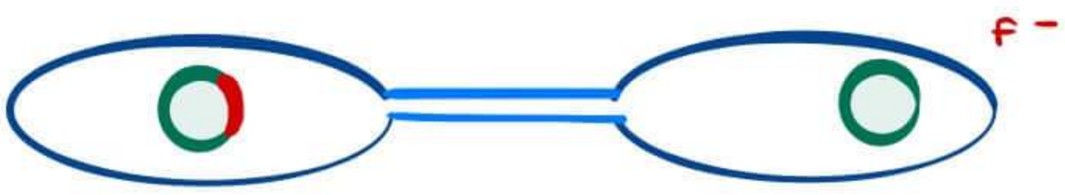
→ Mc method of resistance transfer in lysogenic bacteria

→ POSSIBILITY 1



POSSIBILITY 2 / EPIsome | Hfr [High frequency Recombination] CELL

EPIsome | Hfr [High frequency recombination] CELL



Hfr × F⁻ → F⁻

POSSIBILITY 3

$\times \quad F^- \quad \Rightarrow \quad F'$

→ POSSIBILITIES

F^+	x	F^-	⇒	F^+
HFr	x	F^-	⇒	F^-
F'	x	F^-	⇒	F'

TRANSFERABLE DRUG RESISTACE

- → Example of conjugational transfer
- → **RTF + r = RESISTANCE**
- → RTF - Resistance transfer factor
- → r - Resistant determinant [main factor]
- → Leads to MDR [multidrug resistance] → difficult to treat

- → MUTATIONAL DRUG RESISTANCE → SINGLE DRUG RESISTANCE → EASY TO R
- → TRASFERABLE DRUG RESISTANCE → MULTI DRUG RESISTANCE → Difficult to R

- → In MTB, mc method of resistance is Mutational drug resistance

TRANSPOSONS | JUMPING GENES

- → They are responsible for intracellular transfer
- → Transfer happens b/w
  - chromosome - chromosome
  - chromosome - Plasmid
  - Plasmid - Plasmid

- → work in a cut & paste manner
- → have inverted repeat sequences & insertion sequence
- → method is known as TRANSPOSITION
- → Resistance is transferred mainly t transposons

BIOTERRORISM AGENTS

CATEGORY A → carry highest risk

CATEGORY B

CATEGORY C → Emerging threats

CATEGORY A

Play → Plague

Vith → viral Haemorrhagic fevers [Marburg, Ebola, KFD, Yellow fever]

Small → Small Pox

B → Botulism

A → Anthrax

T → Tularemia

CATEGORY B

C → Clostridium perferingens [Epsilon toxin]

B → Brucella

S → Salmonella, Shigella, Staphylococcus [enterotoxin B]

E → E. coli [O_{157} : H_7]

Vill → Vibrio cholerae

take → Typhus fever

Questions for → Q fever

Medical → Meliodiosis

P → Psittacosis

G → Glanders [non motile]

CATEGORY C

N → Nipah

H → Hunta Virus

S → SARS