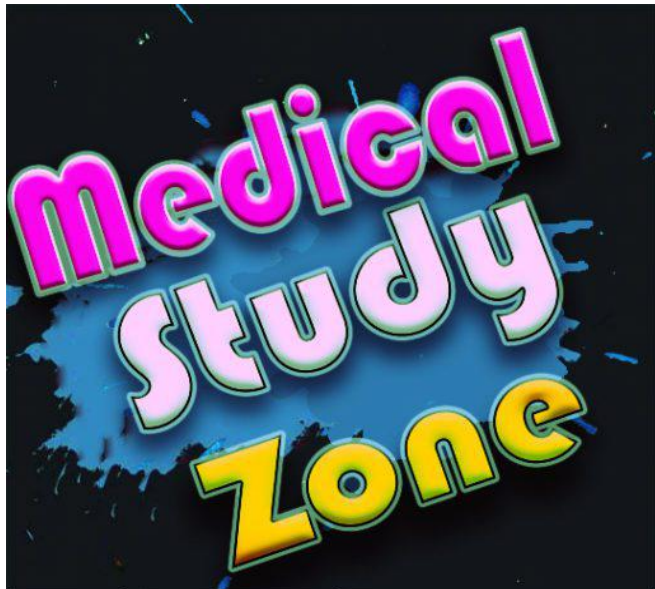
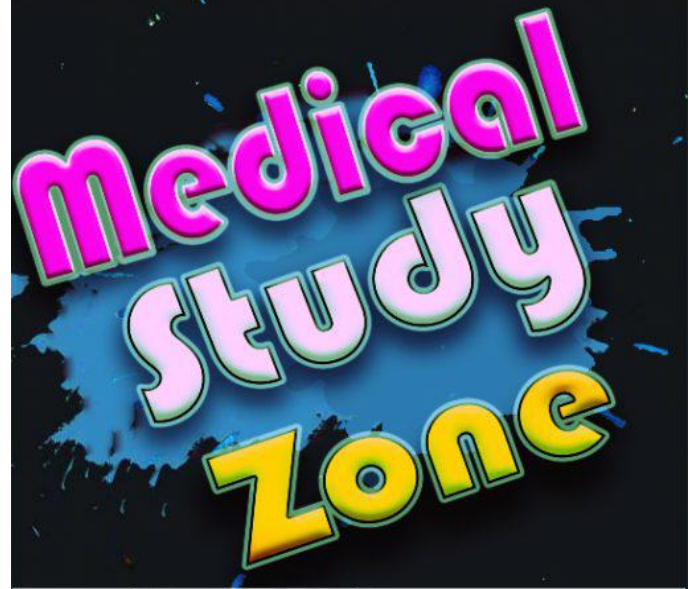


# PrepNotes





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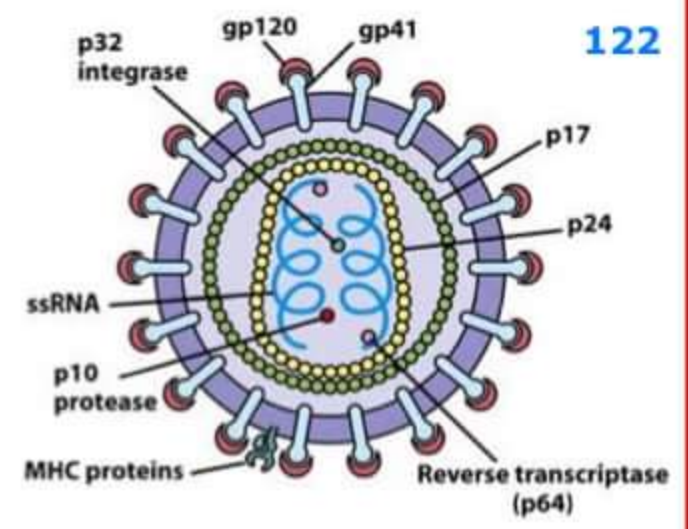
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# HIV



HIV

→ Family → Retroviridae  
 → Sub family → Lenti viralae

→ 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 / Gp41

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

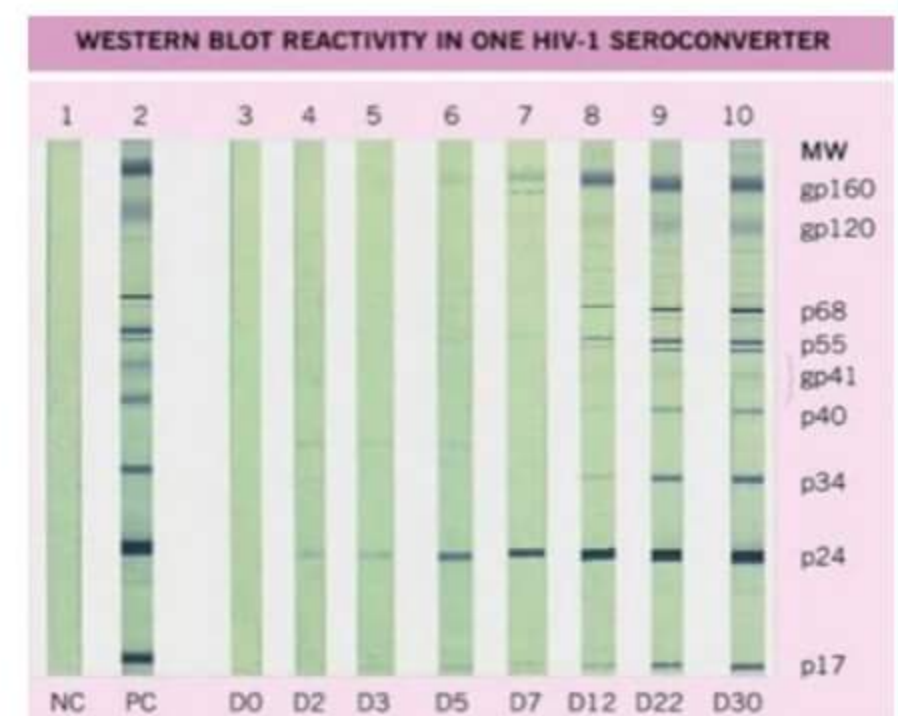
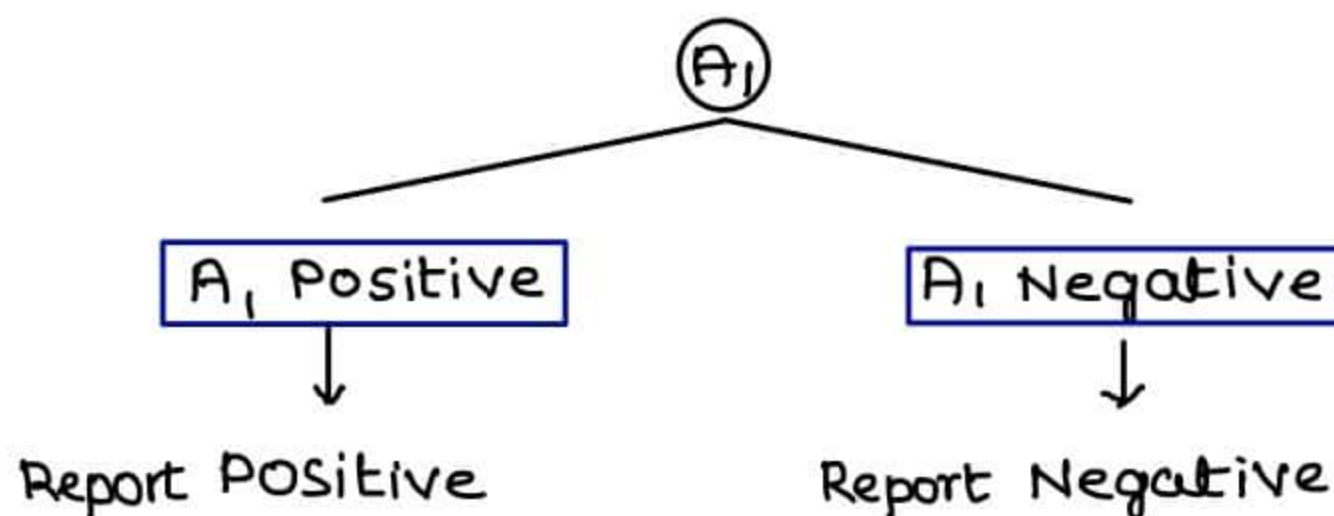


### NACO STRATEGIES

- 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 I / ALGORITHM 1

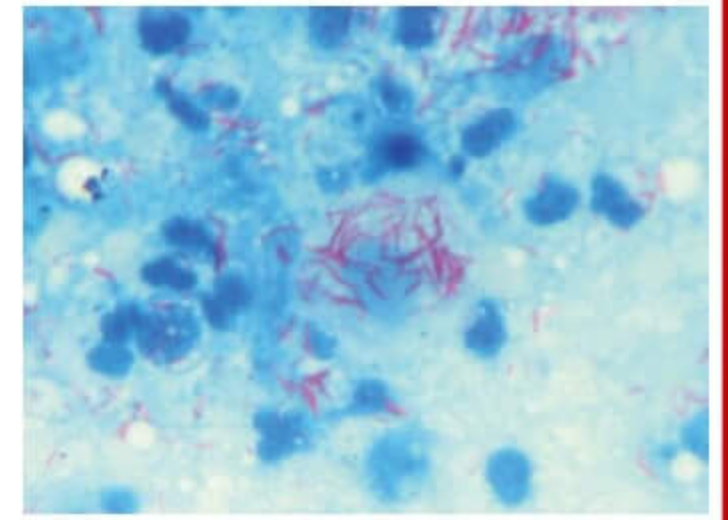
- done for transfusion & transplantation safety
- one test kit is required





## 2. ACID FAST STAINING [ ZIEHL NEELSEN STAINING ]

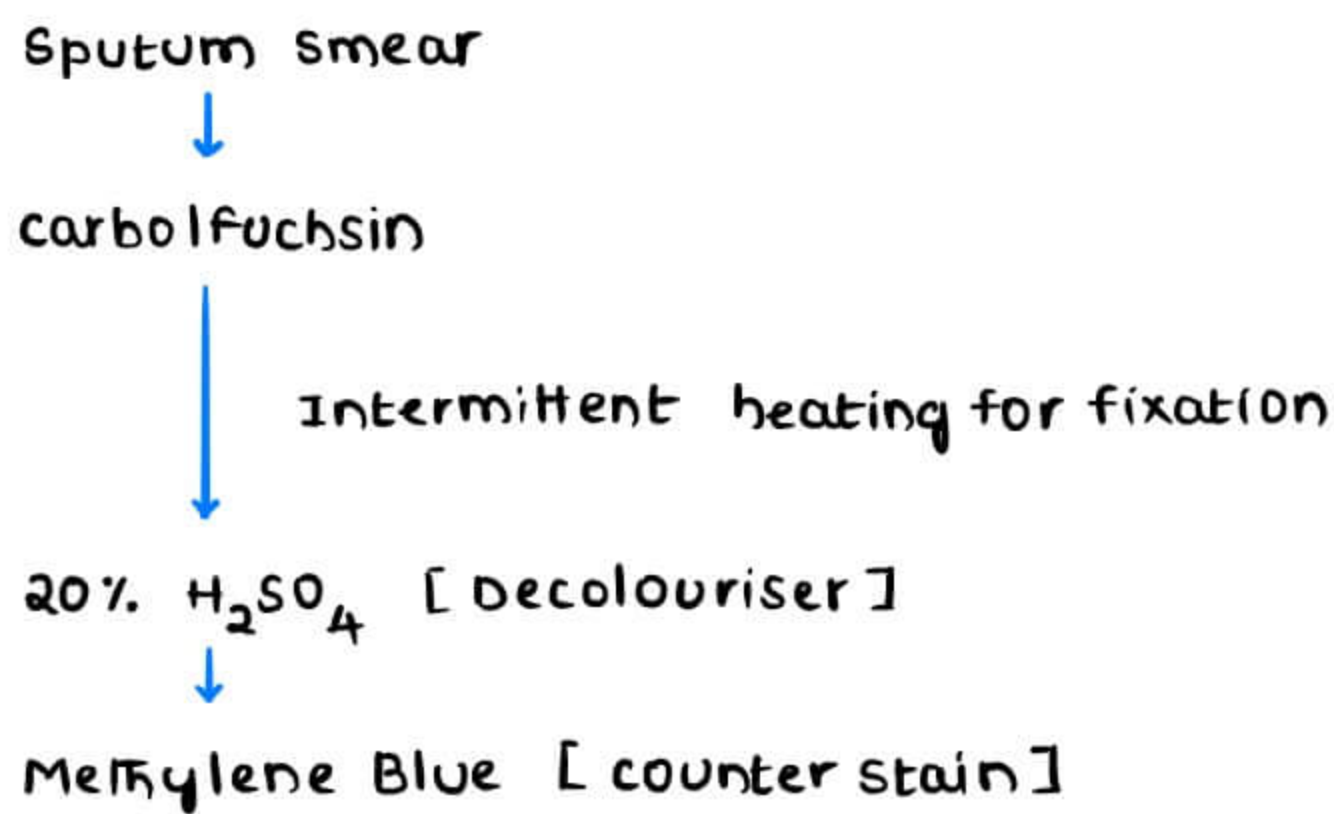
- differentiates b/w Acid fast / Non - Acid fast
  - Based on ACID FASTNESS [ Ability to resist decolorisation ]
- ↓
- due to MYCOLIC ACID [ Content ]



ZN STAINING

- Mycobacterium Tuberculosis → 20% Acid fast
  - Mycobacterium Leproue → 5% Acid fast
  - Nocardia → 1% Acid fast
- 
- Bacterial spores
  - Oocyst of
    - ↳ cryptosporidium parvum
    - ↳ cyclospora
    - ↳ isospora
- } 0.25 - 1% Acid fast

### STEPS [ for MTB ]



### 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
  - ↳ Haemophilus ducreyi
  - ↳ Burkholderia mallei
  - ↳ Burkholderia pseudomallei
  - ↳ vibrio parahemolyticus
  - ↳ Francisella Sps.
  - ↳ Pasturella Sps.



## CAPSULATED ORGANISMS

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

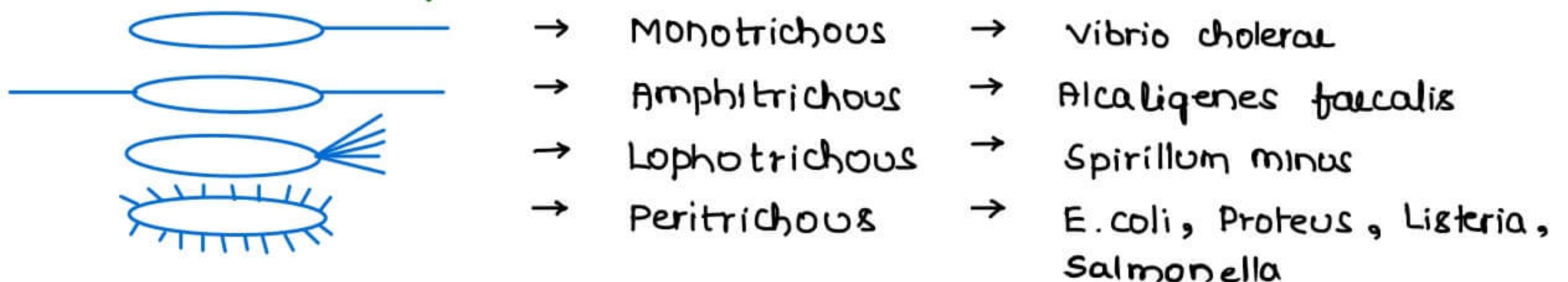
### Capsule

- virulence factor
- Antiphagocytic
- mainly made of polysaccharide
  - Except<sup>n</sup> → Bacillus anthracis capsule [made of Polypeptide]
- Demonstrated by
  1. QUELLUNG REACTION
    - capsular swelling dit serological react<sup>n</sup>
    - seen in Pneumococcus, Haemophilus influenza
  2. Mc FADYEAN'S REACTION
    - observed in Bacillus anthracis
    - culture growth + Loeffler'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

- dit Flagella [Flagellin] → Antigenic

- Demonstrat<sup>n</sup> of Flagella



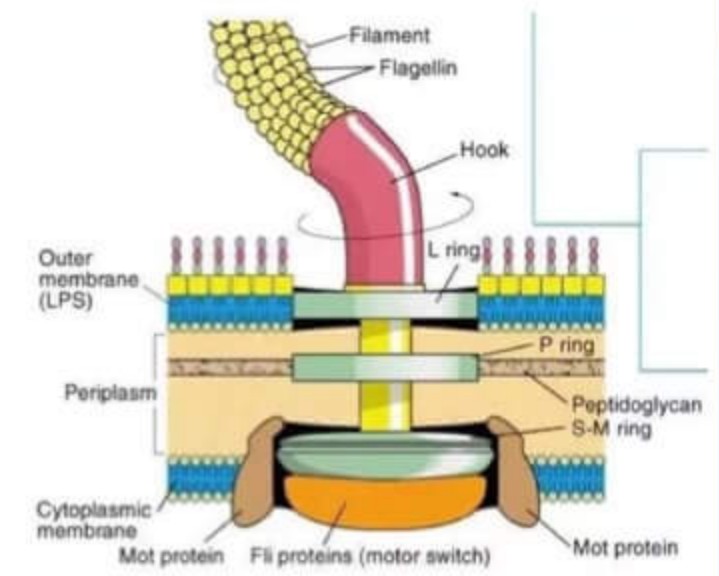
- MOTILITY NAMES

- |   |            |               |
|---|------------|---------------|
| T | → Tumbling | → Listeria    |
| D | → Darting  | → v. cholerae |
| S | → Swarming | → Proteus     |



- Falling leaf like
- Slow form of swarming [stately]
- Gliding motility
- Giardia Lamblia [4 pairs of flagella]<sup>4</sup>
- Clostridium
- Mycoplasma

- Spirochetes have **ENDOFLAGELLA**
  - Arise from periplasmic space
  - Treponema pallidum
  - Borrelia
  - corkscrew motility
  - 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. NEWZEALAND AGAR [more jellifying property]

### CLASSIFICATION

#### A. BASED ON NUTRITIONAL STATUS

- Simple
- complex
- Synthetic

#### C. BASED ON O<sub>2</sub> TENSION

- Aerobic media
- Anaerobic media

#### B. BASED ON CONSISTENCY

- Liquid
- semisolid
- solid

#### 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. Loeffler's serum slope [used for Corynebacterium diphtheriae]



## 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]
- Differentiate b/w
  - Lactose fermenting [E. coli, klebsiella, shigella sonnei (late lactose fermentor)]
  - Non lactose fermenting organisms
- Selective dlt
  - 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 Electrolyte 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 → Saccharolytic sps.

2. THIOGLYCOLATE BROTH

## COMPOSITE MEDIA

→ demonstrates DIFFERENT PROPERTIES using single medium

→ Eg → TRIPLE SUGAR IRON AGAR [TSI]

→ Used to study fermentat<sup>n</sup> of glucose, Lactose, Sucrose & product<sup>n</sup> of H<sub>2</sub>S & gas



## STERILIZATION OF MEDIA

- MOSTLY done by AUTOCLAVE at  $121^{\circ}\text{C}$  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  
[ $80-85^{\circ}\text{C}$  for 20-30 min for 3 days]
- STORAGE OF CULTURE MEDIA → b/w  $4-5^{\circ}\text{C}$  inside refrigerator

## DEHYDRATED MEDIA

- commercially available
- reconstituted before use in distilled water

## CLASSIFICATION

### BASED ON $\text{O}_2$ REQUIREMENT

#### ① OBLIGATE AEROBES

- needs desperate  $\text{O}_2$
- MTB
- Pseudomonas
- Nocardia

#### ② OBLIGATE ANAEROBES

- Does not need  $\text{O}_2$  at all
- A → Actinomyces
- B → Bacteroides
- C → Clostridium species

#### ③ FACULTATIVE ANAEROBES

- changed anaerobe
- Aerobe + Anaerobe
- Most of the medically important bacteria
- Eg. Staphylococcus, Streptococcus species

#### ④ MICROAEROPHILES

- need 5%  $\text{O}_2$  only
- campylobacter
- Helicobacter

#### ⑤ CAPNOPHILES

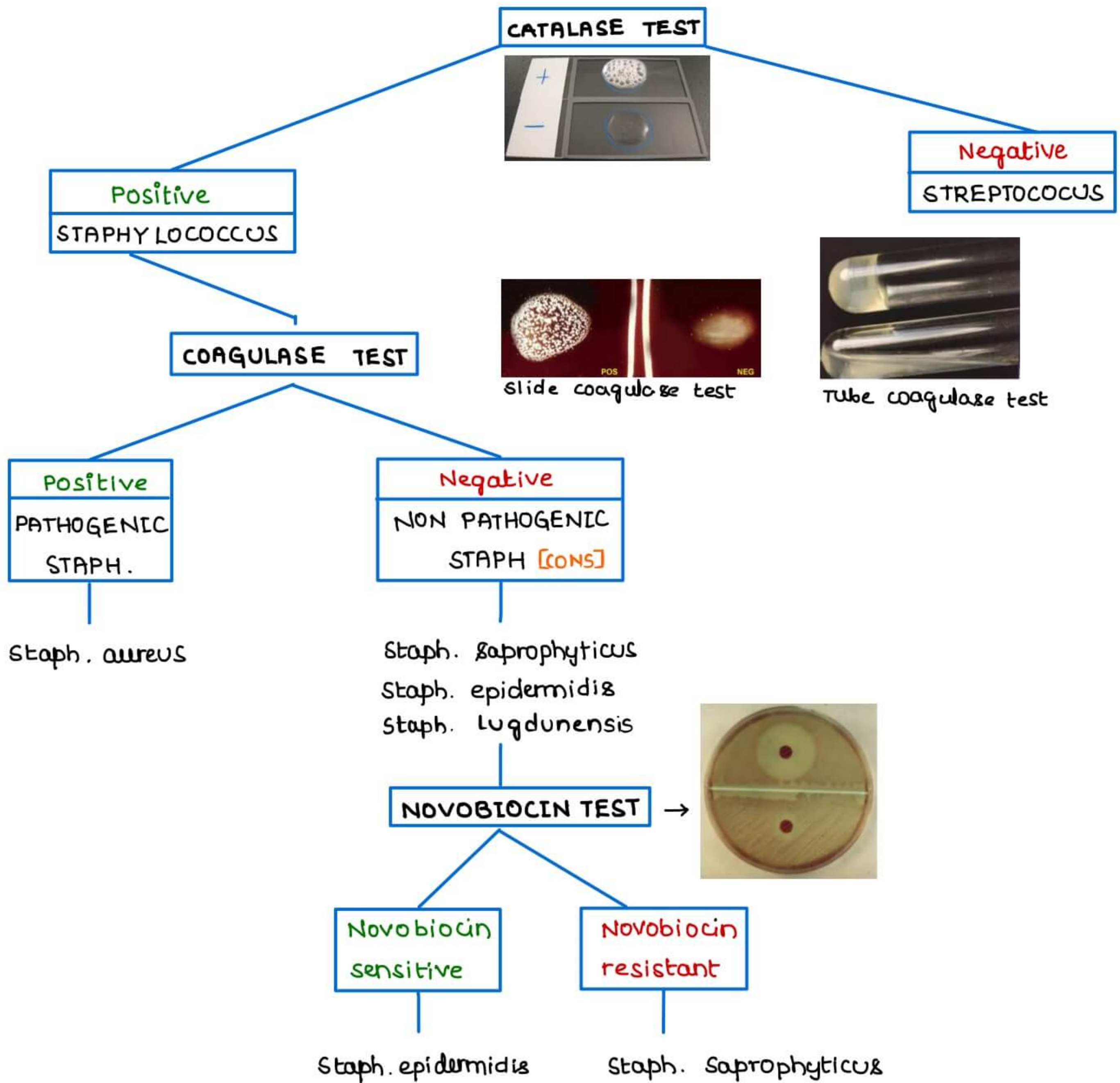
- $\text{CO}_2$  loving
- Brucella abortus
- Pneumococcus
- H. influenzae

### BASED ON TEMPERATURE REQUIREMENT

- P → Psychrophile →  $-20^{\circ}\text{C}$  to  $+20^{\circ}\text{C}$
- M → Mesophile →  $20^{\circ}\text{C}$  to  $40^{\circ}\text{C}$  [medically important bacteria]
- T → Thermophile →  $> 60^{\circ}\text{C}$
- $> 100^{\circ}\text{C}$  [can manage]
- Thermus aquaticus [Typical example]
- Used in PCR
- Father of PCR → KARRY B MULLIS



GRAM POSITIVE COCCI



- Novobiocin test → done for Antibiotic testing
- Slide coagulase test → to determine bound form
- Tube coagulase test → to determine free form
- 3% H<sub>2</sub>O<sub>2</sub> + Staph. colonies → Bubbles [CATALASE POSITIVE]

AST [Antibiotic Sensitivity Testing]

- Eq: 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

- ① 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 inhibit<sup>n</sup> is +ve

### 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 Inhibit<sup>n</sup>

MC ORGANISMS INVOLVED IN EARLY PROSTHETIC VALVE ENDOCARDITIS → CONS

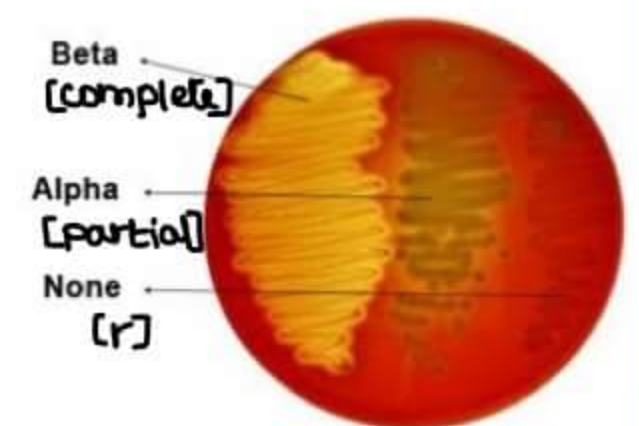
## PATHOGENIC STAPH

### Staph aureus

- Gram positive cocci
- catalase +ve
- coagulase +ve
- Gram staining → VIOLET GRAPE LIKE CLUSTERS
- CULTURE MEDIA

① NA [Nutrient Agar] → GOLDEN YELLOW pigmentat<sup>n</sup>  
↓  
Enhances on milk agar

② BA [Blood Agar]  
- causes  $\beta$  hemolysis  
- pin head size colonies



## HEMOLYSIS

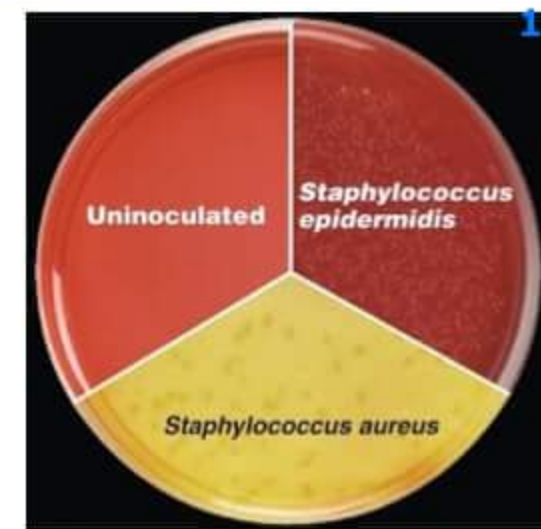
1.  $\alpha$  Hemolysis → Partial hemolysis around colonies  
→ Greenish discoloration is present  
→ unlysed RBCs can be seen under microscope  
→ Eg → streptococcus viridans, streptococcus pneumoniae
2.  $\beta$  Hemolysis → Well defined clear colorless zone of hemolysis around colonies  
→ Eg → streptococcus pyogenes
3.  $\gamma$  Hemolysis → no hemolysis around colonies  
→ Eg → Enterococcus



- ③ MANNITOL SALT AGAR [Best selective media]  
 - In presence of Staph. aureus the agar changes / transforms from pink to yellow

④ LUDLAM'S MEDIA [2nd Best media]

⑤ SALT MILK AGAR



MANNITOL SALT 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 to the Fc portion of IgG



Golden yellow colonies on nutrient agar

⑤ Hemolysins ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ )

→  $\alpha$  hemolysins

→ shows PARADOXICAL ACTION

- inactivates at  $70^{\circ}\text{C}$

- reactivates at  $100^{\circ}\text{C}$

→  $\beta$  hemolysins / sphingomyelins

→ shows HOT & COLD phenomenon

-  $\beta$  hemolysis better seen at  $37^{\circ}\text{C}$

- in S. aureus better seen at  $4^{\circ}\text{C}$

→ 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 cereus emetic strain

- S. aureus → contaminated meat & milk products

- B. emetic → contaminated chinese fast food



→ dlt Preformed toxin → Enterotoxin A

• Heat stable

• Vagomimetic act<sup>n</sup> / vagal Stimulat<sup>n</sup>

② SSSS [STAPHYLOCOCCAL SKIN SCALDED SYNDROME]

→ dlt Exfoliative Toxins A & B

→ aka TEN → Toxin Epidermolytic Neurolysis 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 PNEUMONIA

→ 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<sub>2a</sub> [main mechanism] (PBP - Penicillin Binding Protein)  
 d/tt MEC A gene acquisition; PBP also known as Transpeptidase

#### • MEC A GENE TYPES [6 types]

I	}	causes NOSOCOMIAL MRSA community	IV	}	causes Acquired MRSA
II			V		
III			VI		

② Product<sup>n</sup> of  $\beta$  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/B1A → mc phage



# STREPTOCOCCUS

## CLASSIFICATION

$\alpha$ hemolysis	$\beta$ hemolysis	$\gamma$ hemolysis
<ul style="list-style-type: none"> <li>→ <i>S. pneumoniae</i></li> <li>→ <i>S. viridans</i></li> </ul>	<ul style="list-style-type: none"> <li>→ classified into A-V based upon carbohydrate <math>c</math> in the cell wall [LANCIEFIELD CLASSIFICATION]</li> <li>→ A is divided into 80 types based on M Protein [GRIFFITH TYPING]</li> <li>→ Group A [<i>S. pyogenes</i>] B [<i>S. agalactiae</i>] D [Enterococcus]</li> </ul>	<ul style="list-style-type: none"> <li>→ Enterococcus</li> </ul>

	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*

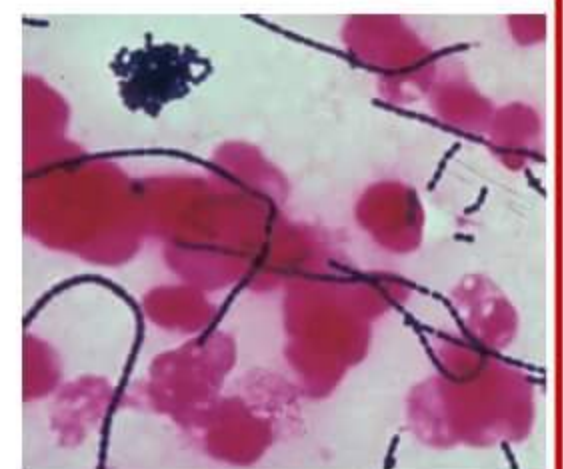
- Gram positive cocci
- catalase -ive
- arranged in chains in Gram staining [violet]
  - Longest chain → *S. salivarius*

#### → CULTURE MEDIA

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

- 2 PIKE'S MEDIA

- Transport media



GRAM STAINING



PINPOINT SIZE COLONIES



## → DISEASES

- ① ACUTE PHARYNGITIS [Sore throat]
- ② CELLULITIS
- ③ IMPETIGO
- ④ ERYSIPELAS [lymphatics involved]
- ⑤ NECROTISING FASCITIS → FLESH EATING BACTERIA
- ⑥ PUERPERAL 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 <sup>n</sup>
→ 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 agalactiae

- Normal commensal 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**
  - $\bar{c}$  Van gene acquisition [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

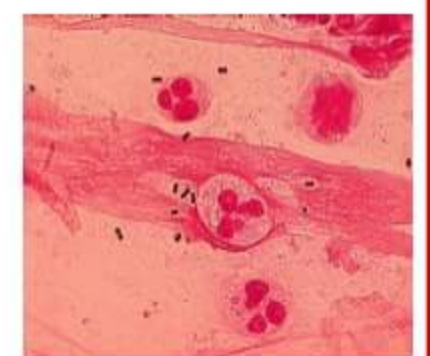
- St. viridans → Leads to SABC, late onset prosthetic valve endocarditis
- St. mutans → Leads to dental caries

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

### Streptococcus pneumoniae [Pneumococcus]

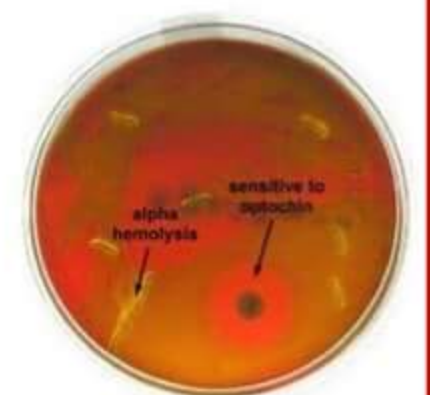
- Gram positive cocci
- Lanceolate [Flame shaped]
- DRAUGHTS MAN / CARROM COIN LIKE COLONIES ON various culture media
- **Capsulated**

- made of Polysaccharide
- demonstrated by **QUELLUNG REACTION**
  - capsular swelling
  - Serological reaction
  - capsular Antigen + Antibody → SWELLING / DELINEATION
  - also seen in Haemophilus influenza



Diplo cocci

- **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 4]

S → SINUSITIS

→ VACCINES

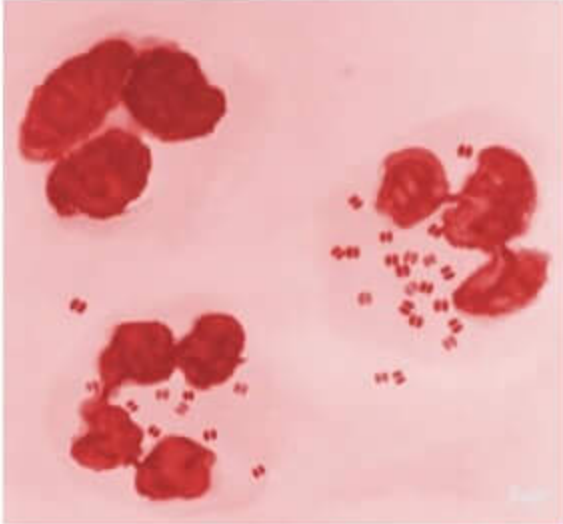
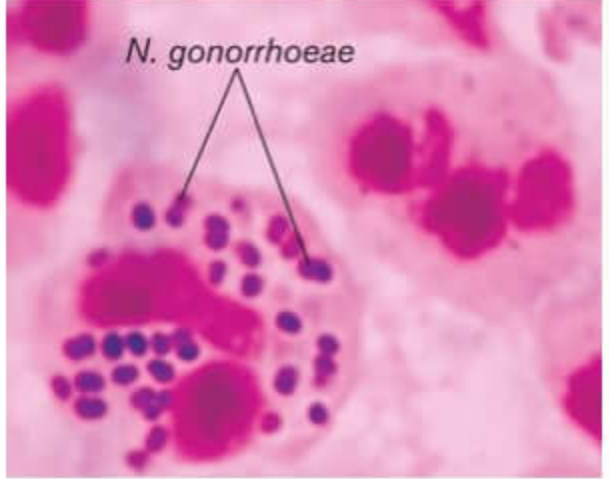
1 23 valent Polysaccharide vaccine

- used for Splenectomized individuals

2 7 valent conjugate vaccine

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



MENINGOCOCCI	GONOCOCCI
<ul style="list-style-type: none"> <li>→ capsulated</li> <li>→ Lens shaped</li> <li>→ RCUT [Rapid carbohydrate utilization test]               <ul style="list-style-type: none"> <li>- ferments Glucose &amp; maltose</li> </ul> </li> <li>→ Plasmid recruit absent [for resistance]</li> <li>→ Extra/Intracellular</li> </ul>	<ul style="list-style-type: none"> <li>→ Non capsulated               <ul style="list-style-type: none"> <li>- some are capsulated</li> </ul> </li> <li>→ Kidney shaped</li> <li>→ RCUT [Rapid carbohydrate utilization test]               <ul style="list-style-type: none"> <li>- ferments only Glucose</li> </ul> </li> <li>→ Plasmid recruit present</li> <li>→ mainly intracellular</li> </ul>
	

## MENINGOCOCCI

### → CAPSULATED

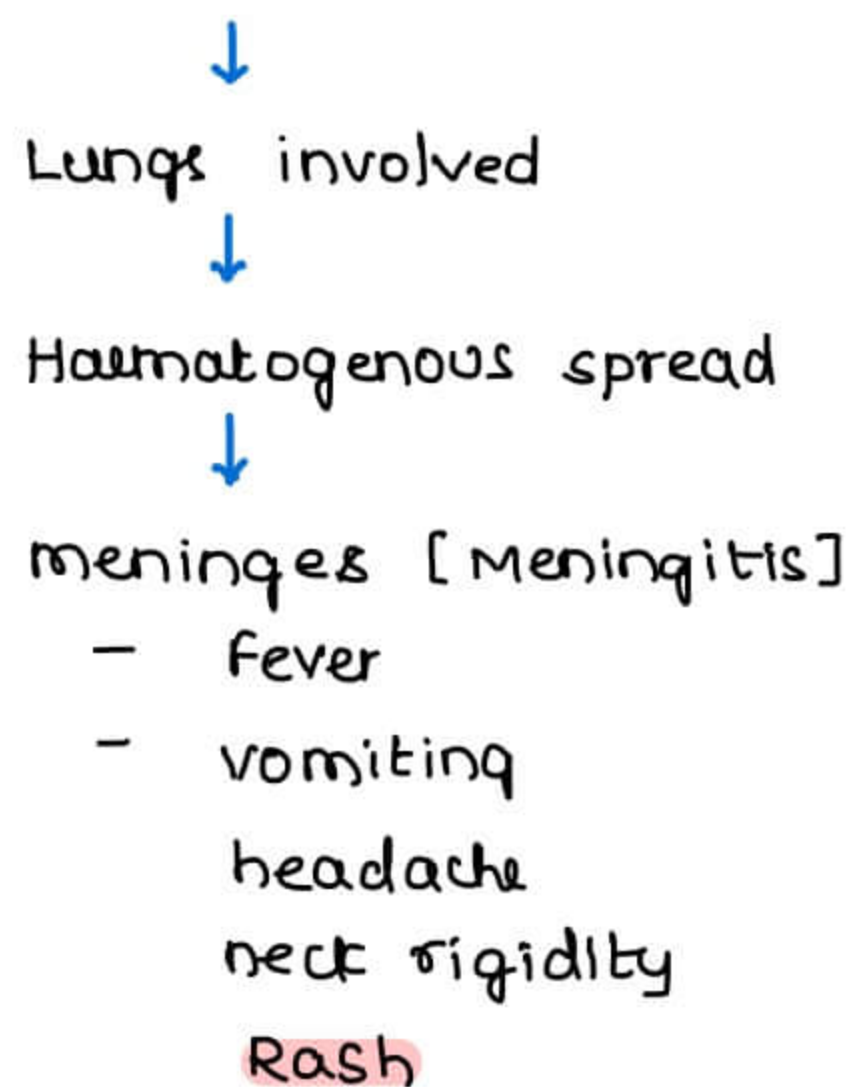
- on the basis of capsular polysaccharides → 13 Serogroups
  - A, B, C, Y, W, E → most important serogroups
  - A, B, C → most infective serogroups

### → VIRULENCE FACTORS

- ① capsular polysaccharide
- ② IgA<sub>1</sub> protease
- ③ Lipo oligosaccharide
- ④ OMP [Outer membrane Proteins]
- ⑤ Pili

→ 5-15% persons are normal nasopharyngeal carriers

→ Spreads by Droplet infections

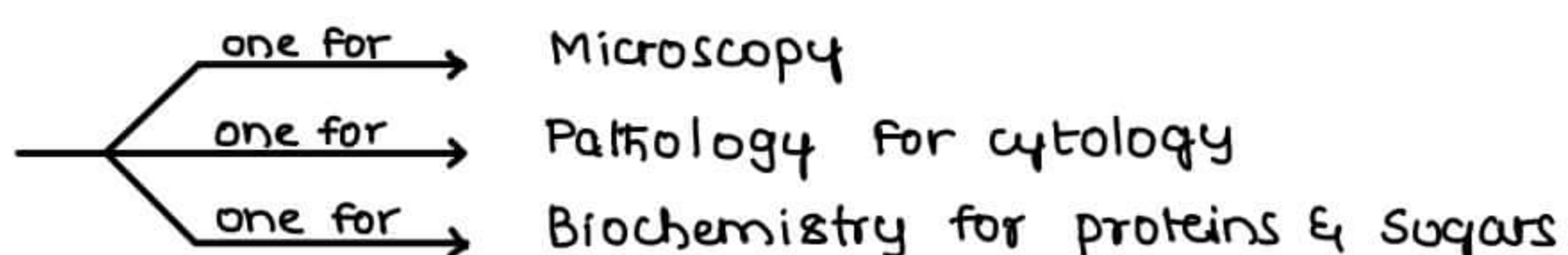




## → LAB DIAGNOSIS

### ① CSF EXAMINATION

→ 3 samples taken



### 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<sub>5-9</sub> 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 generat<sup>n</sup> 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<sub>1</sub> protease

③ Lipo oligosaccharide

④ OMP [Outer membrane Proteins]

⑤ Pili

⑥ Transferrin / Lactoferrin



## → DISEASES

### ① Gonorrhoea

- Males → Urethritis
- Females → Cervicitis
- Newborn → Ophthalmia neonatorum

## → LAB DIAGNOSIS

### I 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



**CORYNEBACTERIUM DIPHTHERIA (Klebloffer's bacillus)**→ **GRANULES**

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

→ These are Energy deposits

→ stained by

**P** → **P**onder's stain

**A** → **A**lbert's stain

**N** → **N**eisser stain

→ cuneiform arrangement / chinese letter pattern

→ **CULTURE MEDIA**

→ LSS [LOFFLER SERUM SLOPE]

→ Potassium tellurite Agar

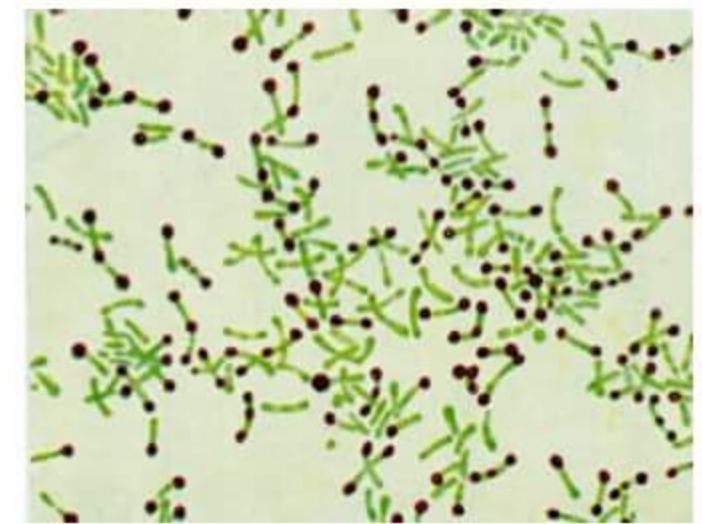
→ **H** Hoyle's media

→ **M** macleod's media

→ **T** Tinsdale media

→ LSS is used for early Dx [6-8 hrs]

→ Best selective media → Potassium tellurite Agar



Chinese letter pattern

→ **BIDYPES**

colonies on PTA

- |               |   |                      |                               |
|---------------|---|----------------------|-------------------------------|
| ① GRAVIS      | → | <b>DAISY'S</b>       | <b>DAISY HEAD APPEARANCE</b>  |
| ② INTERMEDIUS | → | <b>FROG EGGS</b> are | <b>FROG EGG APPEARANCE</b>    |
| ③ MITIS       | → | <b>POACHED</b>       | <b>POACHED EGG APPEARANCE</b> |

→ **DIPHTHERIA TOXIN**

→ made of A+B [Bipartite toxin]

→ Strong exotoxin

→ needs optimum iron for its act<sup>n</sup>

→ **B** → helps in Binding

→ **A** → ADP Ribosylation of Elongat<sup>n</sup> Factor 2



Inhibition of Protein Synthesis of cell



cell Death



→ Every *Corynebacterium diphtheriae* is not pathogenic, but made pathogenic by  $\beta$  phage tox gene → **LYSOGENIC CONVERSION**

Lysogetic Phase	} Part of Transduct <sup>n</sup>
Lytic Phase	

→ **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 immunizat<sup>n</sup>  
 if negative → already immunized  
 neutralizat<sup>n</sup> test

→ Diphtheria is TOXEMIA not Bacteraemia

→ **PRESENTATION**

- **PSEUDOMEMBRANE'S** mc location → faucial [Tonsillar]
- most dangerous location → Laryngeal [resp. obstruct<sup>n</sup>]
- 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

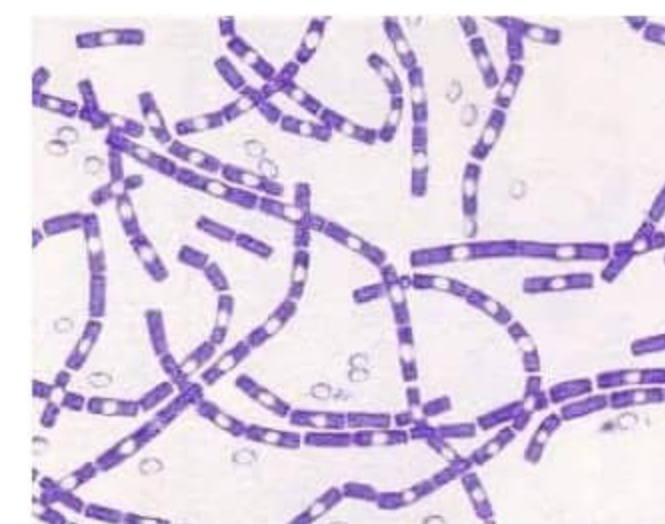
### → APPEARANCES

- 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 (Hand lens) → MEDUSA HEAD / MATTED WOOL LIKE / MATTED HAIR LIKE
  - 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 synergistically → ↑ CAMP

### → DISEASES

CUTANEOUS ANTHRAX [HIDE PORTER'S DISEASE]	PULMONARY ANTHRAX [WOOL SORTER'S DISEASE]
<ul style="list-style-type: none"> <li>→ Eschar</li> <li>→ Pustule with non pitting edema               <ul style="list-style-type: none"> <li>- malignant Pustule</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>→ Occupational Hazard</li> <li>→ causes mediastinal hemorrhagic necrosis</li> <li>→ Prevented by DUCKERING pre treat the wool by formaldehyde (Sporocidal)</li> </ul>

### 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
<ul style="list-style-type: none"> <li>→ IP → 1 to 6 hrs</li> <li>→ causes food poisoning               <ul style="list-style-type: none"> <li>- dit chinese food</li> </ul> </li> <li>→ resembles Heat stable toxin of <i>E. coli</i> in action → ↑ cGMP</li> </ul>	<ul style="list-style-type: none"> <li>→ IP → 8-16 hrs</li> <li>→ resembles Heat labile toxin of <i>E. coli</i> in action → ↑ cAMP</li> </ul>

## → CULTURE MEDIA

→ MYPA [Mannitol Yolk sac Polymyxin Phenol Red Agar] MEDIA

## Obligate Anaerobes CLOSTRIDIUM

- GPB
- Non capsulated
- motile → STAGELY [Slow form of swarming]
- Except<sup>n</sup> → *Cl. perfringens* [capsulated, non motile]



ROBERTSON COOKED MEAT MEDIUM

## CULTURE MEDIA

### ROBERTSON COOKED MEAT MEDIA

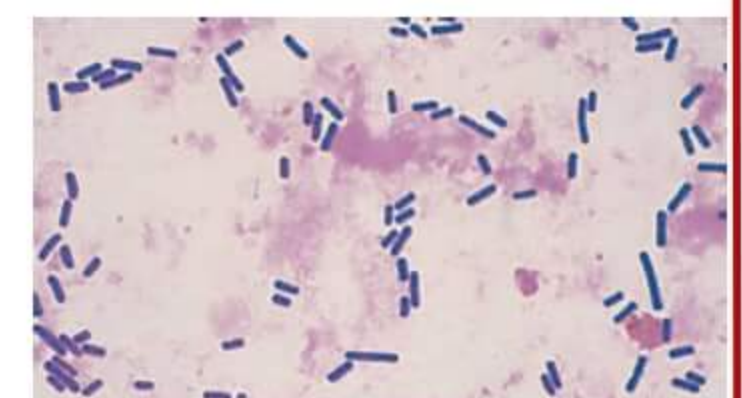
- if meat pieces turn PINK → saccharolytic → *Cl. perfringens*
- if meat pieces turn BLACK → proteolytic → *Cl. tetani*

## 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
  - $\alpha$ ,  $\beta$ , epsilon, theta, iota
  - Type A - E
  - most infections are caused by Type A/ $\alpha$  toxin



*Clostridium perfringens*



## ① GAS GANGRENE / CLOSTRIDIAL MYONECROSIS

- IP → 1-2 days
- C/F → Pain  
crepitus [Gas in the deep compartment]
- TREATMENT
  - For Initial lesion
    - Surgical debridement & Antibiotics
  - For Advanced case
    - Amputation of the limb + Antibiotics

## → GAS GANGRENE CAUSED BY

- P → *Cl. perfringens*
- N → *Cl. novyi*
- S → *Cl. septicum*

## ② FOOD POISONING

- d/t contaminated meat products
- IP → 8-24 hrs
- involves Heat labile enterotoxin
  - but spores are Heat Resistant

## ③ PIGBEL

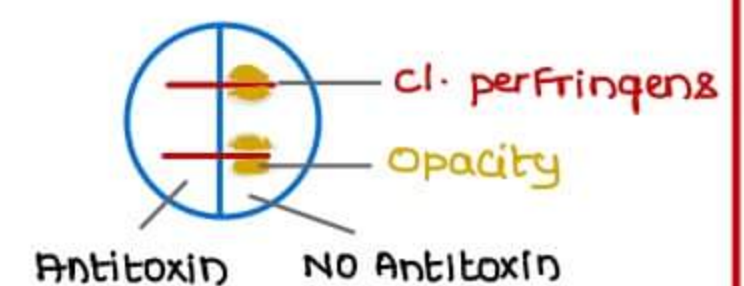
- d/t uncooked pork consumption
- causes necrotising enteritis
- d/t  $\beta$  toxin TYPE C

## 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

- d/t  $\alpha$ -Lecithinase
- it is a neutralizat<sup>n</sup> Reaction
- done on Egg Yolk Agar



## ② STORMY FERMENTATION IN LITMUS MILK

- Immense fermentation of colored milk

## ③ TARGET HEMOLYSIS [DOUBLE ZONE HEMOLYSIS]

- inner zone → d/t  $\theta$  toxin
- outer zone → d/t  $\alpha$  toxin





#### ④ REVERSE CAMP REACTION

→ BOW TIE HEMOLYSIS  $\bar{c}$  St. agalactiae



### Clostridium tetani

#### TOXINS

① TETANOLYSIN

② TETANOSPASMIN

→ more dangerous

→ causes pre synaptic inhibition of Glycine/GABA [inhibitory neurotransmitters]

→ constant stimulation



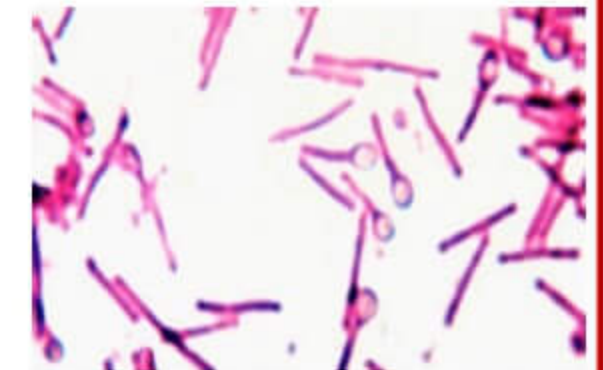
LOCK JAW [TRISMS]



OPISTHOTONUS POSTURE



↑ Deep Tendon Reflexes



DRUMSTICK APPEARANCE OF Cl. tetani

IP → 8 - 10 days

→ if IP

→ < 6 days

→ 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<sub>2</sub> [Enterotoxin] → a/w canned food poisoning

#### CANNED FOOD POISONING

##### CLINICAL FEATURES

→ Dysphagia

→ Diplopia

→ Dysarthria

→ CONSTIPATION [d/t ↓ Ach]

→ ↓ DTR



## INFANT BOTULISM

- a/w Sporulated honey ingestion
- CLINICAL FEATURES
  - Flaccid paralysis → FLOPPY INFANT SYNDROME

## NEUROTOXIN USES

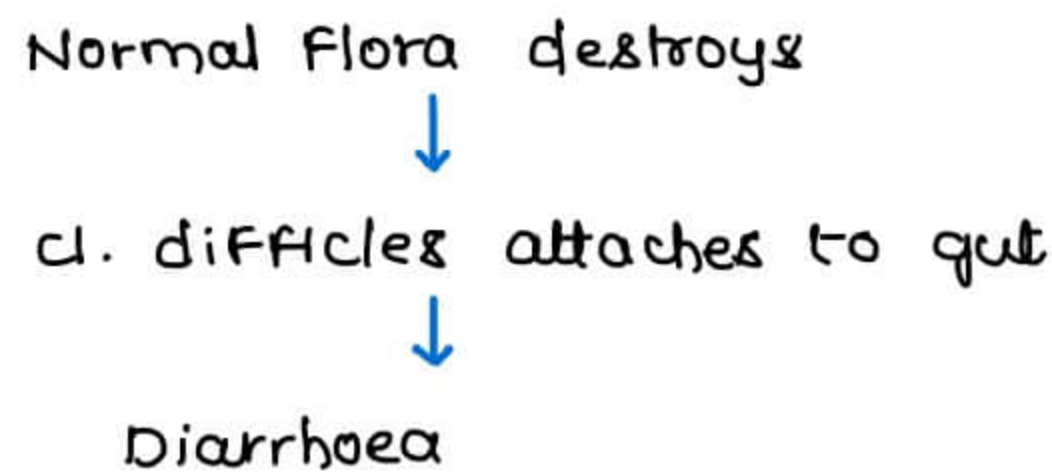
- ① Botox treatment → to remove wrinkles
- ② Eye Surgeries
  - Blepharospasm
  - Strabismus

## DIAGNOSIS

- ① PCR for cl. botulinum
- ② culture for cl. botulinum

## Clostridium difficile

- difficult to isolate
- CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHOEA [CDAD]
  - if not Rx in time, leads to pseudomembranous colitis
  - ④ commensals of gut [cl. difficile]
  - ↑ irrational broad spectrum antibiotic usage.



## LAB DIAGNOSIS

- ① TOXIN DEMONSTRATION [Enterotoxin [A] + cyto toxin [B] ]  
demonstrated on Hep 2 cell lines [commonly used for viral isolat<sup>n</sup>]
- ② PCR for tox gene
- ③ ELISA for toxin production
- ④ SIGMOIDOSCOPY to visualise any ulcer
- ⑤ CULTURE for cl. difficile



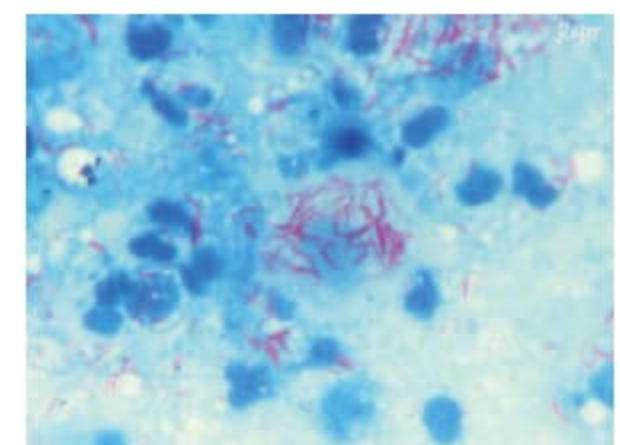
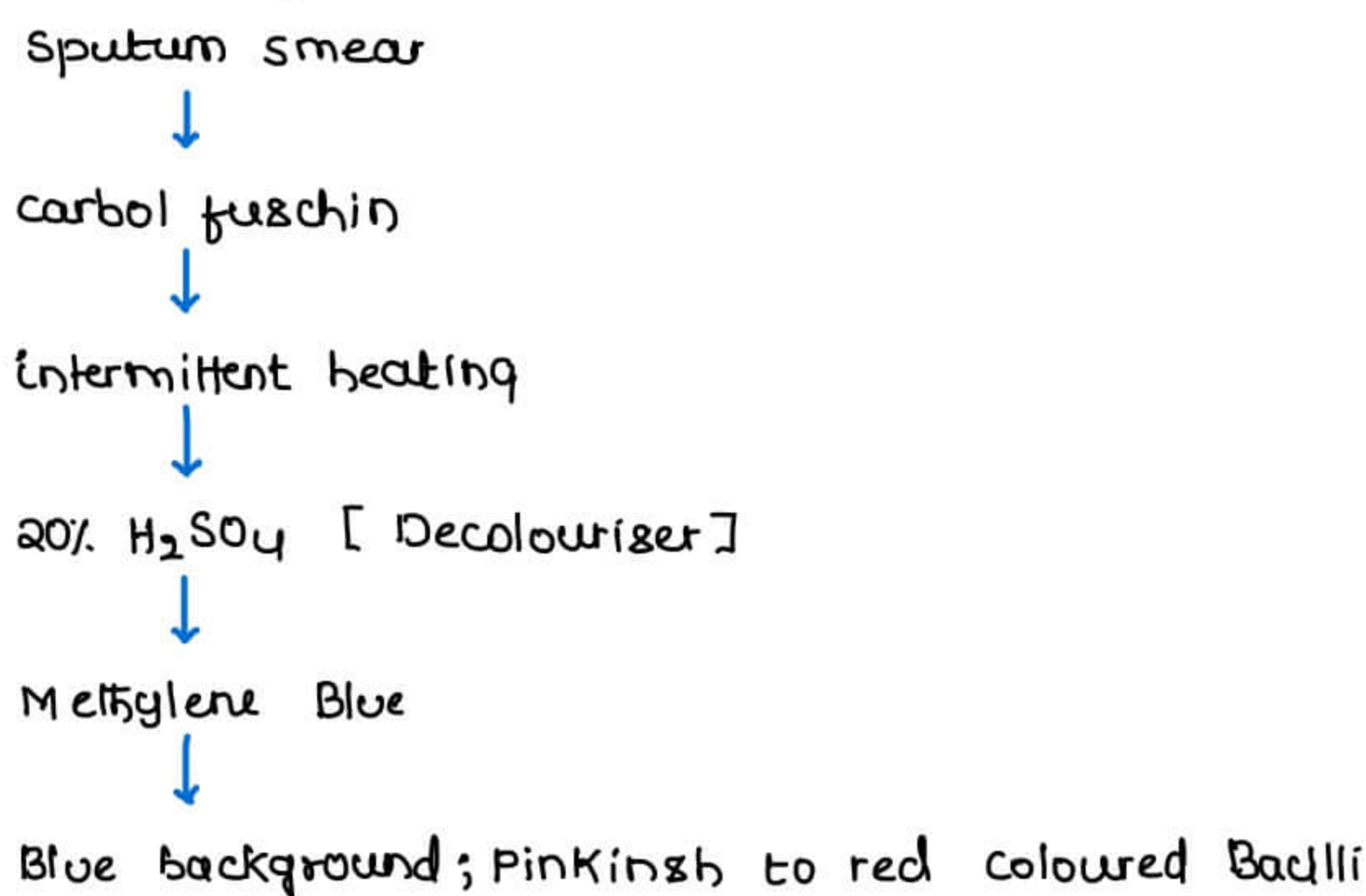
- **ACID FAST**
  - ability to resist decolorization
  - depends on Mycolic Acid content
- **VIRULENCE FACTORS**
  - ① cord factor
  - ② Lipo arabinomannan
- In **children**, Lower lobes are involved
  - GHON'S FOCUS + Hilar lymphadenopathy → **GHON'S COMPLEX**
  - calcified Hilar lymphadenopathy → **RANKE'S 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 DIAGNOSES

### ① SPUTUM EXAMINATION

- 2 sputum samples taken
  - ① onspot sample
  - ② Early morning sample
- concentrat<sup>n</sup> method → **PETROFF'S METHOD**
- Thick sputum + NaOH/HCl → **Liquified sputum**

### ② ZN STAINING



ACID FAST STAINING  
ZN STAINING



## ⑥ LJ MEDIA [LOWENSTEIN JENSEN MEDIA]

- Eggs are used for Solidification
- Selective media agent → Malachite green
- Green colored media
- Rough, Tough, BUFF colonies
  - Rough → wrinkled appearance
  - 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 differentiate b/w Active and Latent TB

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

Mycobacterial Antigens



Stimulate

BLOOD sensitised T lymphocytes



measure Interferon  $\gamma$  activity  
[Activity of sensitised T lymphocytes]



Prior exposure will be known

- Drawback → does not differentiate b/w Active and Latent TB

## ⑧ AURAMINE - RHODAMINE STAINING

- Fluorescent staining
- Done in cases of more load
- Drawback → false positive Rates 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<sup>n</sup>
  - Atypical mycobacteria infection
- False negative
  - HIV +ve [Advanced stage]
  - Miliary TB
  - Malignancy
  - Immunosuppression
- 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. chelonii, 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 scrofulaceum
- G Mycobacterium goodii



## NON CHROMOGENS

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

## RAPID GROWERS

- M. chelonii
- 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. kansasii

## Mycobacterium leprae

- causes HANSEN'S DISEASE
- Not cultured in pure culture media [doesn't follow Koch'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 <sup>n</sup> Diameter	→ measures Nodular Diameter

## LEPRA REACTIONS

TYPE 1 LEPRA REACTION	TYPE 2 LEPRA REACTION
→ 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 $\alpha$ ]



- can occur spontaneously or after the treatment
- Lepra React<sup>n</sup> is a case of emergency
  - DOC → Gluco corticoids

## LAB DIAGNOSIS

### SLIT SKIN SMEAR EXAMINATION

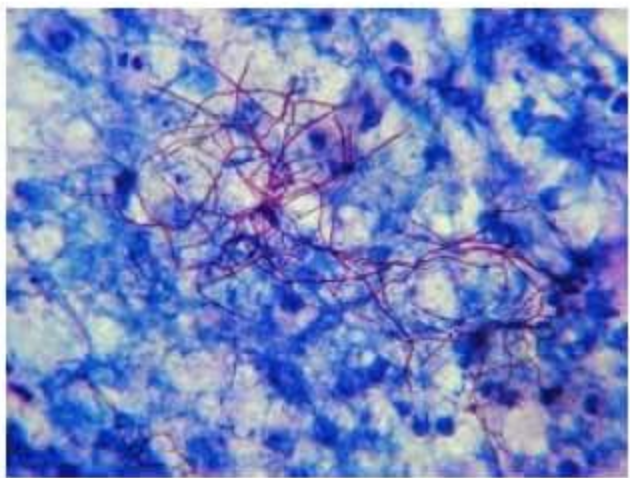
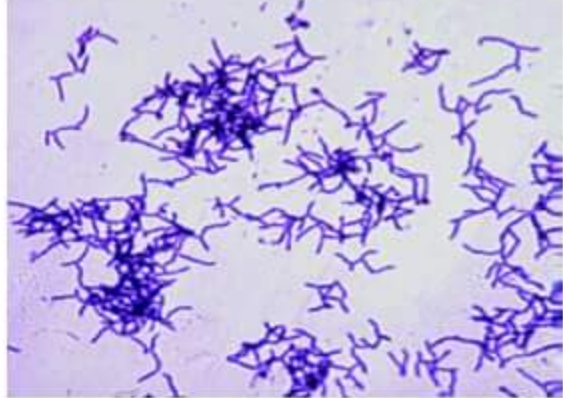
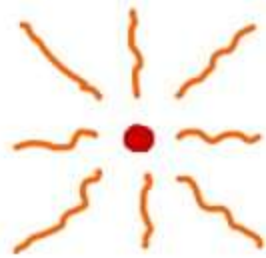

- Taken from
  - Bil ear lobes
  - forehead
  - chin
  - Buttocks
  - ⊕ Nasal mucosal swabs
- ZN Staining
  - add carbol fuschin , do intermittent heating
  - add 5% H<sub>2</sub>SO<sub>4</sub>
  - add Methylene Blue
  - Blue Background
  - cigar bundle shape / Globi like arrangement of leproae seen



## TREATMENT

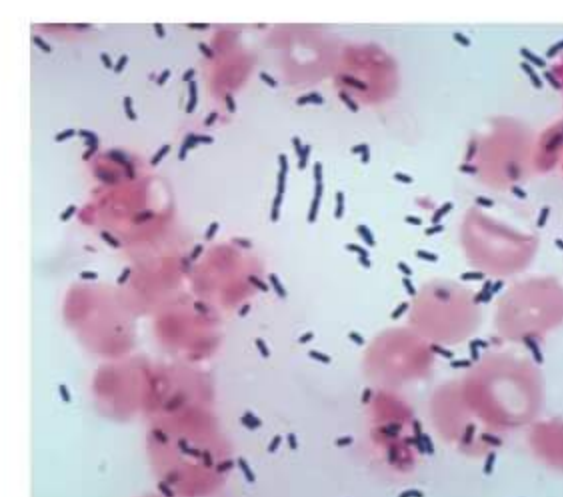
- ① PAUCI BACILLARY → D + R
    - Dapsone
    - Rifampicin
    - For 6 months
  - ② MULTI BACILLARY → D + C + R
    - DAPSONE
    - CLOFAZIMINE
    - RIFAMPICIN
    - For 1 year
- For Prognosis of leprosy
    - MORPHOLOGICAL INDEX → Better
      - measures % of solid stained bacilli [Live bacilli]



NOCARDIA	ACTINOMYCETES
→ Obligate Aerobe	→ Anaerobe
→ 1% Acid fast	→ mostly non Acid fast but some species are acid fast
<p>→ causes PULMONARY NOCARDIOSIS</p>  <p><b>NOCARDIA</b></p>  <ul style="list-style-type: none"> <li>• antler like branching [filamentous &amp; branching]</li> <li>• ACTINOMYCETES</li> </ul>  <p><b>Sulphur granule</b></p>  <p><b>Mycetoma</b></p>	<p>→ causes ORAL ACTINOMYCOSIS</p> <ul style="list-style-type: none"> <li>→ Sinus</li> <li>Swelling</li> <li>Sulphur granules</li> </ul> <p>} TRIAD</p> <p>→ mc site → cervico facial</p> <p><b>SULPHUR GRANULES</b></p> <ul style="list-style-type: none"> <li>→ crush &amp; see under microscope</li> <li>→ SUN RAY APPEARANCE</li> <li>Rays → organisms</li> </ul> <p>→ ACTINOMYCES ISRAELII</p> <ul style="list-style-type: none"> <li>→ causes PID</li> <li>→ Acid fast in nature</li> </ul>
<p>Both Nocardia &amp; Actinomycetes combine to cause → ACTINOMYCETOMA</p> <ul style="list-style-type: none"> <li>→ Nocardia caviae</li> <li>→ Nocardia asteroides</li> <li>→ Actinomadura</li> <li>→ Actino madurae</li> </ul>	
<p>→ CULTURE OF NOCARDIA</p> <ul style="list-style-type: none"> <li>→ PARAFFIN BAIT TECHNIQUE</li> </ul>	<p>→ CULTURE OF ACTINOMYCETES</p> <ul style="list-style-type: none"> <li>→ mostly non cultured</li> <li>→ But some can be cultured</li> </ul>
<p>→ DOC → COTRIMOXAZOLE</p>	<p>→ DOC → PENICILLIN</p>

**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



① GRANULOMATOSIS INFANTISEPTICA [ in neonates ]

② MENINGITIS [ children (mainly), Adults ]

③ CHORIOAMNIONITIS [ in pregnancy ] → PROM



④ SEPSIS [ in immunocompromised ]

→ DOC → AMPICILLIN

→ Listeria shows DIFFERENTIAL MOTILITY based on temperature

- 37°C → Non motile

- 20 - 25°C → Motile



ENTEROBACTERIACAE

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



LACTOSE FERMENTERS
E.coli
Klebsiella
Shigella sonnei

E.coli

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

→ 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 SCROMBOLD FISH POISONING [ E.coli > Morganella ]
- ⑧ Histamine Production +nt

UTI

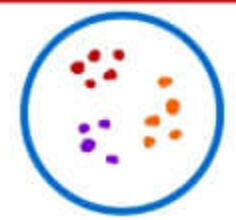
\*P fimbriae possessing E.coli attach to uroepithelial cells  
 > 10<sup>5</sup> E.coli/ml of urine → SIGNIFICANT BACTERIURIA

→ Criteria for diagnosis of UTI

- ① > 100 colonies x 10<sup>3</sup> → > 10<sup>5</sup> / ml
- ② > 10 colonies x 10<sup>3</sup> → > 10<sup>4</sup> / ml
- ③ Symptoms / signs of UTI [Dysuria, burning micturition]
- ④ Even 1 bacteria / ml in supra pubic Aspirate

[10<sup>3</sup> - 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
- culture 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 Hep2 cell lines



## NEONATAL MENINGITIS

common causes of Neonatal meningitis :

- E. coli [ i 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
Kleb	→	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
  - Rx by NaHCO<sub>3</sub> douches
- MODIFIED YOUNG'S OPERATION



## Klebsiella rhinoscleromatis

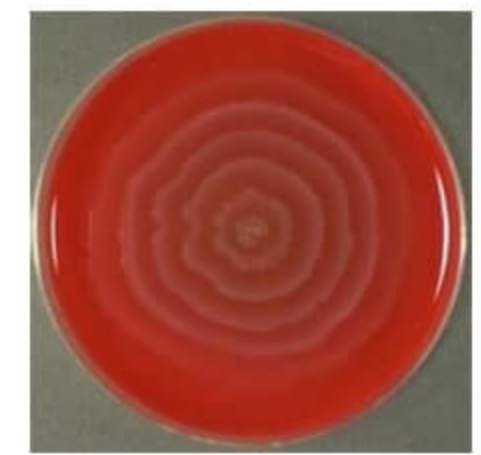
- causes Rhinoscleroma [WOODY NOSE]
- MIKULIZ CELLS } Seen
- RUSSEL BODIES }

## Klebsiella granulomatis

- causes Granuloma inguinale or DONOVANOSIS
- DONOVAN BODIES are seen
- BIPOLAR SAFETY PIN APPEARANCE
- aka SUPER BUG
- K. pneumoniae strain
- resistant to almost all the drugs
- dlt metallic enzyme → Metallic Action → ↑sed virulence
- now termed as METALLO BETA LACTAMASE 1

## PROTEUS

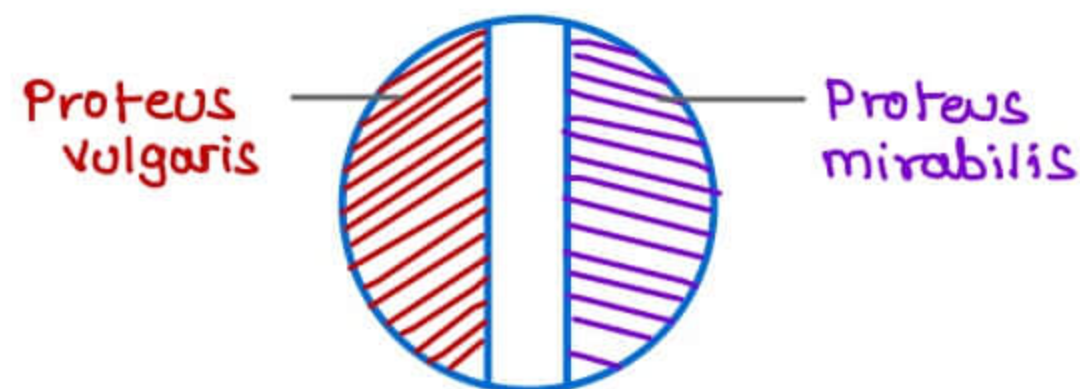
- GNB
- SWARMS
  - inhibit swarming by
    - ↑ % of Agar from 2% to 6%
    - instill some chemicals [Boric acid & chloral hydrate]
- can be cultured on
  - Mac - conkey Agar → produce Non Lactose fermenting [NLF] colonies
  - Phenyl Pyruvic Acid Agar



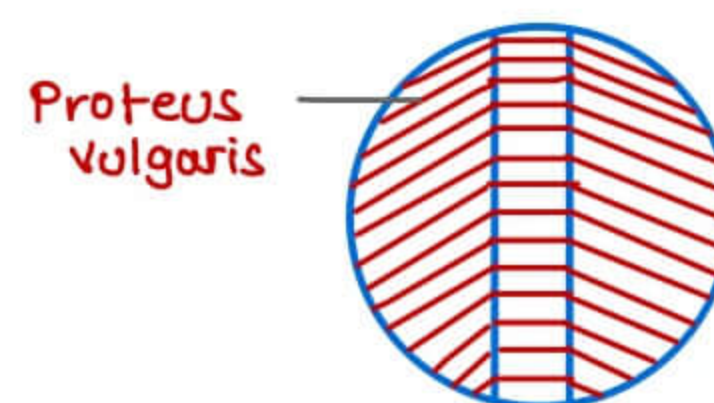
swarming

FISHY SMELL / SEMINAL SMELL produced on both media

## DIENELS PHENOMENON



Border not crossed



crossed borders

## PROTEUS ANTIGEN INTERACTIONS

- OX 19, 2 & K → cross reacts  $\bar{c}$  Rickettsial family members
- Gives WEIL - FELIX REACTION [Heterophile agglutination test]
- OX K is strongly positive for scrub typhus
- Proteus a/w chronic UTI
  - Leads to Struvite | Triple Phosphate stones / Straghorn 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 contamination
- Stepladder pattern fever
- ROSE SPOTS seen
- Longitudinal ulcers seen
- PEA SOUP DIARRHOEA seen



ROSE SPOTS

## LAB DIAGNOSIS

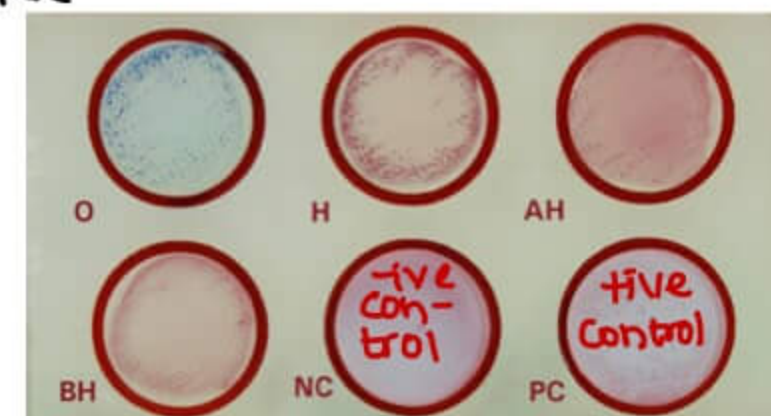
- |   |   |                    |   |                      |
|---|---|--------------------|---|----------------------|
| B | → | Blood culture      | → | 1 <sup>st</sup> Week |
| A | → | Agglutination Test | → | 2 <sup>nd</sup> Week |
| S | → | Stool culture      | → | 3 <sup>rd</sup> Week |
| U | → | Urine culture      | → | 4 <sup>th</sup> 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 Polyanethanol 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	}	POSITIVE
H	→	> 1:200		
- Flagellar antigen is more immunogenic

- Paired testing should be done, after 2 weeks of initial testing
- 2<sup>nd</sup> test - 4 fold ↑ in titre should be present → Positive



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

→ TYPES

① SLIDE WIDAL TEST

② TUBE WIDAL TEST

- serial dilution in Normal saline

PROZONE PHENOMENON is avoided

- Prozone phenomenon → Antibody excess

### STOOL & URINE CULTURE

→ Enrichment media [SELENITE F BROTH] is used

→ Selective media

DCA [Deoxycholate Agar]

XLD [Xylose Lysine Deoxycholate]

SS [Salmonella Shigella Agar]

HE [Hektoen enteric agar]

ABOVE MEDIA 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. typhimurium*

*S. choleraesuis*

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

→ *S. typhimurium*

- a/w contaminated Poultry Products

- can enter the intact egg shell

### DOC

→ 3rd Generation CEPHALOSPORINS



## SHIGELLA

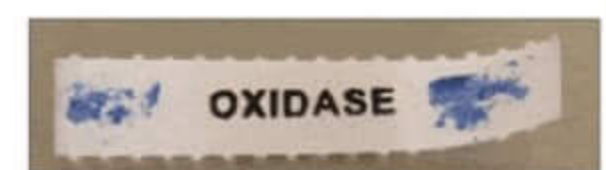
- GNB
  - non - motile
  - Mannitol fermentat<sup>n</sup> Based classificat<sup>n</sup> used
    - non mannitol fermenter → Shigella dysenteriae
  - catalase -ive → S. dysenteriae type 1
  - VMA [virulence Marker Antigen] → responsible invasiveness
  - Secrets Endo & Exotoxins
  - **SHIGA TOXIN**
    - Exotoxin
    - by S. dysenteriae
  - mcc of bacillary dysentery in India → S. flexneri
  - mcc of bacillary dysentery 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

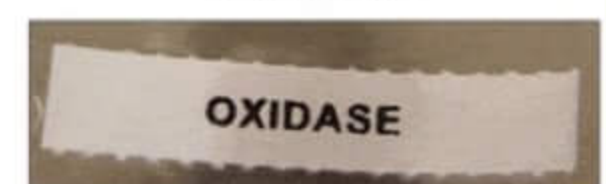
- ① Pseudomonas
- ② Burkholderia mallei
- ③ Burkholderia pseudo mallei
- ④ Acinetobacter baumannii

## PSEUDOMONAS

- 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



Oxidase positive



Oxidase negative

- **VIRULENCE FACTORS**
  - ① Capsular Polysaccharide
  - ② Alginate Acid
  - ③ Pigments

- a. Pyocyanin → Blue
- b. Pyorubrin → Red
- c. Pyomelanin → Brown Black

MULLER HINTON  
AGAR



d. Pyoverdin → Green

- mc

- mostly found in *Pseudomonas aeruginosa* [verdigris]

These pigments enhance on KING'S media

→ Best selective media → ceftrimide Agar

### DISEASES

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



*Pseudomonas* infect'n

→ 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



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

	classical	Eltor
Polymyxin B sensitivity	sensitive	Resistant
Mukherjee Phage $\square$ susceptibility	Susceptible	Resistant
Mukherjee Phage $\Sigma$ susceptibility	Negative	Positive
VP [voges Proskauer]	Negative	Positive
CAMP	Negative	Positive
Chick Erythrocyte Agglutination 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 → Hajj
- Quarantine station

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

→ CHOLERA TOXIN

- A → ADP ribosylation of GTP → ↑ in adenylate cyclase activity → ↑ cAMP
- B → Binds to GM<sub>1</sub> 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 multiplication]

- ① Venkat Raman Ramakrishnan Media
- ② Cary Blair media

Enrichment Media → Alkaline Peptone water

Selective media

- ① TCBS [Thiosulphate Citrate Bile Salt Sucrose]
  - Green → Yellow [due to sucrose lysis]
  - Best selective media



TCBS MEDIA



## HALOPHILIC VIBRIDS

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

## V. PARA HEMOLYTICUS

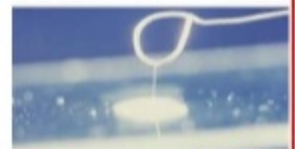
- causes SEA FOOD POISONING
- also KANAGAWA PHENOMENON on WAGATSUMA AGAR [enhanced hemolysis]

## VIBRIO CHOLERA

lmarsmf@hi2.in  
+14386004539

- 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, PARTING MOTILITY seen



STRING TEST POSITIVE

## → STRING TEST

- sodium deoxycholate [Bile salt] is added to stool sample
- try to lift  $\bar{c}$  loop → string is formed

## HAEMOPHILUS

### HAEMOPHILUS DUCREYI

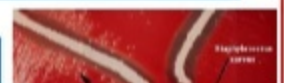
- causes CHANCROID [soft sore]
  - painful genital ulcer
- Grows on chocolate agar → CULTURE MEDIA
- TISSUE BIOPSY + STAINING → TRAMTRACK APPEARANCE OR SCHOOL OF FISH APPEARANCE

### HAEMOPHILUS INFLUENZAE

CAPSULATED	NON CAPSULATED
→ causes invasive diseases <ul style="list-style-type: none"> <li>- Meningitis</li> <li>- Epiglottitis</li> </ul>	→ Non invasive Diseases <ul style="list-style-type: none"> <li>- Otitis media</li> <li>- Sinusitis</li> <li>- LRTI</li> </ul>

- Show SATELLITISM

*H. influenzae* *Staph. aureus*  
satellitism  $\alpha$  hemolysis





## H. AEGYPTI CUS

- causes
  - ① PINK EYES
  - ② BRAZILIAN PURPURIC FEVER

### NORMAL MOUTH COMMENSALS

H	→	H. parainfluenzae, H. aphrophilus, H. para aphrophilus
A	→	Aggregatibacter [Actinobacillus mycetocomitans]
C	→	Cardiobacterium hominis
E	→	Eikenella corrodens [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 PAROXYSMAL → 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
- ACCELLULAR PERTUSSIS VACCINE
  - used now [equal in efficacy to cellular vaccine]
  - made up of
    - Pertussis Toxin
    - 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 / MEDITERRANEAN / 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<sup>n</sup> 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]

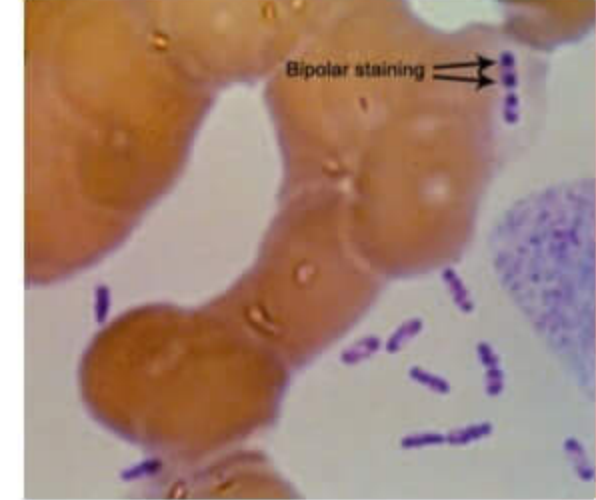
### → CULTURE MEDIA

GHEE BROTH - STALACTITE TYPE OF GROWTH 

→ Rat Flea index → cheopis index is used

### → LAB DIAGNOSIS

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



→ DDC → 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 LEGGIONAIRE'S PNEUMONIA
    - Atypical pneumonia
    - complicates & Encephalitis & Diarrhoea
- } LEGIONNAIRE'S DISEASES  
- alw 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
- FONTANA & LEVADITI STAINING done
  - silver impregnation method
  - makes them thick



Dark Field Microscopy

## 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 Syphilis
        - presents i GPI [General Paralysis of Insane]
        - Tabes dorsalis
      - Cardiovascular Syphilis
        - 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 [venereal Disease Research Lab]
    - Ⓑ RPR [Rapid Plasma Reagent]

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



## ② TREPONEMAL TESTS

- Ⓐ FTA - ABS [Fluorescent Treponemal Antibody Assay]
- Ⓑ TPI [Treponema pallidum Immobilizat<sup>n</sup> Assay]
- Ⓒ TPHA [Treponema pallidum Haemagglutin<sup>n</sup> Assay]
- Ⓓ TPPA [Treponema pallidum Particulate agglutin<sup>n</sup> 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. Pertuonal	→	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 INTERROGENS**

- causes WEIL'S DISEASE [ICTERO HAEMORRHAGIC FEVER]
- CULTURE MEDIA
  - ① EMJH
  - ② FLETCHER
  - ③ KORTAOKOFF
- LAB DIAGNOSIS
  - ① SEROLOGICAL TEST → MAT
    - Microscopic Agglutinat<sup>n</sup> Test [preferred]
    - Macroscopic Agglutinat<sup>n</sup> Test
  - ② PCR for Leptospira species
- |   |   |                            |   |                   |
|---|---|----------------------------|---|-------------------|
| R | → | Rat urine                  | } | a/w LEPTOSPIROSIS |
| R | → | Rice [Paddy field workers] |   |                   |
| R | → | Rainy water                |   |                   |

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

- National Referral Centre for Leptospira located at → Port Blair

- ③ A 20 yr old male who works as truck driver & 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 ?



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



- 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 w/ 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 guinea pig → Endemic typhus
- No such reaction & inflammation in guinea pig → Epidemic typhus

QUERY FEVER / Q FEVER

- caused by Coxiella burnetii
- contaminates milk  
Killed by Flash method of pasteurization
- MOT → Aerosol transmission [no arthropod transmission]
- No Rash occurs
- a/w 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 chaffinensis → causes Human monocytic ehrlichiosis
- EHRlichia phagocytophila → causes Human granulocytic ehrlichiosis



→ 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 CONJUNCTIVITIS INFANTILE PNEUMONIA GENITAL CHLAMYDIASIS
→ Serotypes L <sub>1</sub> , L <sub>2</sub> , L <sub>3</sub>	→ causes	LYMPHO GRANULOMA VENERUM [L <sub>2</sub> >L <sub>1</sub> ]

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 McCoy 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
- Complicat<sup>n</sup> → 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 Agglutinat<sup>n</sup> Test] → type of Heterophile agglutinat<sup>n</sup> test
  - 2 Streptococcus MG Agglutinat<sup>n</sup> Test → type of Heterophile agglutinat<sup>n</sup> test
  - 3 PCR for M. pneumoniae

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

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<sub>2</sub> required]
- motile
- GULL WING SHAPED BACTERIA
- a/w Poultry bird contaminat<sup>n</sup>
- causes Inflammatory Diarrhoea
  - fecal leucocytes are +ve

- In children, Diarrhoeal episodes  $\xrightarrow[\text{By}]{\text{Followed}}$  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 aerophile
- motile
- Strongly urease positive [yellow → pink]

### → DISEASES

PUD [PEPTIC ULCER DISEASE]

MALTOMA

CA STOMACH

### → LAB DIAGNOSIS

NON INVASIVE TESTS → Urea Breath Test ± radio labelled  $C_{13}$  or  $C_{14}$

INVASIVE TEST → Endoscopic Biopsy

- stain ± WARTHIN STARRY SILVER STAIN
- demonstrate H. pylori
- Bx tissue + urease media → Yellow → PINK

→ Rx → HELIBACT / PYLO KITS for eradication of H. pylori

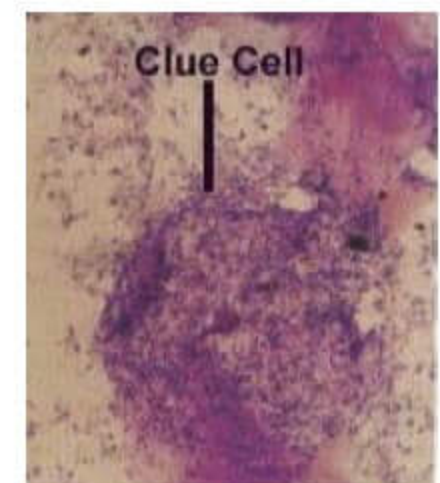
→ cag & vac → ↑ses virulence of H. pylori

## BACTERIAL VAGINOSIS

caused by

- Gardnerella vaginalis
- Mobiluncus
- Prevotella

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





**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**

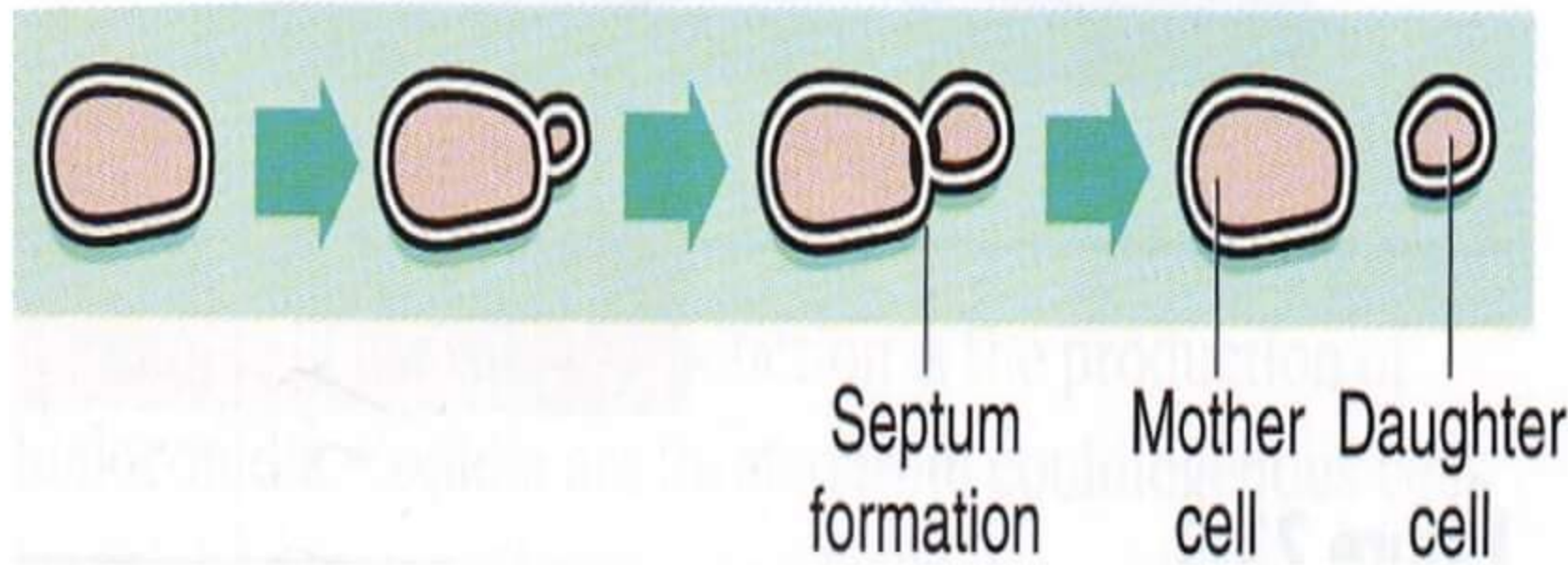
- a/w Eikenella cordens
- a/w anaerobic streptococci

**CAT & DOG BITES** → a/w Pasteurella multocida

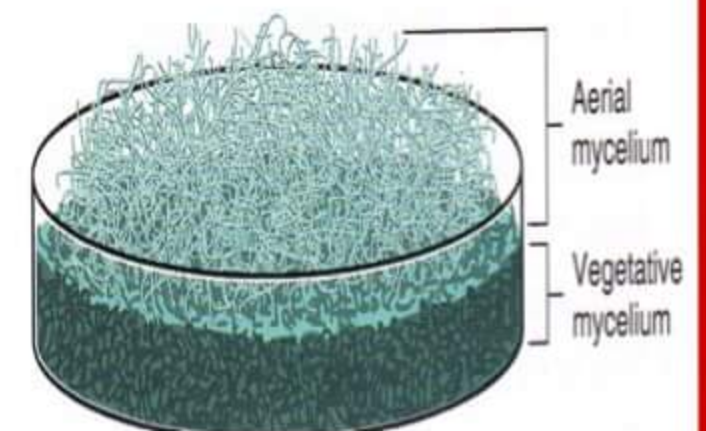
**SCROMBID FISH POISONING** a/w → E.coli > Morganella  
[NOTE - AS per new data → Morganella > Proteus > E.coli]



- 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**

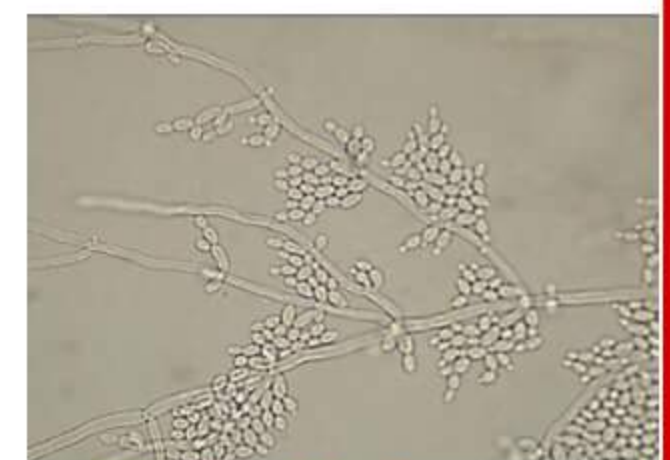
- spherical or ellipsoidal cells
- reproduce by budding
- Ex: Cryptococcus neoformans



YEASTS

**2. YEAST LIKE**

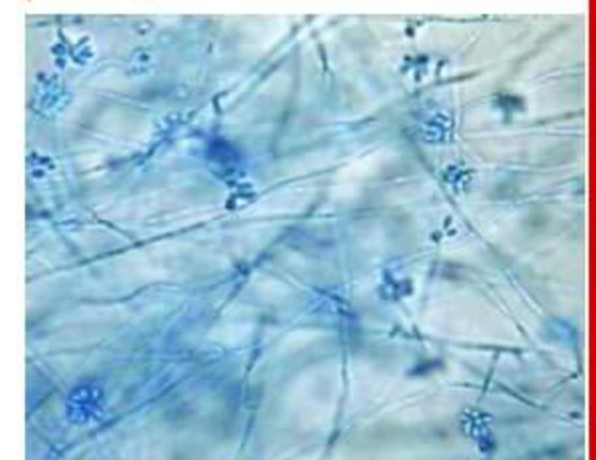
- Partly as yeast
- partly as elongated cells: PSEUDOHYPHAE
- Ex: candida albicans
  - ↳ can show true hyphae / pseudohyphae
  - ↳ can show yeast / yeast like [main form]



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  | → | Coccidiomycosis      |
| S | → | Sporotrichosis | p2 | → | Para coccidiomycosis |
| B | → | Blastomycosis  |    | → | Penicilliosis        |



## BASED ON SEXUAL SPORE FORMATION

### 1. PHYCOMYCETES / ZYGOMYCETES

- non-septate hyphae
- obtuse angled
- forms asexual spores : Sporangia
- Sexual spores → Zygosporangia

### 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

- Z → Zygosporangia
- A → Ascospore
- B → Basidiospore

#### ASEXUAL SPORES

- A → Arthrospore
- B → Blastospore
- C → Chlamydospore

## LAB DIAGNOSIS

### By microscopic examination of materials

1. Wet mounts in 10% KOH → can see fungal elements
  2. Tease mounts in Lactophenol cotton blue [LPCB]
    - calcofluor white staining [Fungal fluorescent staining]
  3. Slide culture
  4. Periodic Acid Schiff [PAS]
  5. Gomori methanamine silver stains
    - used for Pneumocystis carinii / jiroveci
    - stains green
    - Trophozoites stained green
    - cysts stained black
- PNEUMOCYSTIS CARINII
- ↳ causes Pneumocystis carinii pneumonia in HIV +ve with 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 & condensat<sup>n</sup>  
→ rectangular  
→ Eg: coccidiomycosis

## CLASSIFICATION BASED ON LOCATION

### MYCOSES

1. superficial mycoses → superficial layer of skin involved  
Subcutaneous mycoses → skin & subcutaneous tissue involved
2. Deep mycoses / systemic mycoses
3. Opportunistic mycoses

## CLINICAL CLASSIFICATION OF MYCOTIC INFECTIONS

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



Area of Predominant involvement	MYCO SIS	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	Phialophora verrucosa Fonsecaea pedrosoi & others
	mycetoma	Pseudallescheria boydii, Madurella mycetomatis & others
	<b>Mnemonic</b> M - mycetoma R - Rhinosporidiosis C - chromomycosis S - Sporotrichosis	
4. SYSTEMIC MYCOSES [HSBC P <sup>2</sup> ]		
PRIMARY MYCOSES	Coccidioidomycosis	Coccidioides immitis
	Histoplasmosis	Histoplasma capsulatum
	Blastomycosis	Blastomyces dermatitidis
	Paracoccidioidomycosis	Paracoccidioides brasiliensis
OPPORTUNISTIC MYCOSES	Candidomycosis	Candida albicans, other candida sp.
	Cryptococcosis	Cryptococcus neoformans
	Aspergillosis	Aspergillus fumigatus, other asp. sp.
	Mucormycosis	Species of Rhizopus, Absidia, Mucor & others



## SUPERFICIAL MYCOSES

60

Include

- A. SURFACE INFECTIONS
- B. CUTANEOUS INFECTIONS

### SURFACE INFECTIONS

#### 1. TINEA VERSICOLOR

- caused by *malassezia furfur*
- presents  $\bar{c}$  discrete hyperpigmented or hypopigmented macules | scales on chest or upper back
- colony → yeast like consistency
- microscopy → spherical, oval or cylindrical cells



Tinea versicolor



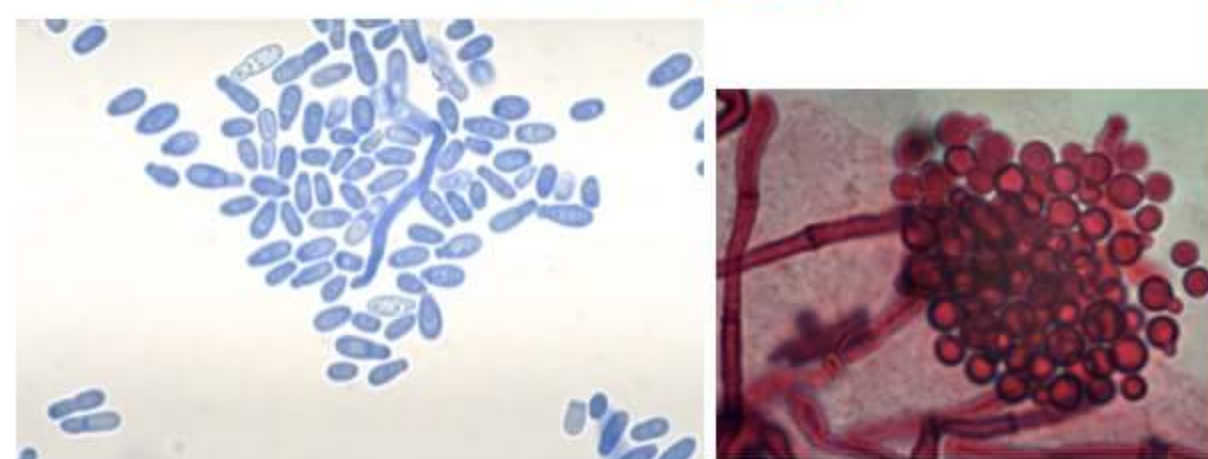
Tinea versicolor

#### CLINICAL FEATURES

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



Tinea versicolor



#### DIAGNOSIS

##### 1. MICROSCOPIC EXAMINATION

###### → SKIN SCRAPINGS

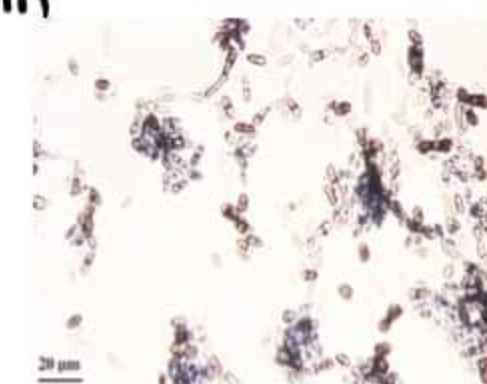
- ↳ scales + 10% KOH → SPAGHETTI & MEATBALL APPEARANCE
- ↳ scales + Wood lamp → YELLOW FLUORESCENCE
- ↳ scales + SDA & olive oil overlay [Lipophilic fungus] → FRIED EGG COLONIES

#### 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 *Cladosporium wernickii* or *Exophiala wernickii*
- Saprophyte & dimatiaceous [pigmented hyphae]
- microscopically → Budding yeasts & chains of yeast cells
- LESIONS
  - ↳ solitary, innocuous maculae  $\bar{c}$  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 beigellii*
- 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 colonial appearance & microscopy

### TRICHOPHYTON

- cylindrical / pencil shaped, smooth walled macro conidia & microconidia



T. rubrum



microconidia



macroconidia

### TRICHOPHYTON MENTAGROPHYTES





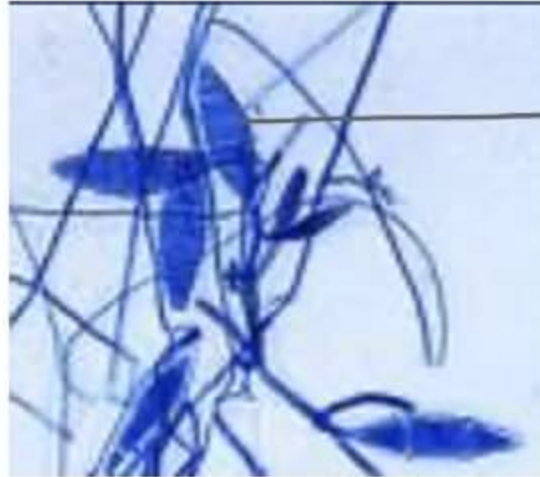
## TINEA VIOLACEUM



## MICROSPORUM

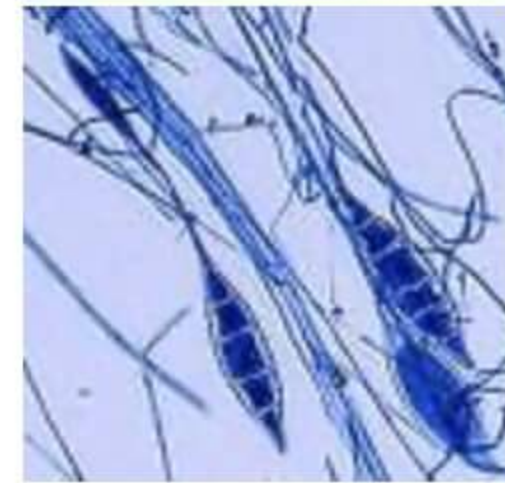
→ multicellular macroconidia [spindle shaped] with rough walls & few microconidia

### M. gypseum



→ Spindle shaped macroconidia

### M. canis



## EPIDERMOPHYTON

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



## PATHOGENESIS & IMMUNITY

- By contact with 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

**TINEA CORPORIS**

- Lesions are annular, sharply marginated with raised border
- This pustular well circumscribed, elevated, crusted lesion is called as MAJOCCHI'S GRANULOMA
- caused by → T. rubrum



Majocchi's Granuloma

**TINEA CAPITIS**

→ Infection 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

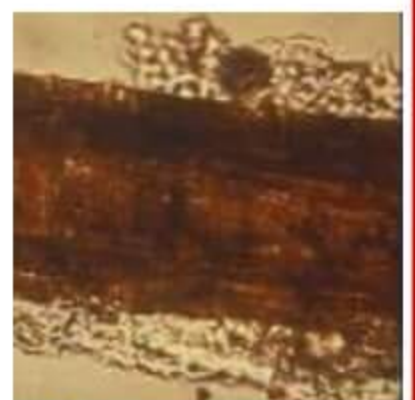


**b. FAVUS**

- dense crust formation on scalp
- caused by Trichophyton schoenleinii

**ECTOTHRIX**

- sheaths of spores around the shaft
- caused by microsporum canis, M. audonii



Ectothrix

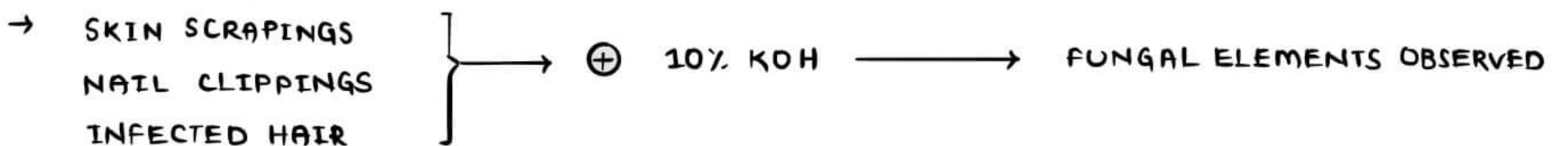
**ENDOTHRIX**

- Arthroconidia within the hair shaft
- caused by
  - T → Trichophyton tonsurans
  - V → Trichophyton violaceum
  - S → Trichophyton schoenleinii



Endothrix

**LAB DIAGNOSIS**



→ 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 keratosis 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<sup>n</sup> 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 & Subcutaneous tissues
- Eg: M → Mycetoma
- R → Rhinosporidiosis
- C → Chromoblastomycosis
- S → Sporotrichosis [dimorphic]

### MYCETOMA / MADURA FOOT

ACTINOMYCETOMA	EUMYCETOMA
<ul style="list-style-type: none"> <li>→ Bacterial</li> <li>→ numerous sinus &amp; swelling</li> <li>→ Purulent discharge</li> <li>→ White/yellow granules               <ul style="list-style-type: none"> <li>• Actinomyadura madurae</li> <li>• Nocardia caviae</li> <li>• Nocardia asteroides</li> <li>• Nocardia brasiliensis</li> </ul> </li> <li>→ Red granules               <ul style="list-style-type: none"> <li>• Actinomyadura pelletri</li> </ul> </li> <li>→ Crush granules &amp; see under microscope → <math>&lt; 2\mu</math> bacilli</li> <li>→ On x ray → osteolytic lesions</li> <li>→ Responds well to antibiotics</li> </ul>	<ul style="list-style-type: none"> <li>→ Fungal</li> <li>→ Single sinus &amp; swelling</li> <li>→ serous discharge</li> <li>→ Black granules               <ul style="list-style-type: none"> <li>• Madurella grisea</li> <li>• Madurella mycetomatis</li> </ul> </li> <li>→ white granules               <ul style="list-style-type: none"> <li>• Actinomyadura</li> <li>• Pseudallescheria boydii</li> </ul> </li> <li>→ Crush granules &amp; see under microscope → <math>&gt; 2\mu</math> hyphae / <math>&gt; 2\mu</math> Fungal colonies</li> <li>→ On x ray → osteosclerotic lesions</li> <li>→ Responds very poorly to anti fungus [amputat<sup>n</sup> may required]</li> </ul>

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

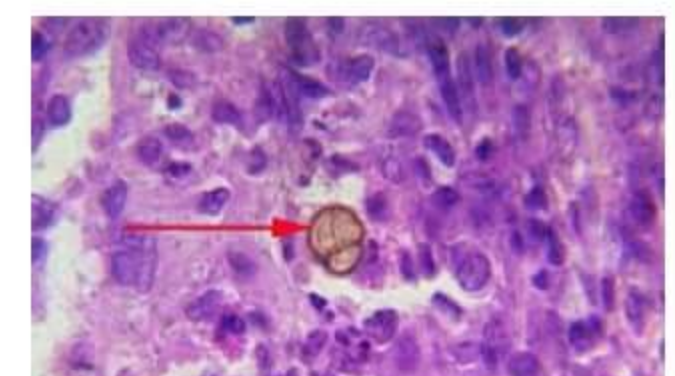
### RHINOSPORIDIOSIS

- caused by Rhinosporidium seeberi [AQUATIC PROTOZOA]
- presents w/ B/L Nasal polyps [mc presentat<sup>n</sup>]
- POLYP BIOPSY → shows SPHERULE & endospores
- Rx → Sx Polypectomy [Best]
- Non cultured on SDA



### CHROMOBLASTOMYCOSIS / DERMATACEOUS 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



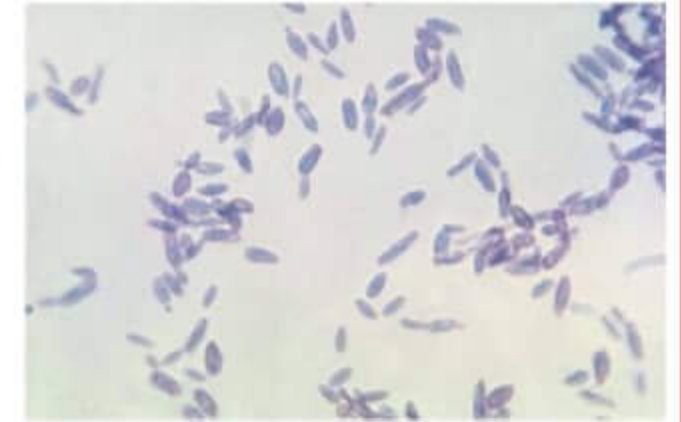
MEDLAR/SCLEROTIC BODY



- Examples →
- |               |                 |
|---------------|-----------------|
| 1. Alternaria | 4. Phialophora  |
| 2. Bipolaris  | 5. Cladosporium |
| 3. Curvularia | 6. Exophiala    |

### SPOROTRICHOSIS | ROSE GARDENER'S DISEASE

- caused by *Sporothrix schenckii*
- spread by thorn prick [Gardener's disease]
- ↑ incidence in & around valentine day
- lymphocutaneous 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 schenckii*

- ASTEROID BODIES are seen
  - Basophilic material is surrounded by Eosinophilic material → SPLENDORE - HOPELLI 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                       | → Chronic form    |                |
| → influenza like disease           | → haemoptysis     | } resembles TB |
| → resolves within 2-3 weeks        | → apical cavities |                |
| → shows lymphocyte rich infiltrate | → calcification   |                |

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 + Actidione
  - white cottony mycelial growth + tuberculate spores/macroconidia [mold]
- Potato dextrose agar

**SEROLOGY**

- Immunodiffusion
- Latex agglutination
- complement fixation test
- Histoplasmin skin test - DTH [delayed type hypersensitivity]

**MOLD** → **TUBERCULATE MACROCONIDIA**

 narrow base yeast cell

**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 & 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

 Broad Base yeast cell

## COCCIDIOIDOMYCOSIS

- caused by *Coccidioides immitis*
- Pathogenic dimorphic fungi
- In culture, it produces arthroconidia within the mycelia at 25°C
- In tissue, it produces spherules & 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
- due to Inhalation of conidia
- 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* & endospores

### 2. CULTURE

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

### MICROSCOPY MORPHOLOGY

- single celled, Hyaline
- rectangular to barrel shaped, alternate arthroconidia

At 25°C → Arthroconidial arrangement [MOLD]

At 37°C → Spherule & endospore [Yeast]

### 3. SKIN TEST → coccidioidin

### 4. SEROLOGICAL TESTS

- Immunodiffusion test
- complement fixation test

## PARACOCIDIIDOMYCOSIS | 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

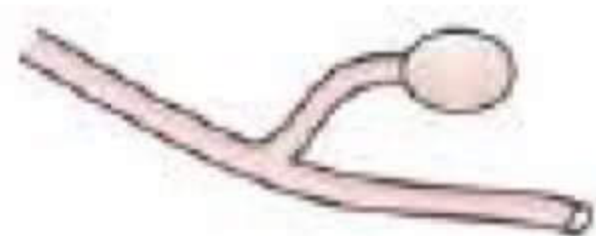
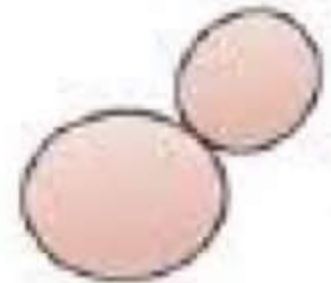
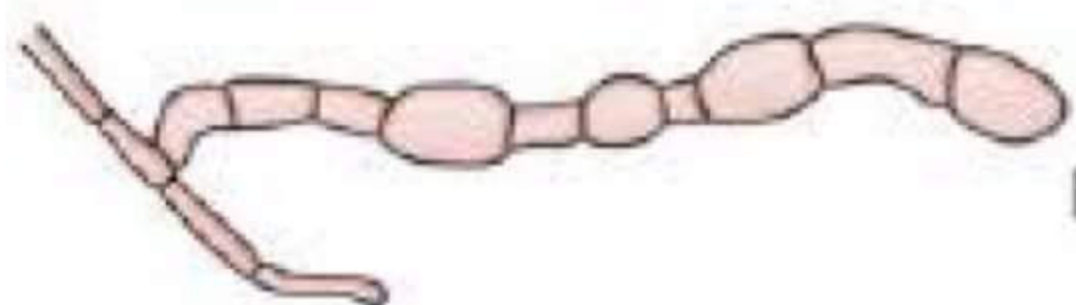

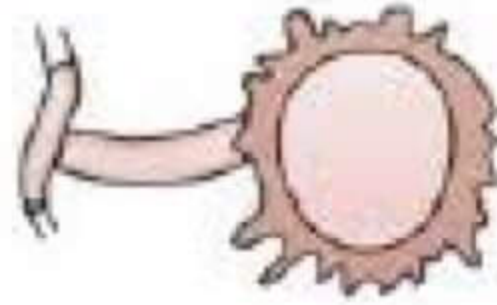
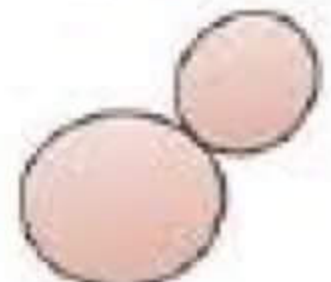
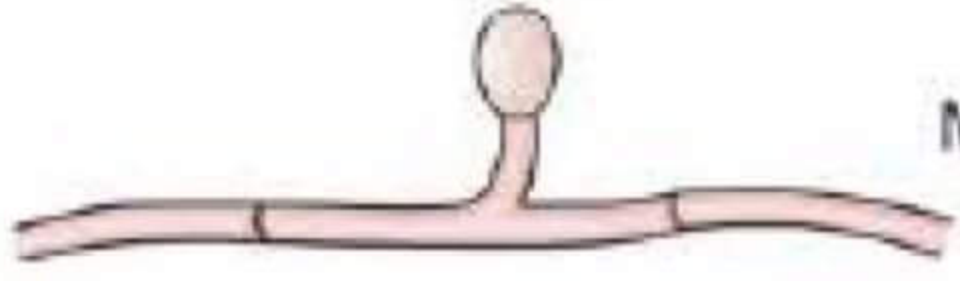
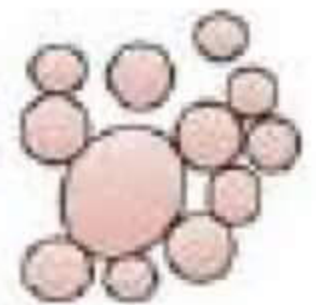
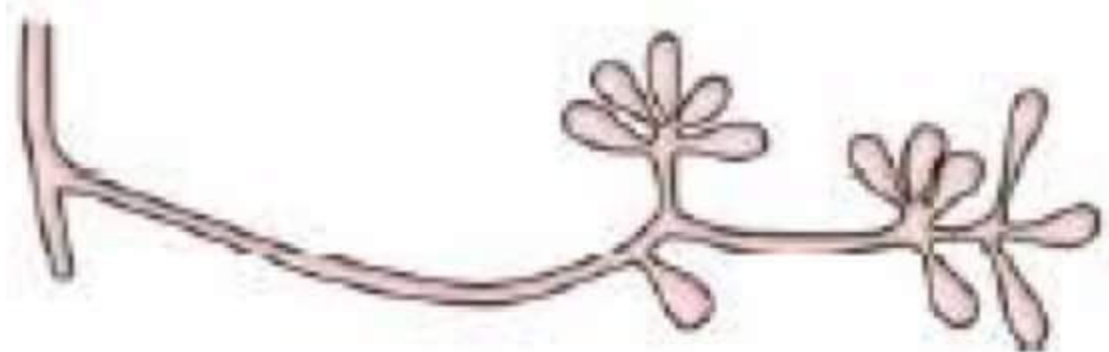
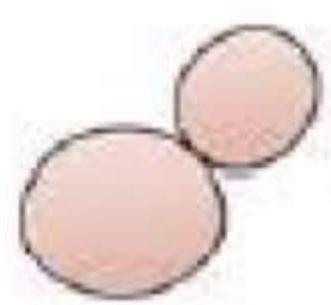
- Spreads by lymphatic or hematogenous route
- Lung (90% of cases)
- CNS, Bones
- BIL patchy pneumonia & nodular miliary shadows



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

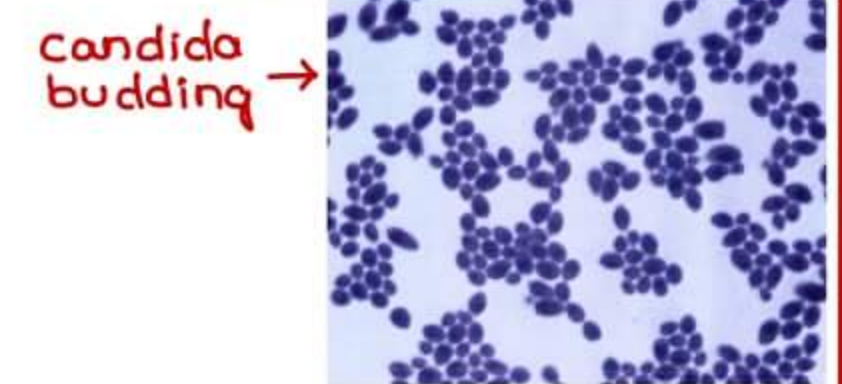
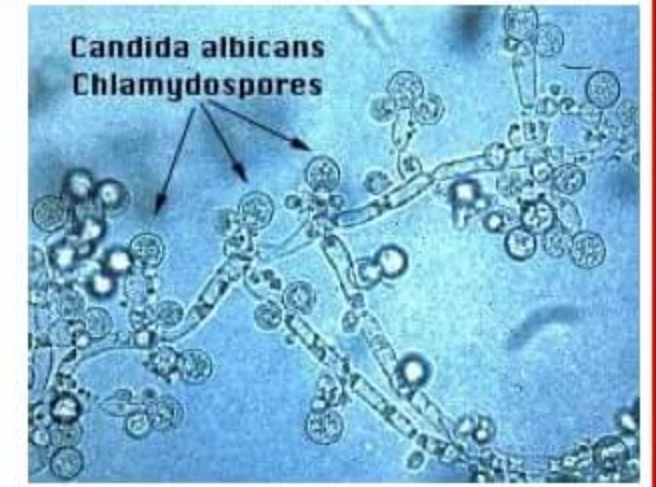
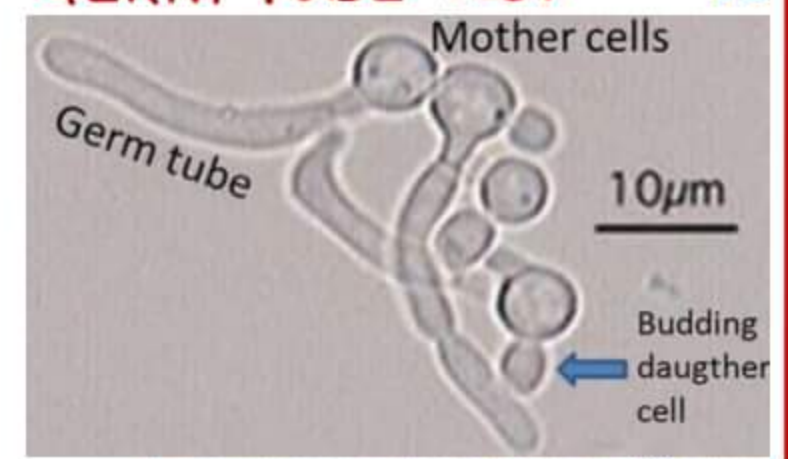


## CANDIDA [YEAST LIKE]

### CANDIDA ALBICANS

- Shows
  - ① REYNOLD BRAUDE PHENOMENON [Germ tube formation]
  - ② CHLAMYDOSPORE FORMATION ON CORN MEAL AGAR
- REYNOLD BRAUDE PHENOMENON [C<sub>x</sub> growth + human plasma]
  - shows germ tube formation [GERM TUBE TEST]
- on corn meal agar (nutrient deficient medium), chlamydo spores are formed [at 20°C]

### GERM TUBE TEST 71



### NON - CANDIDA ALBICANS [NCA]

- C. krusei → naturally resistant to Azoles
- C. kefyr
- C. glabrata
- C. stellatoidea
- C. guilliermondii
- C. tropicalis
- C. viswanthii
- C. aureus → caused worldwide infections mostly in immunodeficient

### 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 mucocutaneous candidiasis [T-cell disorder]

### LAB DIAGNOSIS

- Tissue Biopsy ; samples
- Gram stain → yeast cells are seen
- C<sub>x</sub> on SDA → creamy, pasty colonies
- Chromagar method → colourful agar
  - used for CA | NCA identification
- SUGAR FERMENTATION METHODS
- β GLUCAN TESTING FOR INVASIVE CANDIDIASIS
- PCR for candida species



chromagar



ON SDA



## CRYPTOCOCCUS NEOFORMANS

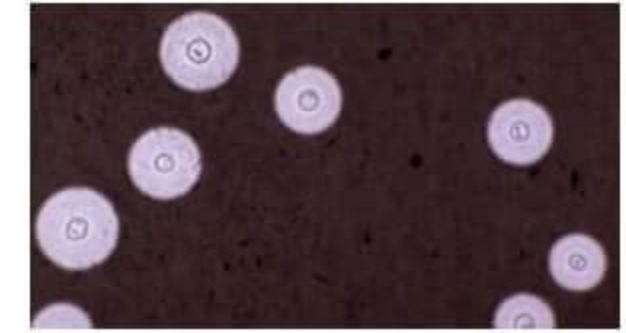
- present in pigeon faeces → Aerosol transmission
  - LUNG INVOLVEMENT
  - ↓ Haematogenous spread
  - MENINGES INVOLVED [meningitis]
- } **NEUROTROPISM**  
[for HIV +ve individuals]
- mc cause of Meningitis in HIV +ve → Cryptococcus neoformans

### 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

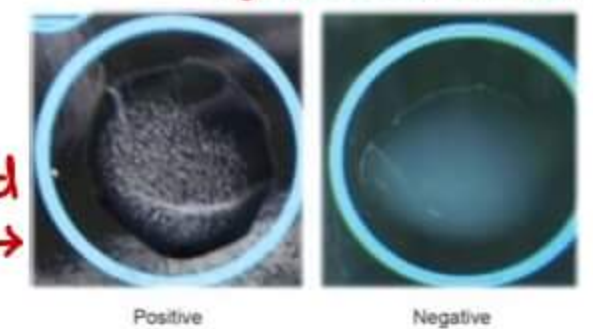


Cryptococcus on SDA

#### 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



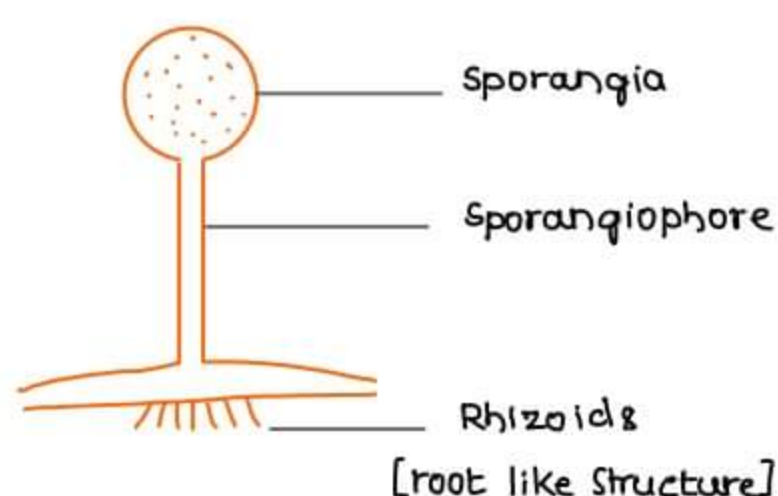
urea hydrolysis test [+ive] for cryptococcus

## RHINO CEREBRAL ZYGOMYCOSIS

- caused by Rhizopus species
  - fulminant disease
  - Tissue Biopsy + H&E staining
  - Tissue Biopsy + SDA
- Aseptate hyphae obtuse angled

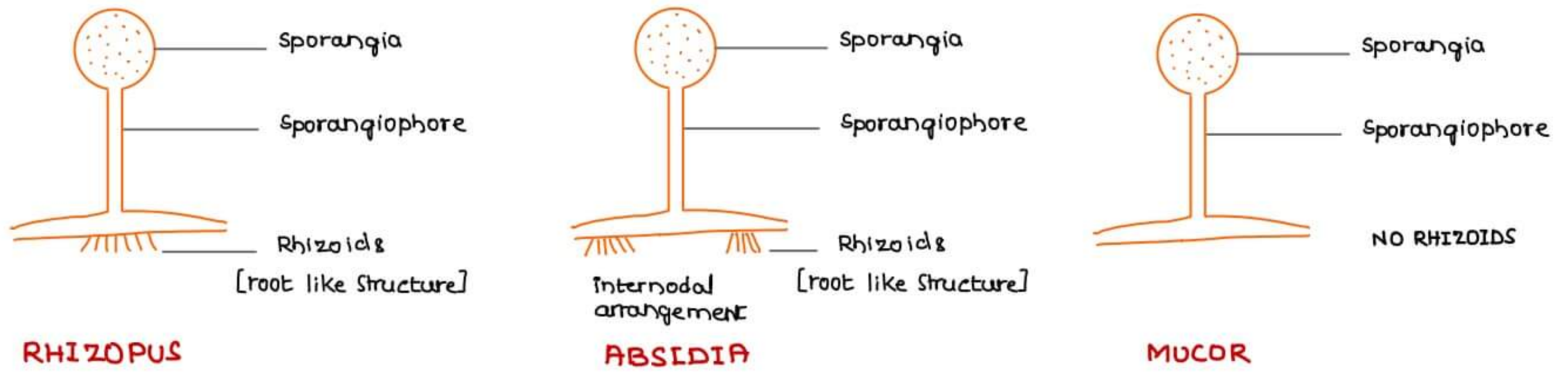


↓  
culture growth  
↓  
LPCB mount [Lactophenol cotton blue mount]  
↓



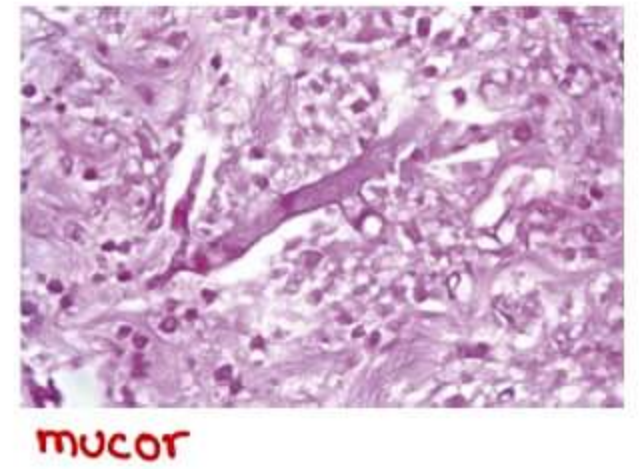


DIFFERENT ZYCOMYCETES



MUCOR MYCOSIS

- Predisposes in DM
- Frequent blood transfusions
- chronic dialysis



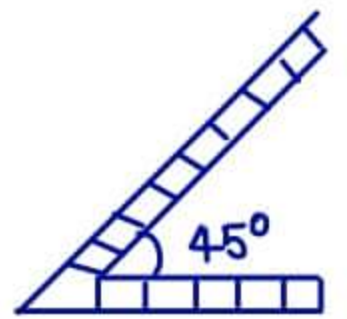
ASPERGILLUS

- A. fumigatus** → causes ABPA (Allergic Broncho Pulmonary Aspergillosis)  
Aspergilloma (fungal ball)  
Oculomycosis  
Otomycosis
- 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°)



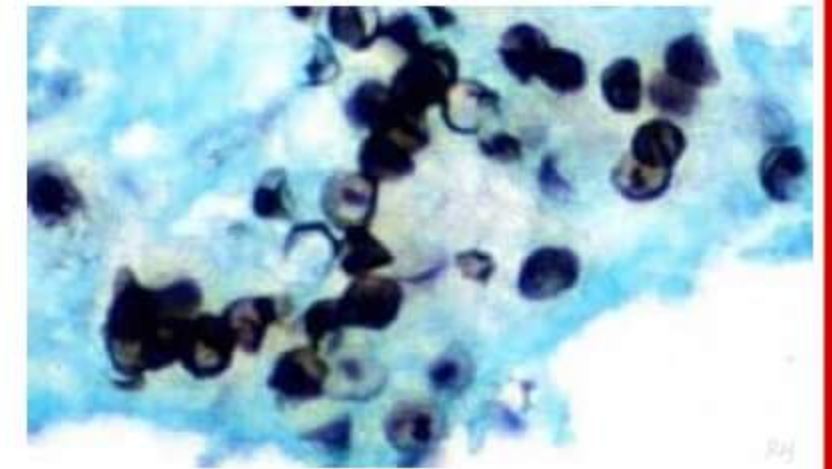
ERGOTOXICOSIS [ERGOT POISONING]

- Edibles + claviceps purpurea seeds → Ergototoxicosis
- consumpt<sup>n</sup> of contaminated edibles leads to
  - ① St. Vitus Dance E
  - ② St. Anthony's fire
- Gastric irritation occurs
- ↓
- Restlessness [ St. vitus dance ]



## PNEUMOCYSTIS CARINII / PNEUMOCYSTIS JIROVECI

- Protozoa → cyst / Trophozoite + nt
  - fungus → chromosomal studies favours fungus
  - **Pneumocystis carinii pneumonia**
    - occurs in persons  $\bar{c}$  CD<sub>4</sub> → < 200 [HIV +ve]
    - DOC → COTRIMOXAZOLE
    - LAB DIAGNOSIS
      - ① BAL [Broncho Alveolar Lavage] Fluid
      - ② Gastric washings (children)
- ↓
- GMS [Gomori's Methanamine silver]
- ↓
- Trophozoites → Green  
Cysts → Black
- Non cultured on SDA



Pneumocystis carinii  
Cyst

## OCCUPATIONAL DISEASES

### FARMER'S LUNG

- dit Hay dust
- caused by Micro monospora / micropolyspora faenii, faenia rectivirgula  
Thermo actinomyces vulgaris
- Type III hypersensitivity

### BAGGASSOSIS

- dit sugarcane dust
- caused by Thermo actinomyces sacchari

### MALTSTER'S LUNG

- dit Barley & malt → caused by Aspergillus clavatus

### CHEESE WASHER'S LUNG

- also cheese factor workers
- caused by Penicillium caseocolum

### MAPLE BARK STRIPPER'S LUNG

- dit maple tree bark
- caused by cryptostroma corticale

### SEQUIOIOSIS

- dit redwood saw dust
- caused by aureobasidium pullulans



**SUBEROSIS**

- dlt cork
- caused by *Penicillium frequentans*

**WOOD PULP WORKERS**

- dlt wood pulp
- caused by alternating species

**HUMIDIFIER'S LUNG**

- dlt humidifier
- caused by *T. vulgaris* , *T. candidus*



**PROTOZOA**

**Classification**

<p><b>I AMOEBAE</b></p>	<p><b>II CILIATES</b></p>
<p><b>a Free living</b></p> <ul style="list-style-type: none"> <li>- Naegleria fowleri</li> <li>- Acanthamoeba balamuthia</li> </ul> <p><b>b Intestinal</b></p> <ul style="list-style-type: none"> <li>- Entamoeba histolytica</li> <li>- E. coli</li> </ul>	<p>Balantidium coli</p>
<p><b>III FLAGELLATES</b></p>	<p><b>IV SPOROZOA</b></p>
<p><b>a Intestinal</b></p> <ul style="list-style-type: none"> <li>- Giardia lamblia</li> </ul> <p><b>b vaginal</b></p> <ul style="list-style-type: none"> <li>- Trichomonas vaginalis</li> </ul> <p><b>c Blood &amp; Tissue</b></p> <ul style="list-style-type: none"> <li>- Leishmania</li> <li>- Trypanosoma</li> </ul>	<p><b>a Blood Species</b></p> <ul style="list-style-type: none"> <li>- Plasmodium</li> <li>- Babesia</li> </ul> <p><b>b Tissue species</b></p> <ul style="list-style-type: none"> <li>- Toxoplasma</li> </ul> <p><b>c Intestinal species</b></p> <ul style="list-style-type: none"> <li>- Isospora</li> <li>- Cyclospora</li> </ul>

**Entamoeba histolytica**

QUADRI NUCLEATE CYST [Infective form]



Ingested in contaminated food & water



Bypasses gastric acidity

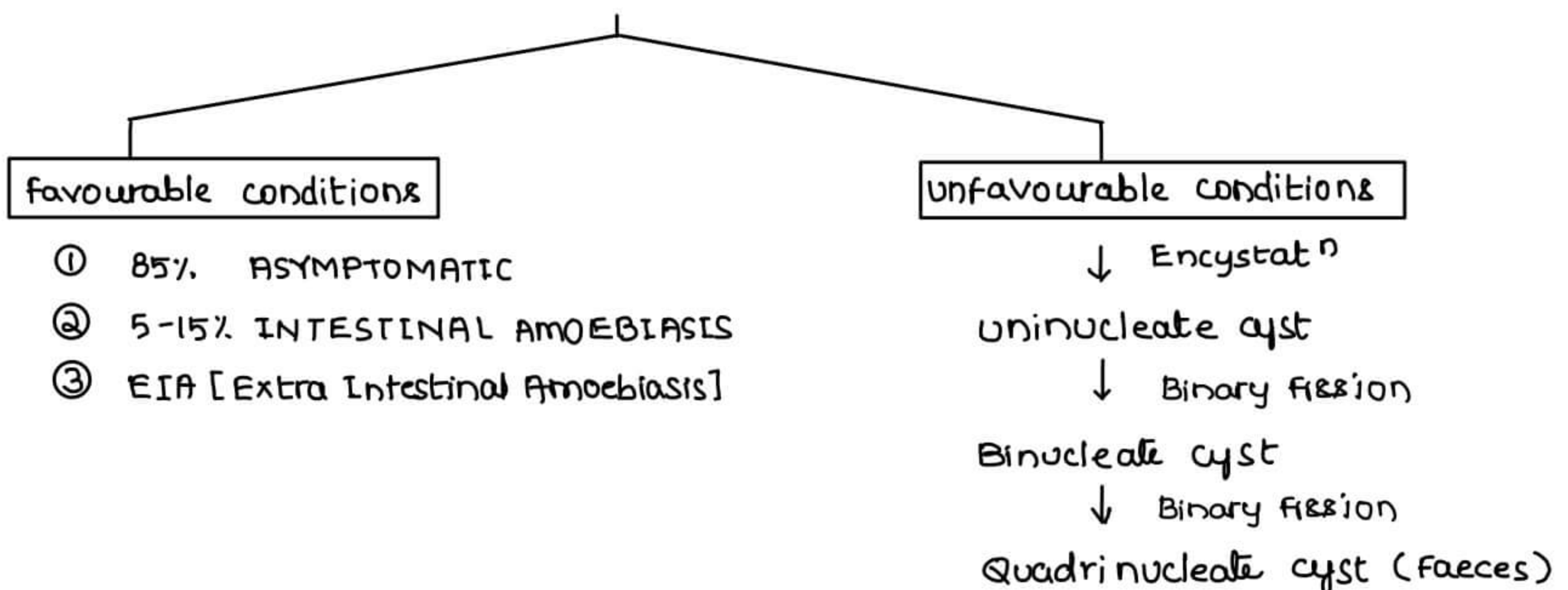


Excystation in small intestine / large intestines



TROPHOZOITES

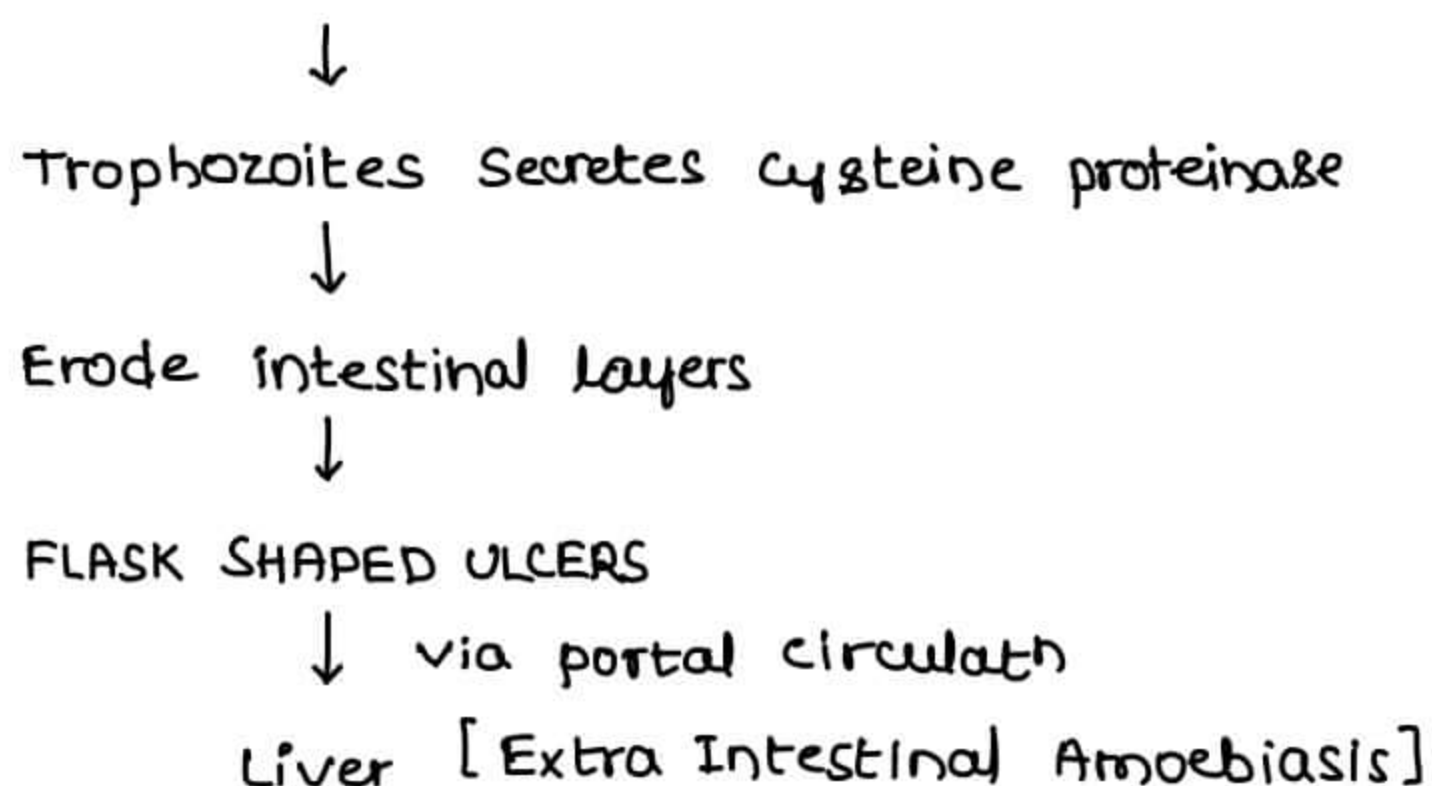
- active feeding & growing stage
- 8 Metacystic trophozoites are formed





## Favourable conditions

### ③ EIA [EXTRA INTESTINAL AMOEBIASIS]



- mc site for EIA → Liver > Lungs > Brain
- chocolate pus / Ancovy sauce pus formed due to damage to hepatocytes
- Right lobe, posterior 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 & central karyosome is present
- ERYTHROPHAGOCYTOSIS seen
  - Engulfed RBCs
  - seen in fresh stool samples
  - characteristic feature of *E. histolytica*

#### Ⓑ Stool sample + Iodine mount → cyst is seen under microscope

- 4 nuclei cyst (mostly)
- uninucleate or binucleate cysts & chromatoidal bars & Glycogen
- vacuoles also seen some time

#### ② CULTURE

media used are

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

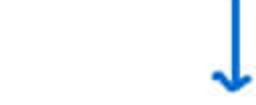
#### ③ PCR for *Entamoeba histolytica*



**NAEGLARIA FOWLERI** [Brain Eating Amoeba]

→ Infective form → Amoeboid form  $\rightleftharpoons$  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
- Neutrophils are observed

**ACANTHAMOEBA SPECIES**

→ causes contact lens keratitis

→

Aerosol 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*

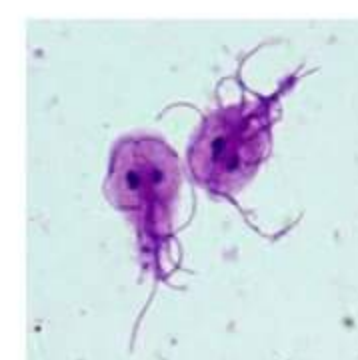
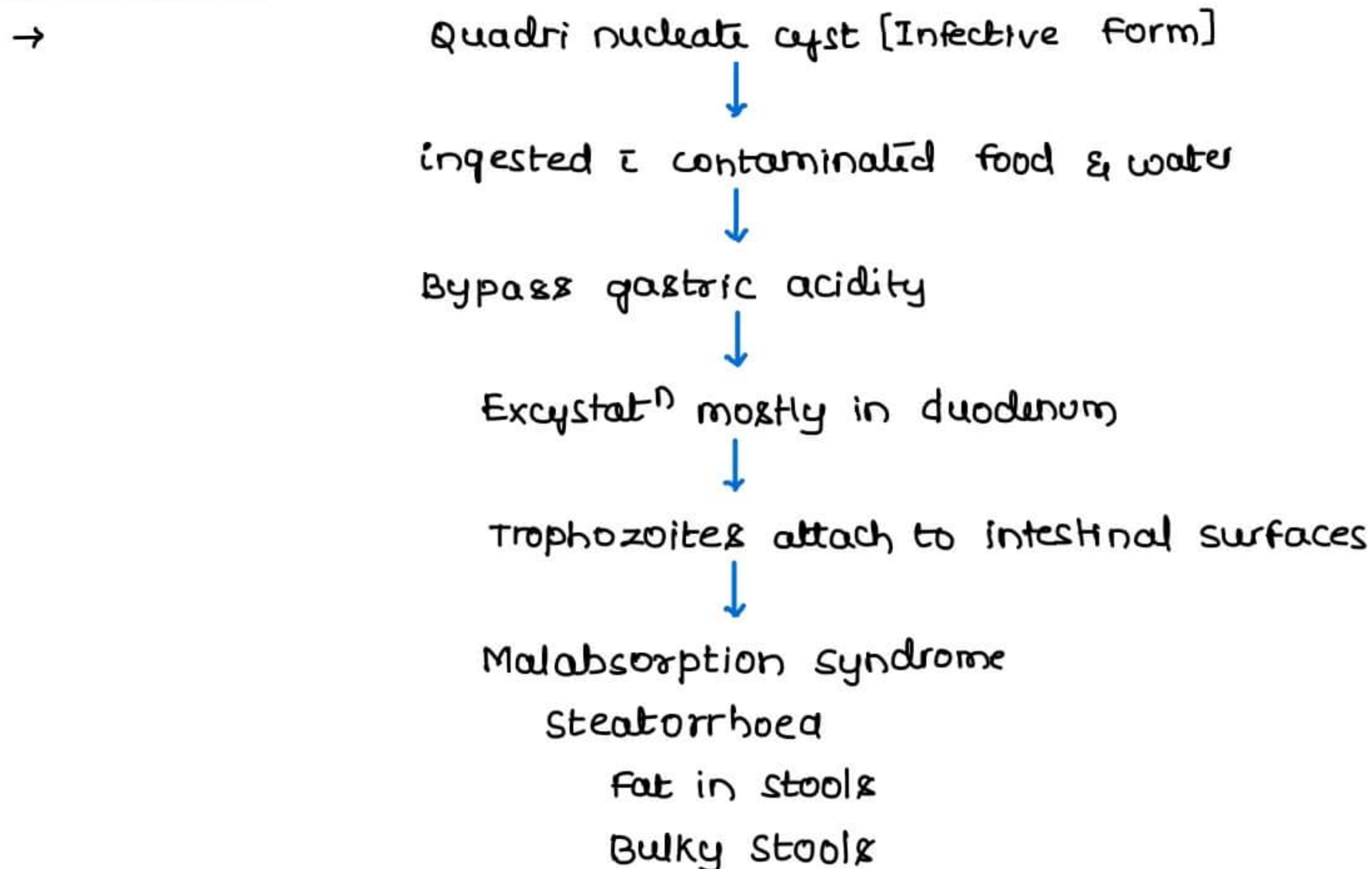
*Balamuthia*

} causes Encephalitis



## INTESTINAL FLAGELLATES

### GIARDIA LAMBLIA



GIARDIA  
TROPHOZOITE

### TROPHOZOITE

- TENNIS RAQUET APPEARANCE OR MONKEY FACE APPEARANCE OR TEAR DROP APPEARANCE
- Axostyles are +ve
- 4 pairs of flagella → FALLING LEAF LIKE MOTILITY
- Sucking disc → helps in adherence to intestine (virulence factor)



GIARDIA CYST

### CYST

- 4 nuclei +ve
- have Axostyle
- ENTERO TEST / STRING TEST done to demonstrate trophozoites
- Giardia lamblia infect<sup>n</sup> a/w CVID [Common Variable Immuno Deficiency]

## VAGINAL FLAGELLATE

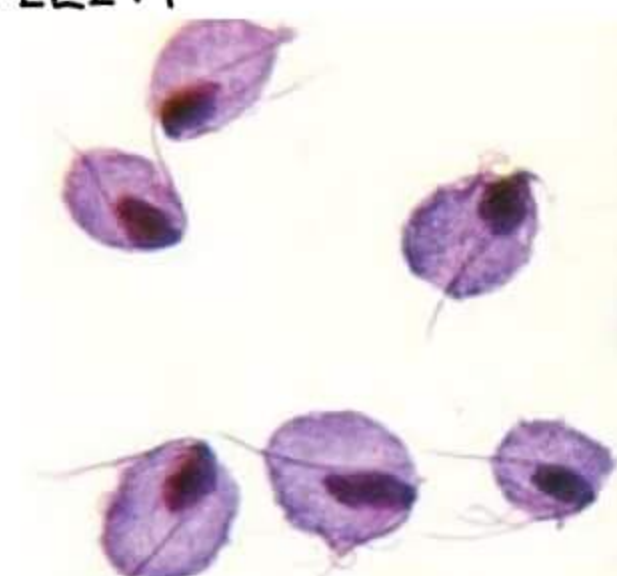
### TRICHOMONAS VAGINALIS

- No cystic stage
  - only Trophozoite stage is present
  - 5 pairs of Flagella +ve → 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 trypanomastigote [infective form]

**LEISHMANIA**

- L. donovani → causes Kala Azar (Phlebotomous (sand fly) transmits)
- L. tropica → causes Oriental sore / Delhi or Baghdad boil / Aleppo button
- L. brasiliensis → causes Espundia [mucocutaneous leishmaniasis]

**KALA AZAR**

- hyper pigmentation + Fever + hepatosplenomegaly seen
- KA & HIV +ve → hepatosplenomegaly absent (atypical presentat<sup>n</sup>)
- Anemia, Thrombocytopenia, Leucopenia seen
- Hypergamma globulinemia seen
- LAB DIAGNOSIS

**① BLOOD SMEAR**

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

- Hypergamma globulinemia 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

A flagellar forms

AKA LD BODIES [LEISHMAN DONOVAN BODIES]

**③ TK-39 ANTIGEN TESTING using CARD METHOD****④ MONTENEGRO TEST [skin testing]****⑤ CULTURE on NNN [Novy Neil Nicholle] media**

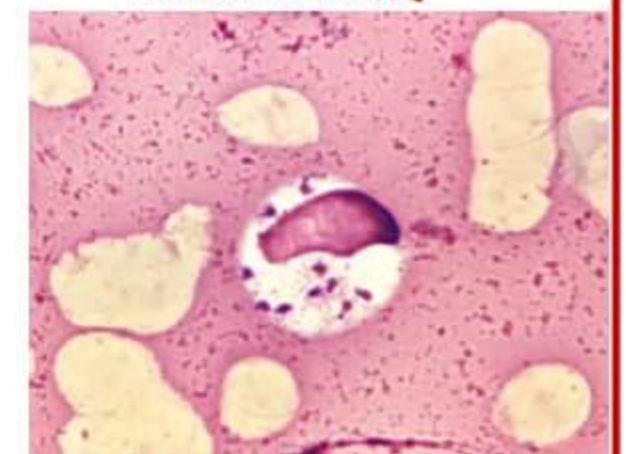
- Promastigote forms (flagellar forms) demonstrated

**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  
 → dit dermatotropic 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

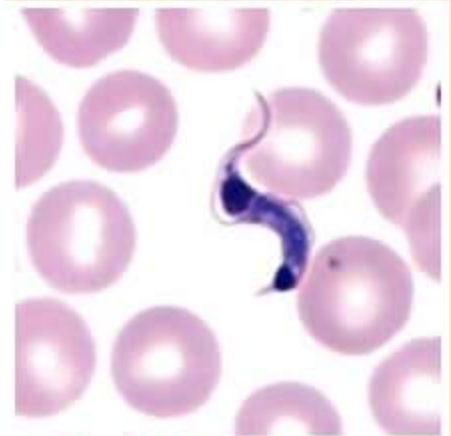
- transmitted by Reduviid bug
- causes CHAGAS DISEASE / AMERICAN TRYPANOSOMIASIS

### FEATURES

- CHAGOMA → entry site edema
- ROMANA SIGN → eye lid swelling
- M → Mega oesophagus
- M → Mega colon
- M → Myocarditis
- M → Meningo encephalitis



REDUVID BUG

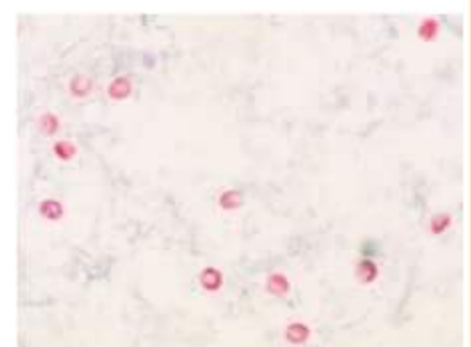
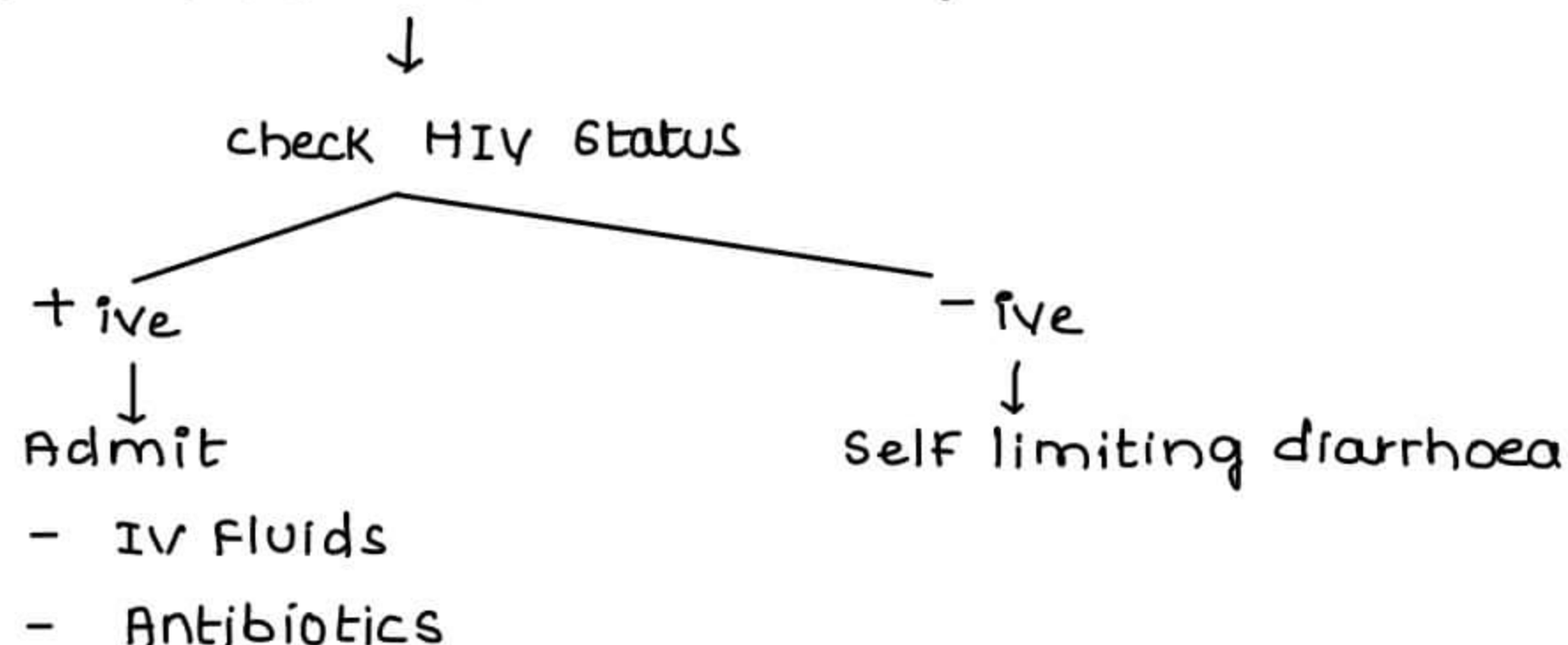


T. cruzi

## COCCIDIAN PARASITES

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

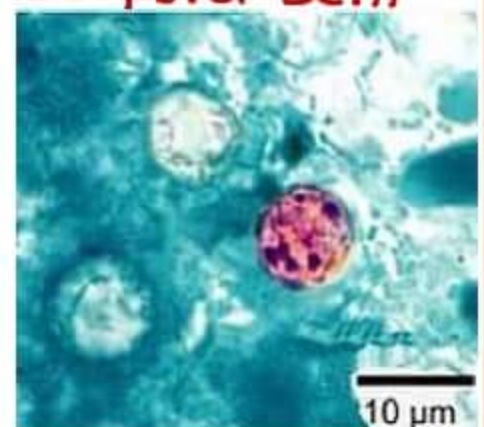
- All are Acid fast
- If Stool Examinat<sup>n</sup> shows coccidian parasites



cryptosporidium parvum oocyst



Isospora belli

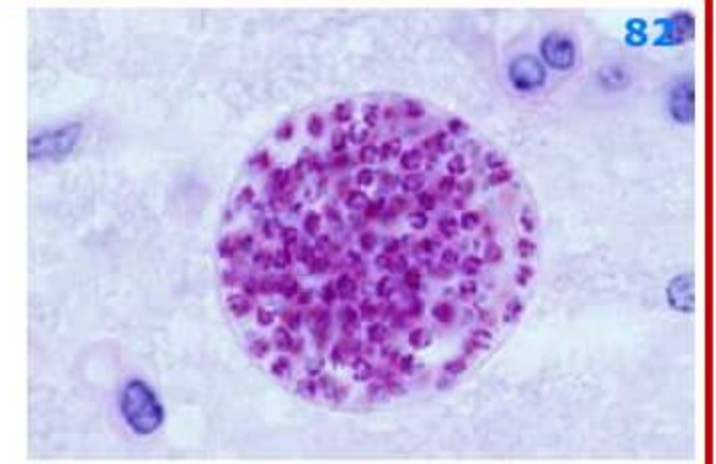


Cyclospora



## TOXOPLASMA GONDII

- causes Toxoplasmosis
- Definite Host → cat
- Intermediate Host → Rat / man



Bradyzoite

- cat → Enteric cyclic occurs → oocyst formed
- Rat / Man → Exoenteric cyclic occurs → No oocyst format<sup>n</sup>

### → 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 → complement 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 (N) individual → mild cervical lymphadenopathy
- Toxoplasmosis in immunodeficiency (HIV+ve) → Seizures
- Toxoplasmosis in pregnancy at 1st Trimester → max damage to fetus
- at 3rd Trimester → max chance of transmiss<sup>n</sup>

## PLASMODIUM SPECIES

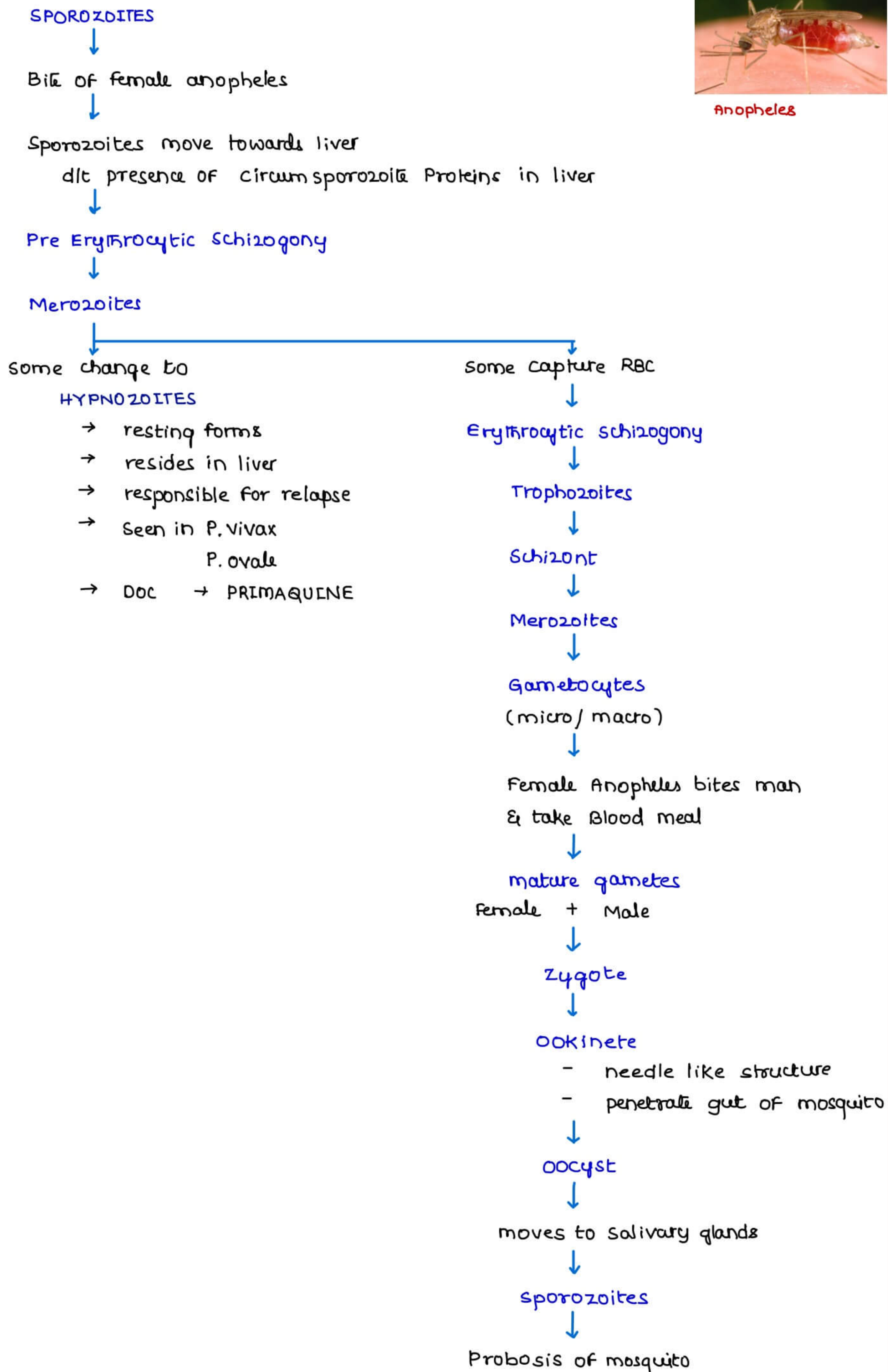
- P. vivax
- P. ovale
- P. falciparum
- P. malariae
- P. knowlesi



## LIFE CYCLE



Anopheles





CONDITIONS PREVENTING P. falciparum infection


- G → G6P Deficiency
- S → Sickle cell anemia
- T → Thalassemic trait
- Excess of fetal Hb

CONDITION PREVENTING P. vivax infect<sup>n</sup> → 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 dlt hypnozoites	RECRUDESCENCE Seen dlt incomplete Py
Single large ring in RBC	multiple rings are seen in 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

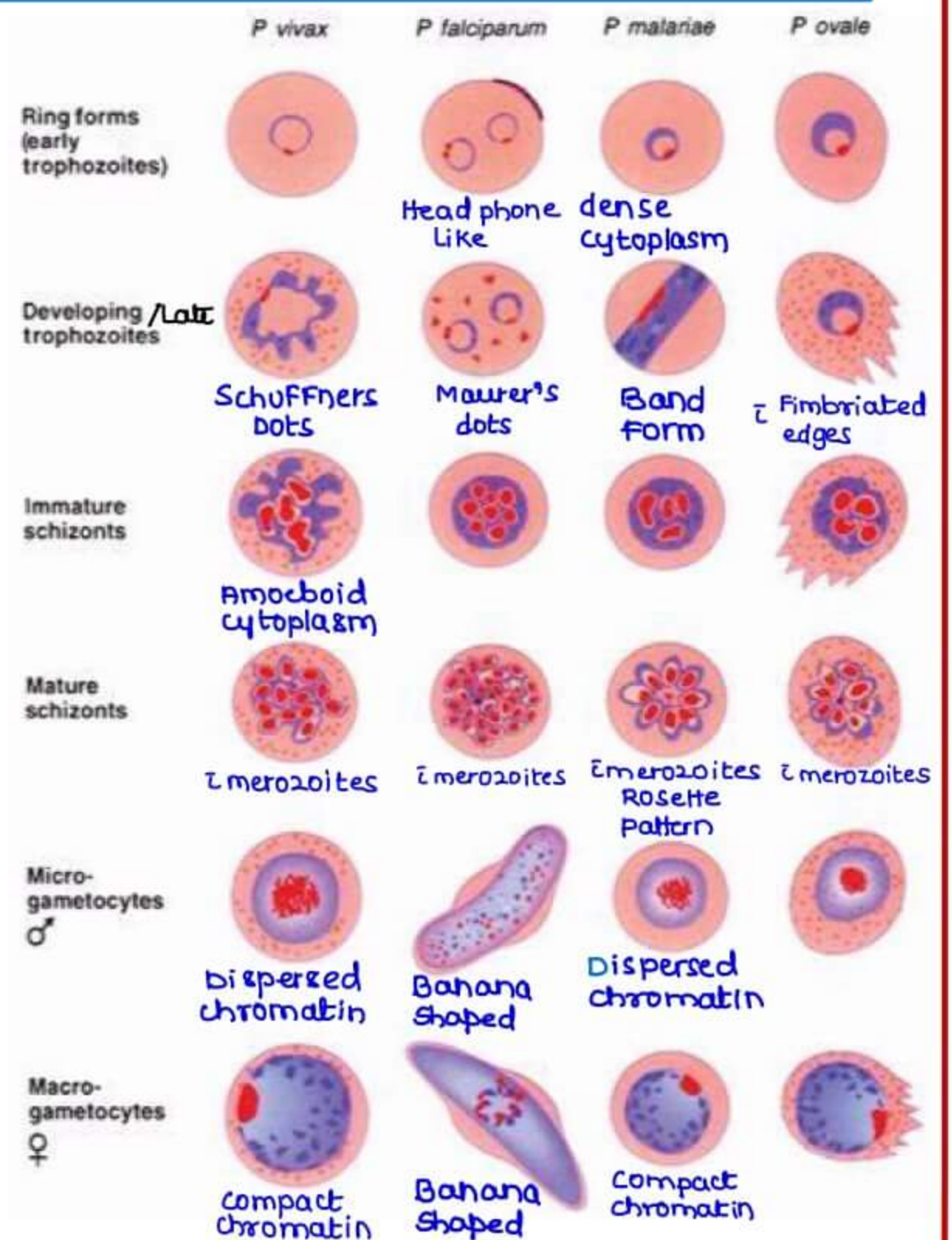
→ using Acridine orange & using fluorescent microscopy

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

⑤ PCR for Malarial Parasites



Malaria card Test



BUFFY COAT EXAMINATION [P. falciparum]



**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

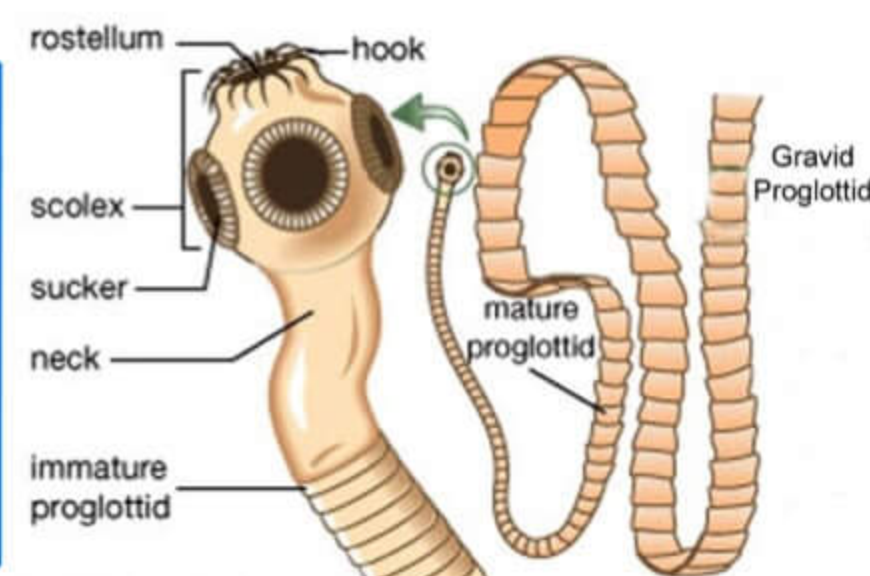
**HELMINTHOLOGY**

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

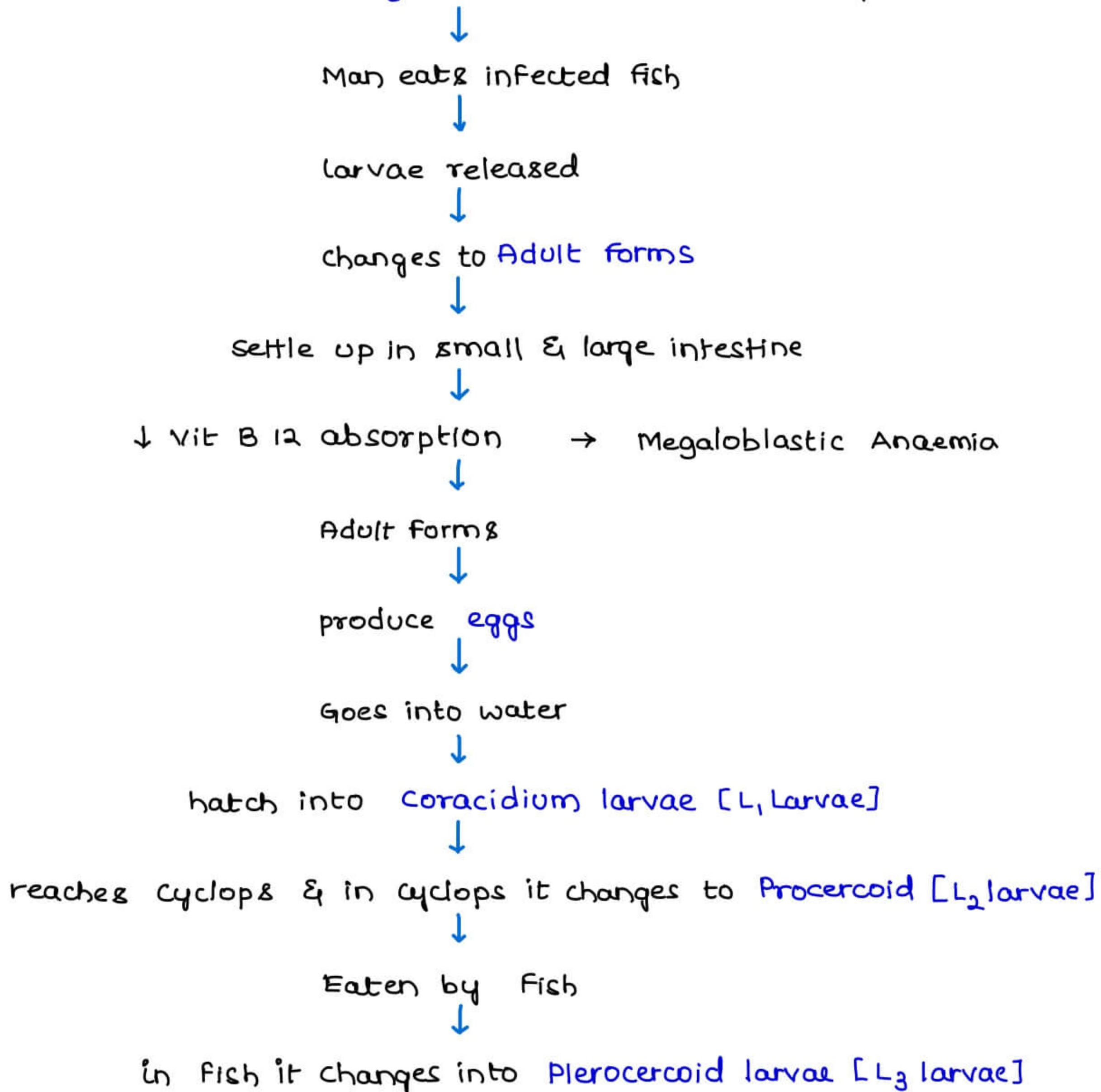




	Definite Host	Intermediate Host
Taenia solium	man	Pig
Taenia saginata	man	cattle
Echinococcus granulosus	Dog	sheep/man
H. nana	man	man
Diphyllobothrium latum	man	1st → Cyclops 2nd → fish

**DIPHYLLOBOTHRIUM LATUM / FISH TAPE WORM**

Plerocercoid larva [L<sub>3</sub> larvae] [Infective form] present in fish

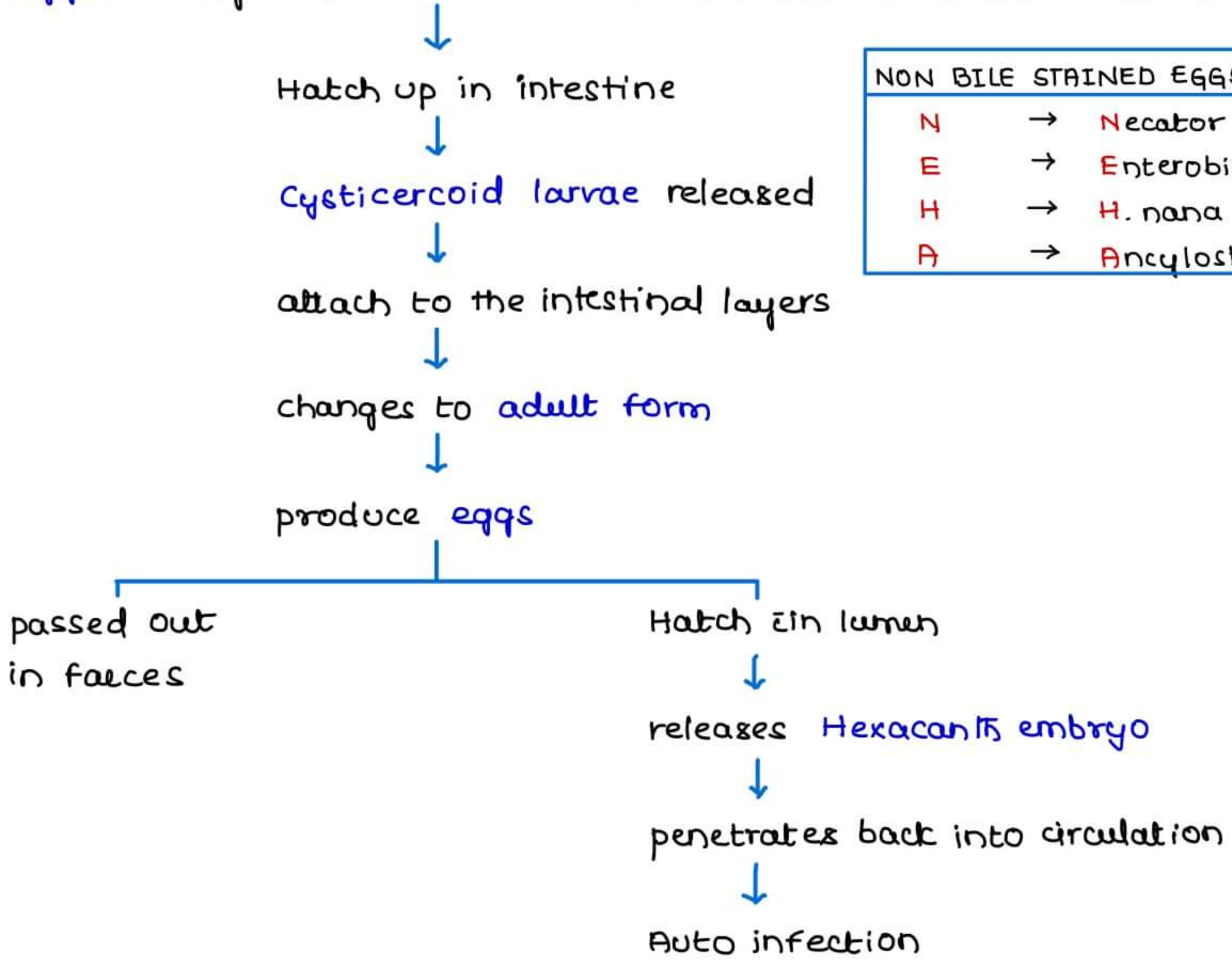


→ Egg of Diphyllobothrium latum → Operculated egg



### H. nana / Dwarf Tape worm

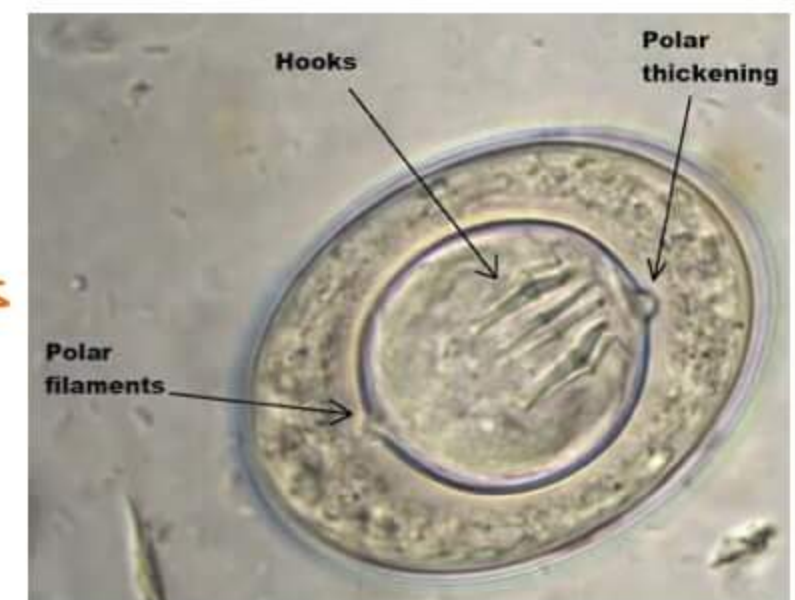
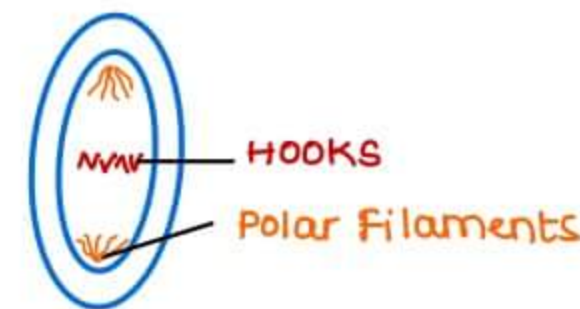
Eggs are ingested in contaminated food & water [infective form]



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

#### EGG

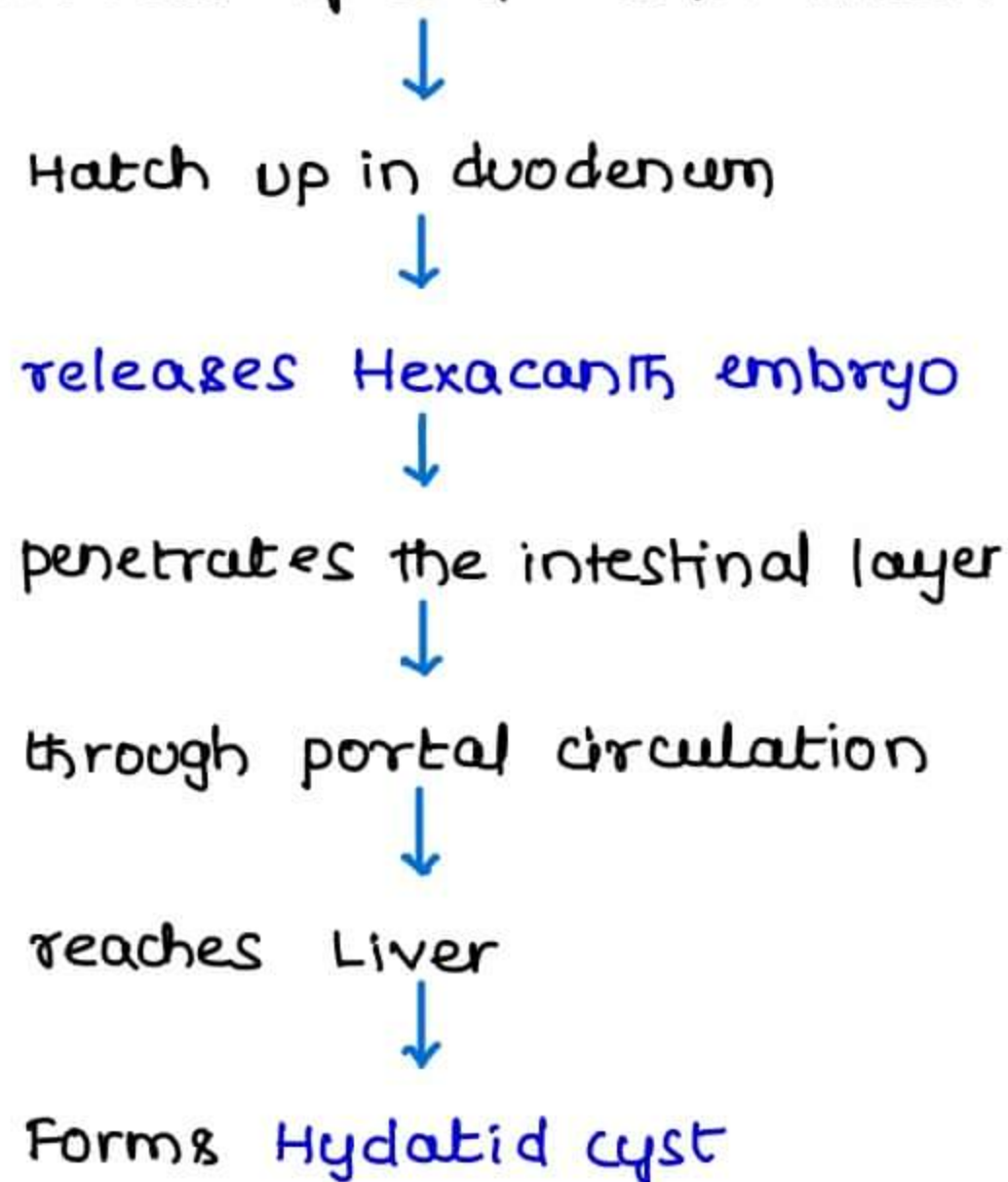
- contains → polar filaments & central hooks
- non bile stained Egg



TREATMENT → DOC → PRAZIQUANTEL

### ECHINOCOCCUS GRANULOSUS / DOG TAPEWORM

Eggs of E. granulosus ingested in contaminated food & water [Infective form]





**HYDATED CYST**

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





HYDATED CYST

**Hydatid Fluid**

- **Casoni's Test**
  - Type 1 hypersensitivity
    - immediate wheal & flare reaction
  - obsolete now
- *E. multilocularis* → causes Malignant Hydatid Diseases

**TREATMENT OF HYDATED CYST**

- ① mild to moderate cyst
  - PAIR THERAPY** → Percutaneous Aspirat<sup>n</sup> Inject<sup>n</sup> Reaspirat<sup>n</sup> Therapy using scolicidal agent
- ② Large size cyst → Surgical Removal

<b>Taenia solium</b>	<b>Taenia saginata</b>
<ul style="list-style-type: none"> <li>→ Shorter</li> <li>→ Armed tapeworm [rostellum &amp; hooklets +nt]</li> </ul> 	<ul style="list-style-type: none"> <li>→ Longer [in metres]</li> <li>→ Unarmed tapeworm [No rostellum &amp; hooklets]</li> </ul> 
<ul style="list-style-type: none"> <li>→ Eggs of both look like same</li> <li>DD → <i>T. saginata</i> eggs hooklets are acid fast</li> </ul>	
<ul style="list-style-type: none"> <li>→ Proglottids number → &lt;1000</li> <li>Proglottids → male &amp; female ill defined organs</li> <li>→ eaten uncooked pork [cesticercus cellulosa]</li> <li style="text-align: center;">↓</li> <li>TAENIASIS [diarrhoeal episodes]</li> <li>CYSTICERCOSIS [few cases]</li> <li>dlt ingestion of eggs</li> </ul>	<ul style="list-style-type: none"> <li>→ Proglottids number → &gt;1000</li> <li>→ eaten uncooked beef [cesticercus bovis]</li> <li style="text-align: center;">↓</li> <li>TAENIASIS [diarrhoeal episodes]</li> <li>→ nothing happens if eggs are eaten</li> </ul>



Portal of entry for cysticercosis

Eggs in contaminated food & water

can lead to

**NEUROCYSTICERCOSIS**

- mc site → subarachnoid > Parenchymal
- presents with atypical seizures
- IOC → CT/MRI scan
- DOC → ALBENDAZOLE



**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 → Metacercariae (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 HAEMATOBIIUM**

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



**S.MANSONI**

- resides in inferior mesenteric plexus
- causes swimmer's itch  
Katayama fever





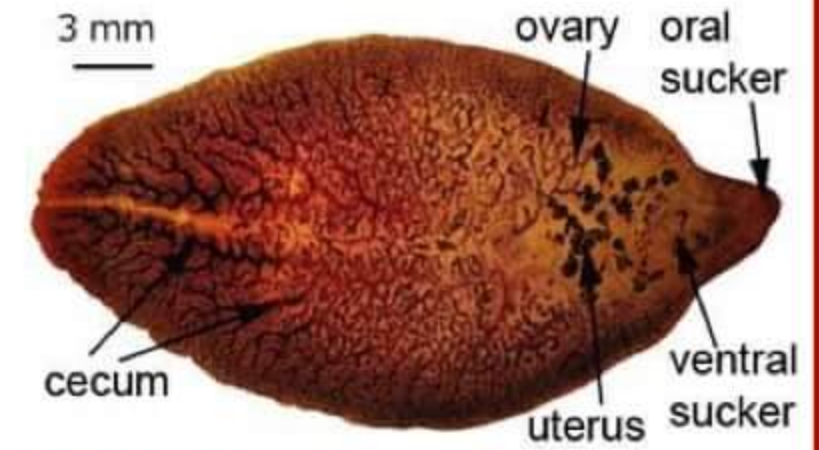
## S. Japonicum

- resides in superior mesenteric plexus
- causes Katayama fever  
Eosinophilic diarrhoea



## Fasciola Hepatica / Sheep liver fluke

- 1st IH → Snail
  - 2nd IH → Aquatic vegetat<sup>n</sup>  
[with metacercariae]
- ↓
- Man eats this aquatic vegetat<sup>n</sup>
- ↓
- 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 with water
- ↓
- Eggs → Miracidium [In SNAIL]
- ↓
- Sporocyst
- ↓
- Radial [1st generat<sup>n</sup> & 2nd generat<sup>n</sup>]
- ↓
- cercariae
- ↓
- Metacercariae [cyst like structure]
- ↓
- Goes & settles on aquatic vegetation



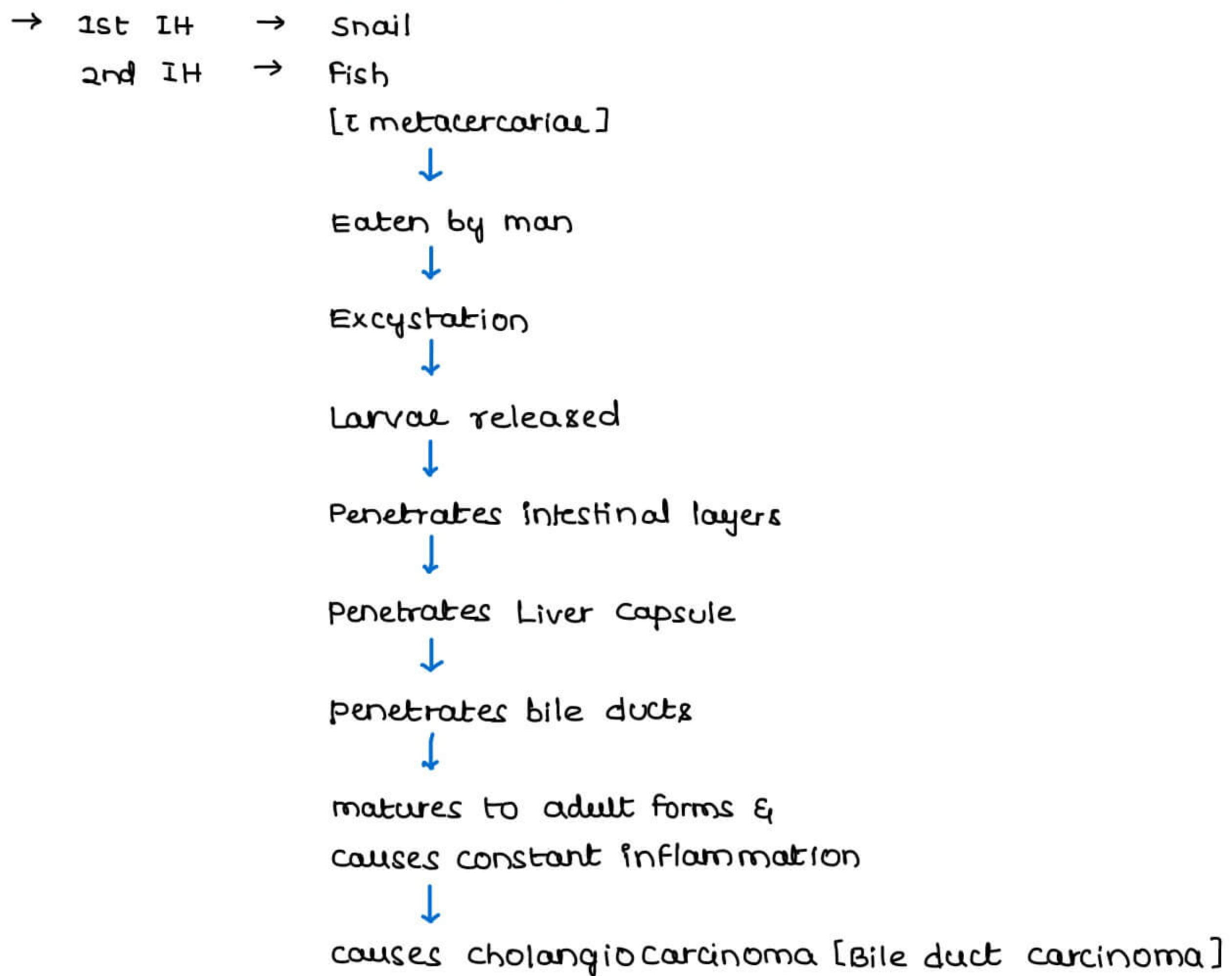
Fasciola hepatica



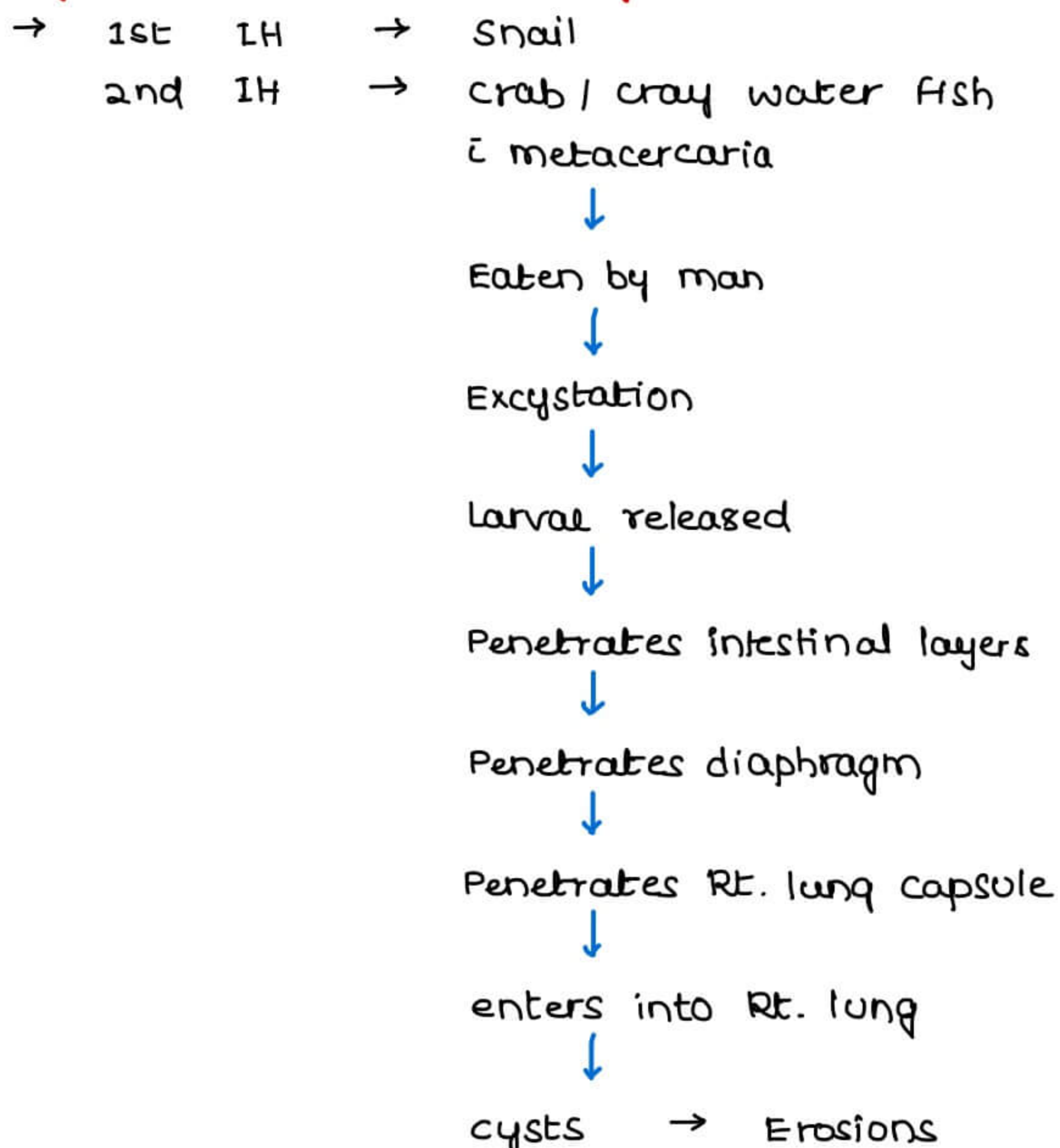
Fasciola hepatica egg  
[operculated]



## Clonorchis sinensis / Oriental / Chinese liver fluke



## Paragonimus westimonii [Lung Fluke]



Red Brown sputum [Endemic hemoptysis]



SMALL INTESTINAL NEMATODES	TISSUE NEMATODES
Ascaris Hook worm Necator americanus Strongyloides stercoralis	Resides in Lymphatics Wucheria bancrofti Brugia malayi Brugia timori
LARGE INTESTINAL NEMATODES	Resides in subcutaneous sites
Trichuris trichiura Enterobius vermicularis	Loa loa Onchocerca volvulus
MISCELLANEOUS NEMATODES	Resides in Serous cavities
Dracunculus medinensis Trichinella spiralis	Mansonella ozzardi Mansonella perstans

TRANSMISSION BY

EATING EGGS	PENETRAT <sup>n</sup> OF SOLE OF FOOT [Filariform larvae]
E → Enterobius vermicularis	A → Ancylostoma duodenale
A → Ascaris	N → Necator americanus
T → Trichuris Trichiura	S → Strongyloides stercoralis

LUNG FOR MATURATION
A <sup>2</sup> → Ancylostoma duodenale Ascaris
N → Necator americanus
S → Strongyloides stercoralis

LARGE INTESTINAL NEMATODES  
TRICHURIS TRICHIURA / WHIP WORM

Eggs are ingested in contaminated food & water

↓  
Hatch up in duodenum or caecum

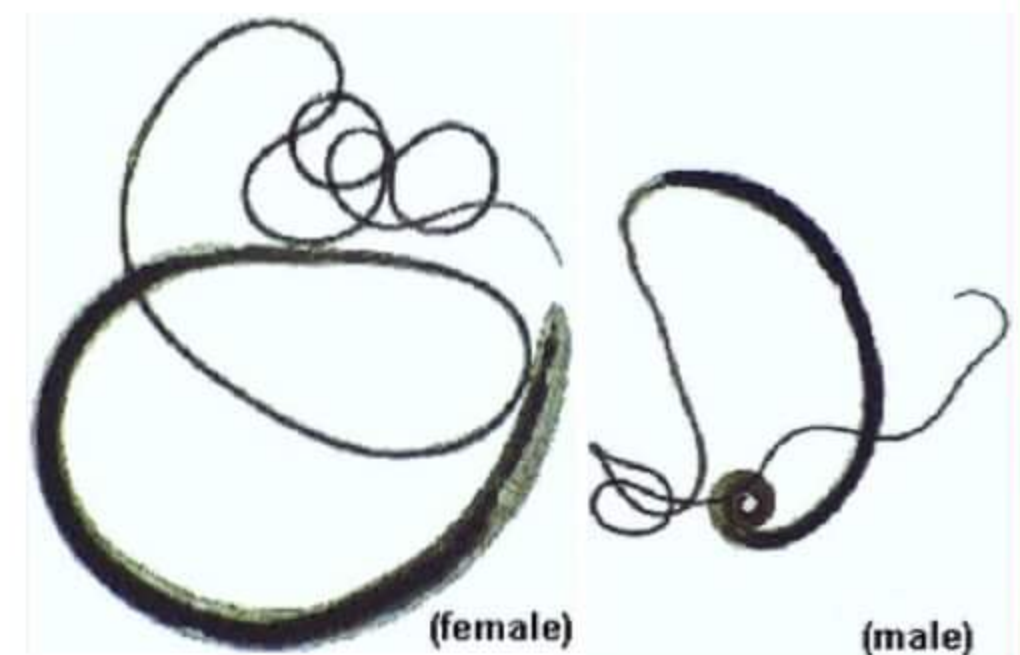
↓  
Larvae released

↙  
Male

↘  
Female

⊕ Fertilise

↓  
Eggs



EGG

- Barrel / dumb bell shaped egg
- have mucous plugs on both sides

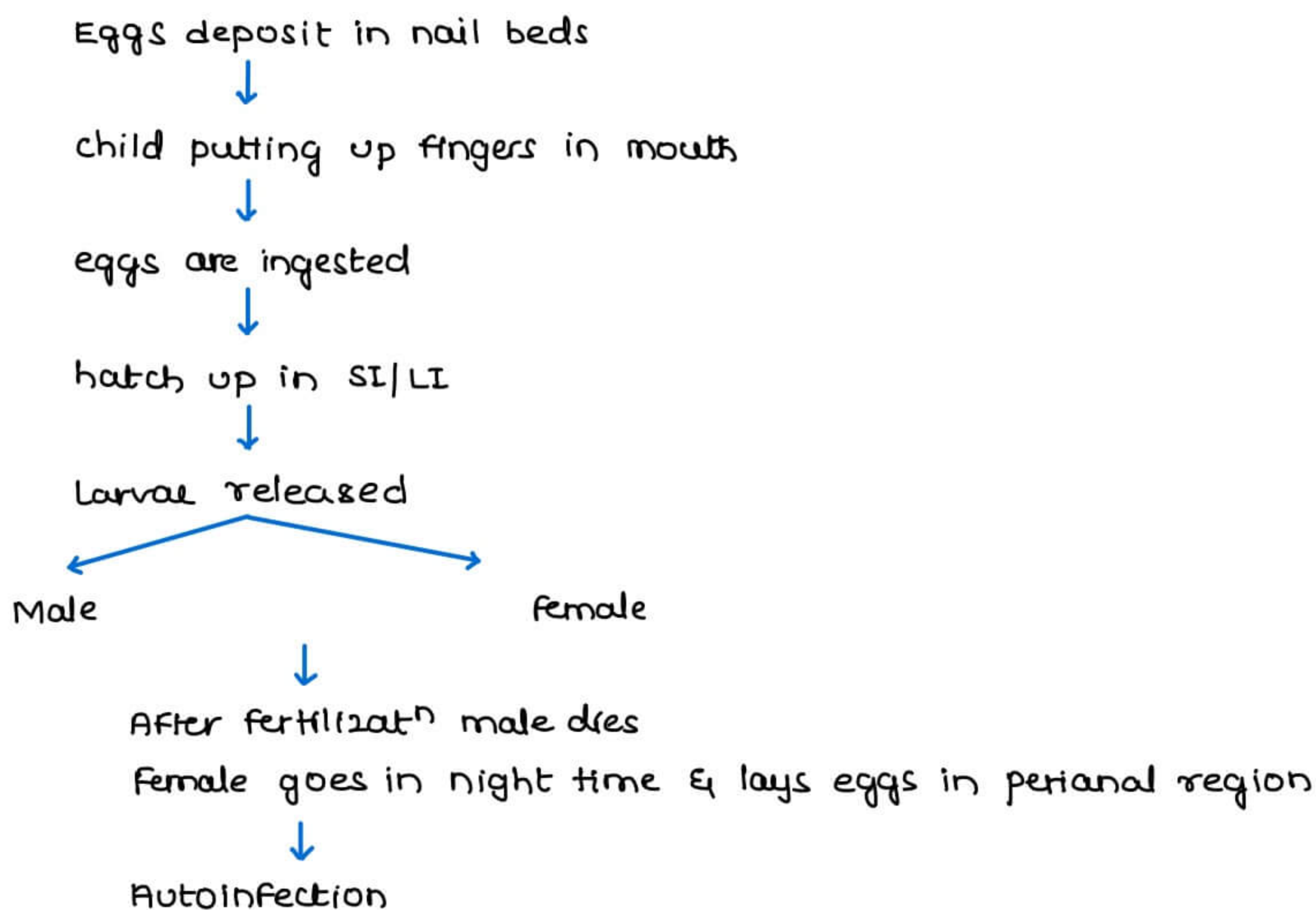




- causes Fe deficiency anaemia
- causes frequent Rectal prolapse in children  
COCONUT CAKE LIKE RECTUM  
Growth retardation

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

- presents  $\tau$  Perianal pruritis



### AUTO INFECTION SEEN IN

- C<sup>2</sup> → Capillaria philipensis  
Cryptosporidium parvum [Sporozoa]
- H → H. nana
- E → E. vermicularis
- S → Strongyloides stercoralis
- T → Taenia solium

### EGG

- Plano convex egg
- having a tadpole like larvae
- non bile stained egg

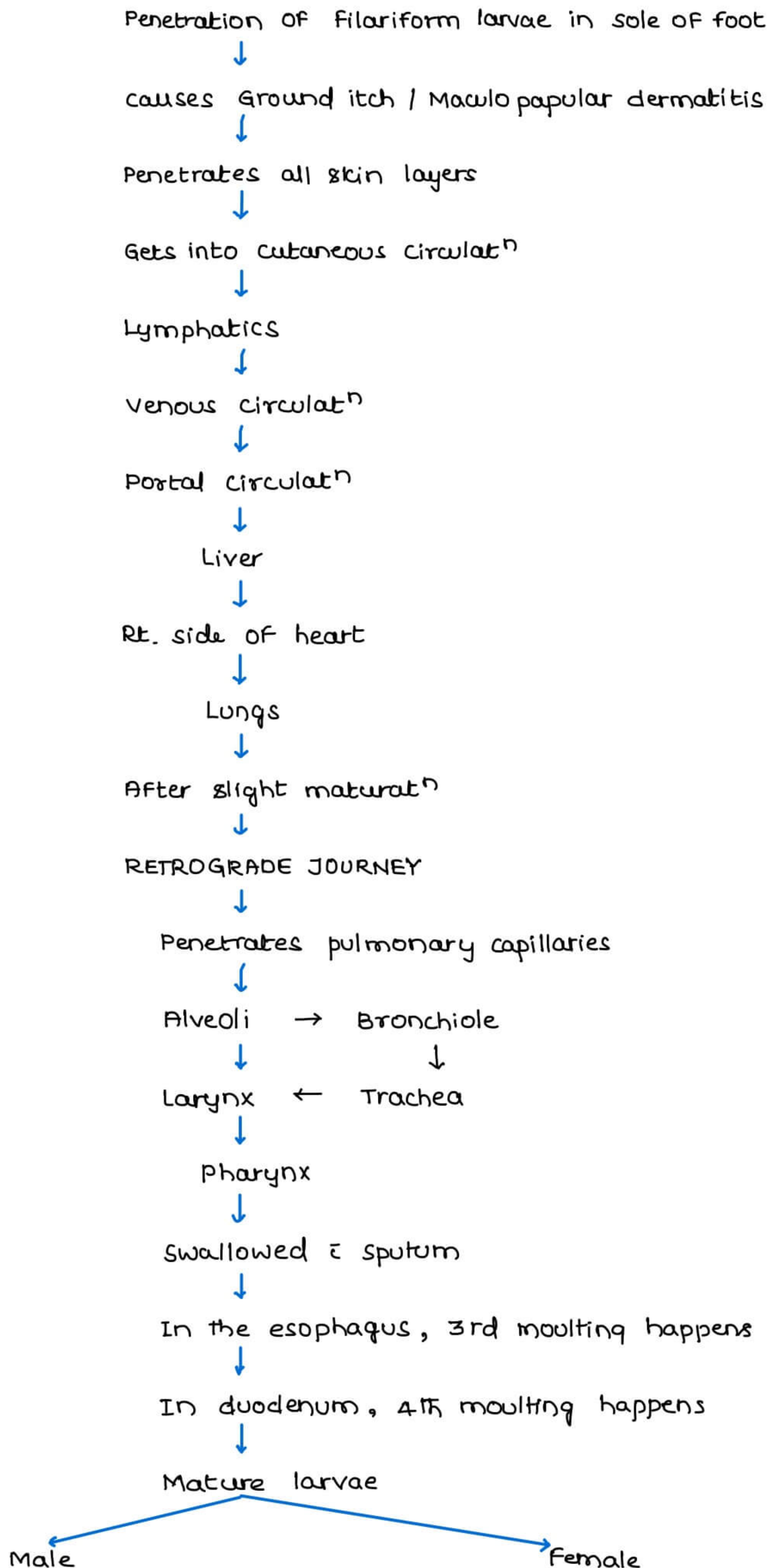


### NIH SWAB

- National Institute Health swab
- made of glass rod  $\tau$  cellophane tape



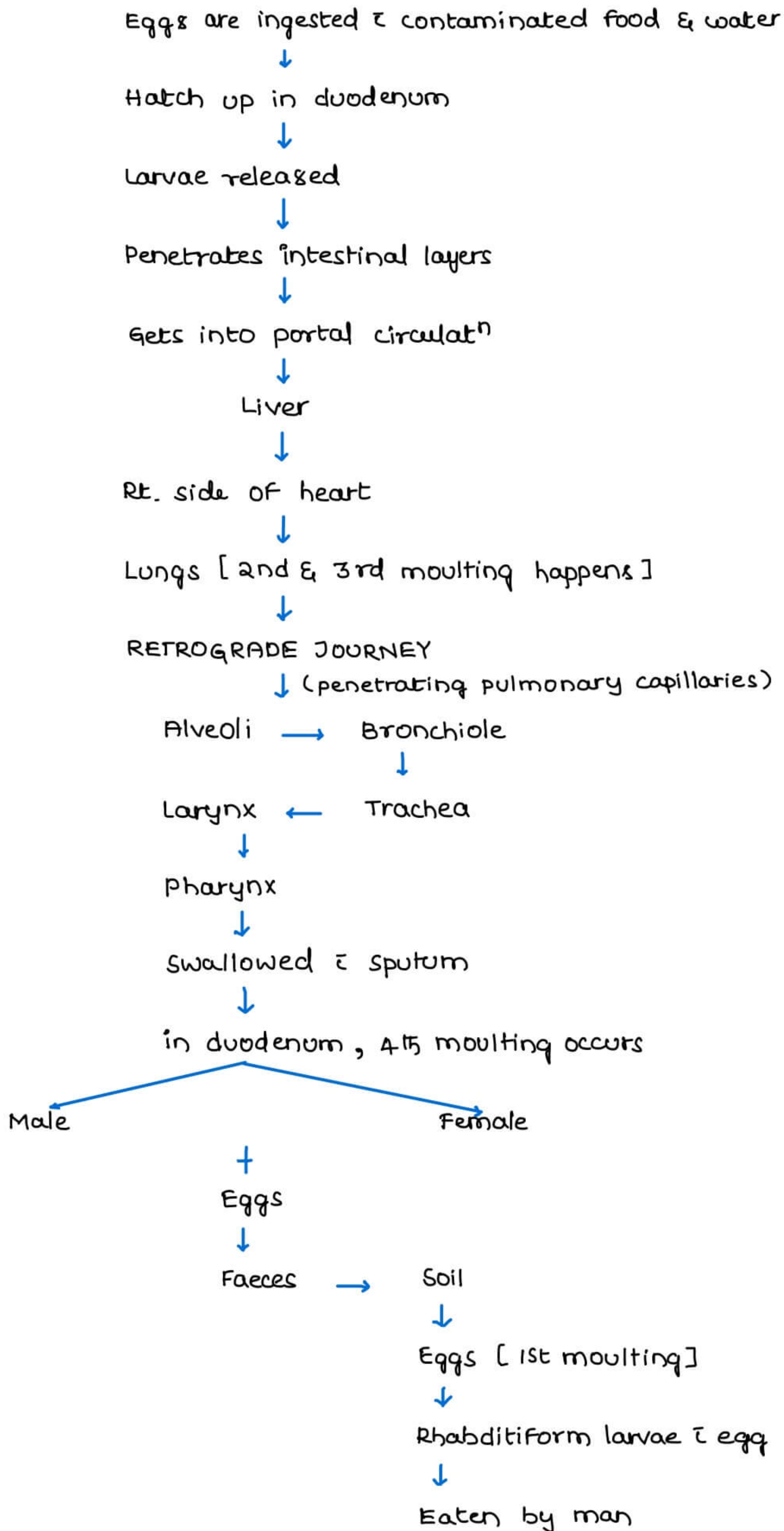
## ANCYLOSTOMA DUODENALE / HOOK WORM







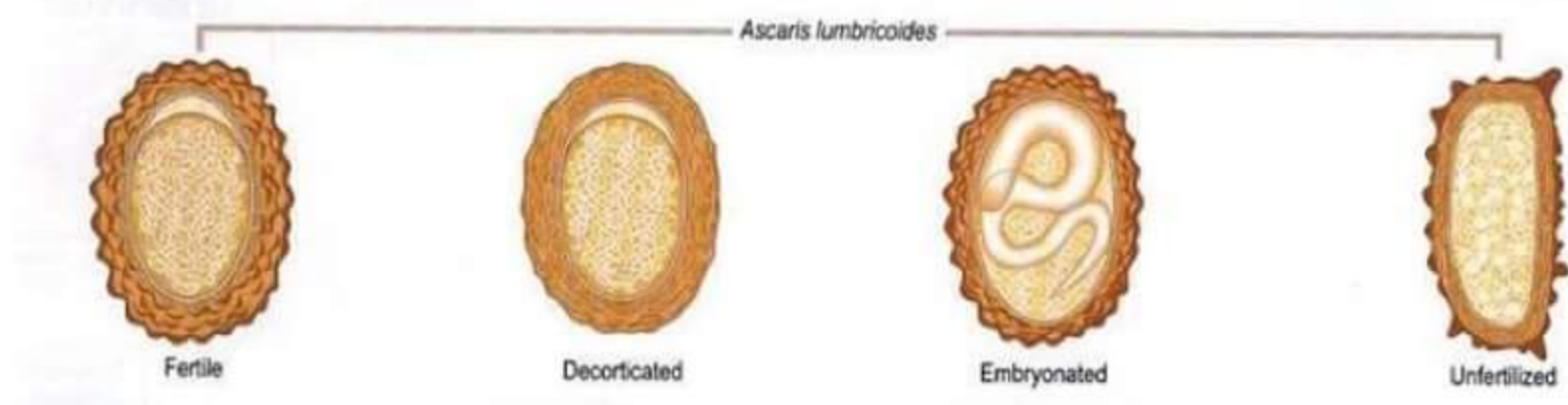




- causes Intussuscept<sup>n</sup> → intestinal obstruct<sup>n</sup>
- a/w Loeffler's syndrome [strong association] → massive eosinophilia



EGG OF ASCARIS



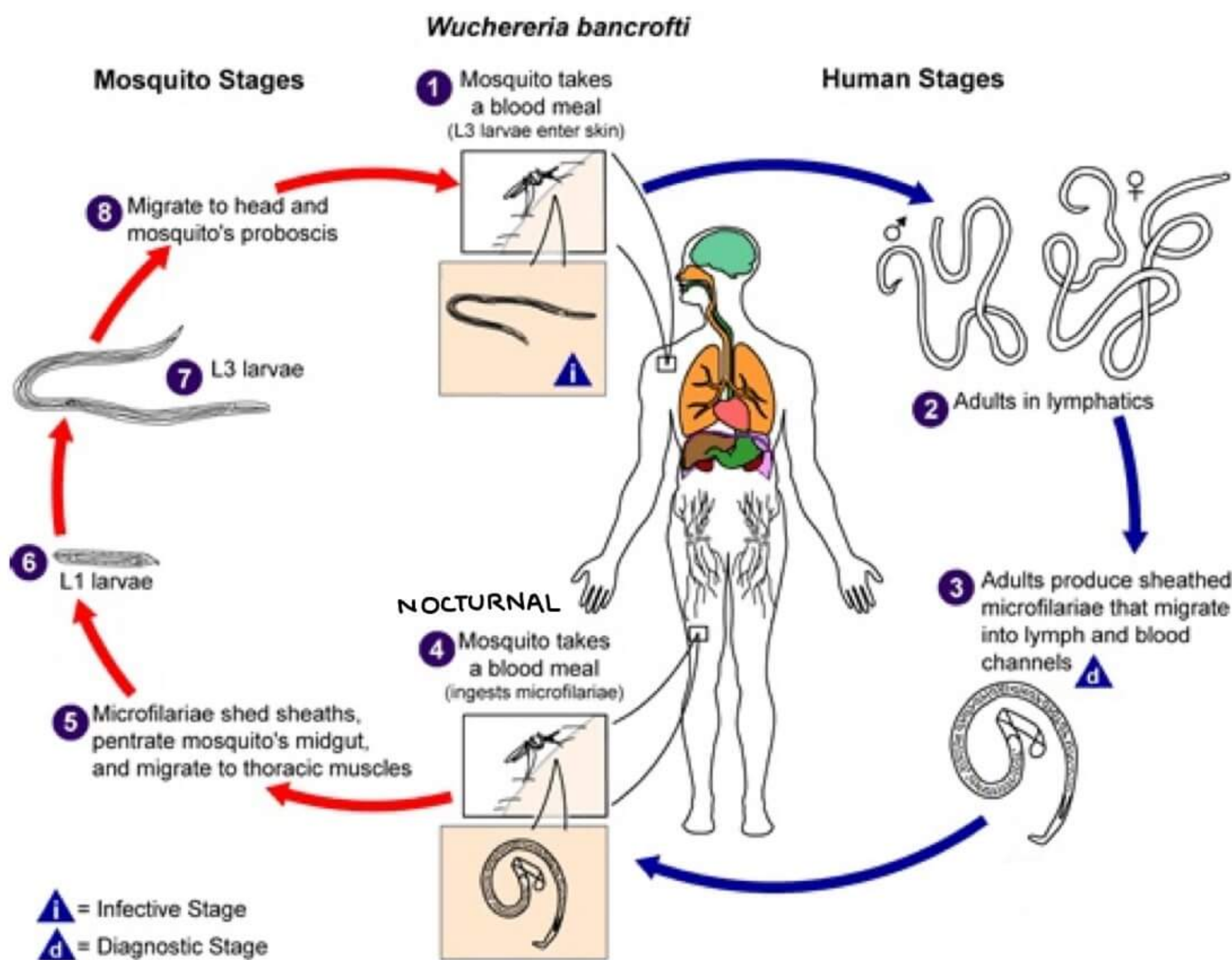
EGGS WHICH FLOAT ON SATURATED SALT SOLUTION

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

TISSUE NEMATODES

WUCHERIA BANCROFTI

- Infective form → L<sub>3</sub> 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

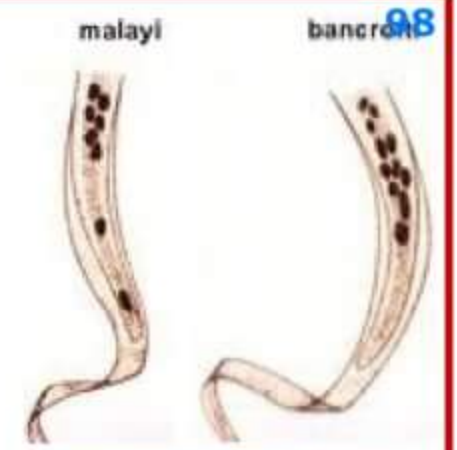


## LAB DIAGNOSTICS

### ① BLOOD SMEAR EXAMINATION

→ taken at night time d/t nocturnal periodicity

### ② DEC PROVOCATIVE TEST



ELEPHANTIASIS

### TAIL TIP

→ For identificat<sup>n</sup> Terminal Tail Tip is examined  
No nuclei seen in W. bancrofti

→ having terminal 2 nuclei → microfilariae of Brugia malayi

### ③ RADIOLOGICAL

→ XRay

→ 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 VOLVULUS

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



Onchocercoma

## 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



## 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 ( 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  $56^{\circ}\text{C} \times 30 \text{ min}$
- Stable & can be stored at  $-40$  to  $-70^{\circ}\text{C}$  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 &  $\text{H}_2\text{O}_2$  kills most viruses
- Enteroviruses are resistant to phenol & chlorination

## Replication

### STEPS

- |               |                           |                       |
|---------------|---------------------------|-----------------------|
| ① Adsorption  | ④ Biosynthesis / Assembly | "A PUB MR" → mnemonic |
| ② Penetration | ⑤ Maturation              |                       |
| ③ uncoating   | ⑥ Release                 |                       |

## ADSORPTION

- comes in contact by RANDOM COLLISION
- depends on presence of SPECIFIC RECEPTORS
- Eg: 1. HIV → Envelope proteins gp 120 / gp 41  
↓  
Binds to CD4 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
- on enveloped viruses, envelop fuses w plasma membrane of host cell  
- releasing the nucleocapsid into the cytoplasm

## UNCOATING

- physical separati<sup>n</sup> 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<sup>n</sup>

↓  
itive Sense RNA

↓ Transcription  
mRNA

↓  
Proteins

- Eg. Rhabdo, Orthomyxo, Paramyxoviridae

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

→ + strand RNA can act as mRNA

↓ Translation  
Proteins

→ Both  $\oplus$  &  $\ominus$  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  $\xrightarrow{\text{using DNA polymerase of same reverse transcriptase enzyme}}$  DS DNA  $\rightarrow$  integrated into the host cell chromosome [nucleus] by viral integrase

- integrated DNA serves as a template to produce mRNA

- mRNA  $\longrightarrow$  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



→ may take place in nucleus or cytoplasm

Eq. 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

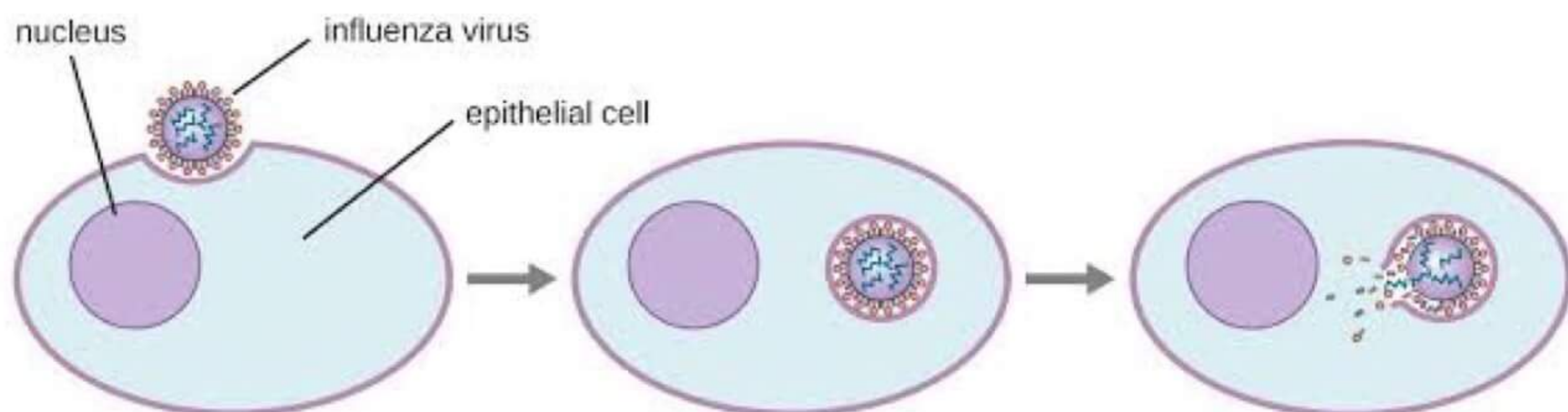
2. BUDDING → happens in enveloped virus - they acquire a part of host cell membrane to form lipid part of envelope

→ Eq: 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

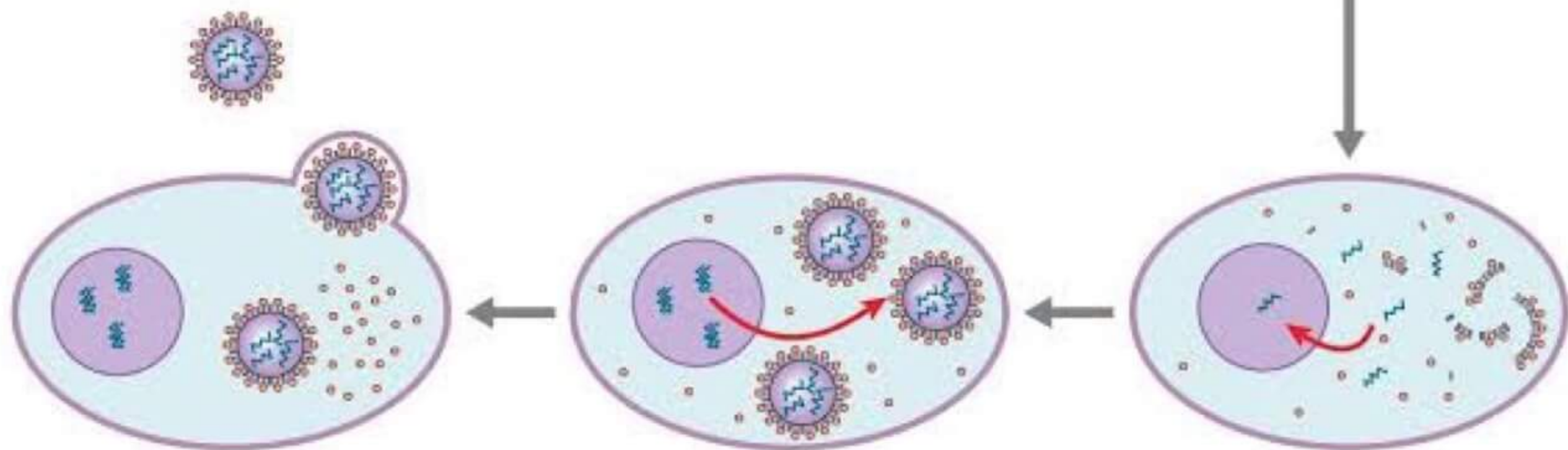
→ so that virus will not stick to host cells following release



**1 Attachment**  
Influenza virus becomes attached to a target epithelial cell.

**2 Penetration**  
The cell engulfs the virus by endocytosis.

**3 Uncoating**  
Viral contents are released.



**6 Release**  
New viral particles are made and released into the extracellular fluid. The cell, which is not killed in the process, continues to make new virus.

**5 Assembly**  
New phage particles are assembled.

**4 Biosynthesis**  
Viral RNA enters the nucleus, where it is replicated by the viral RNA polymerase.



## ABNORMAL REPLICATIVE CYCLES

### INCOMPLETE VIRUSES

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

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

**ABORTIVE INFECTION** → dit 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 b/w 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 w/ 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<sup>n</sup> →
 

Y	→	Yellow fever [17D]
I	→	Influenza
R	→	Rabies
- Amniotic sac → primarily used for isolat<sup>n</sup> 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 in normal power microscope

### INTRA CYTOPLASMIC INCLUSION BODIES [Eosinophilic]

- ① Negri bodies → Rabies
- ② Guarnieri bodies → Vaccinia
- ③ Paschen 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 [Lipschultz 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 NUCLEAR + 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]



FAMILIES

$\alpha$ VIRINAE	$\beta$ VIRINAE	$\gamma$ 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 [dit 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 → causes infection below the waist } not a rule

MUCOSAL INVOLVEMENT

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



Herpes labialis

CUTANEOUS INVOLVEMENT

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



HSV Keratitis

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

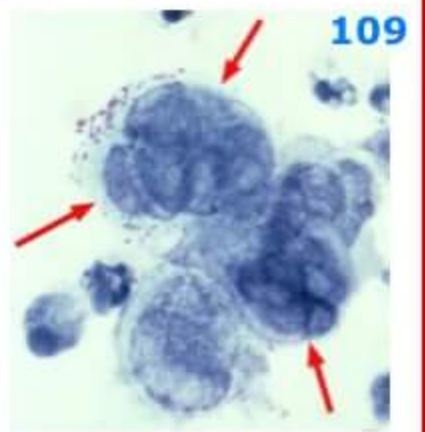
- causes B/L vesicular, pustular, erythematous lesions
- Prevent<sup>n</sup> OF HSV 2 infection to baby → cesarean section done



## LAB DIAGNOSIS

- ① PCR
  - ② ELISA
  - ③ TZANCK SMEAR
- } For HSV1 & 2

Tissue biopsy + toluidine blue → TZANCK CELLS  
[multinucleated 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 +nt, 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
  - cicatrizing lesions
  
- vaccine strain → OKA STRAIN

## LAB Dx

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

## REACTIVATION → Herpes zoster [shingles]

- occurs in stress
- Types

### H2 OTICUS / RAMSAY HUNT SYNDROME

- Bell's palsy
- vesicles over EAC, TM & cheeks
- Geniculate ganglion involved

### H2 Ophthalmicus

- single sided lesion +nt on face
- dermatomal involvement [T<sub>3</sub>-L<sub>3</sub>]
- 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
- chorioretinitis
- calcifications
- convulsions
- microcephaly
- mental Retardation

## MONONUCLEOSIS LIKE SYNDROME

### FEATURES

- Atypical lymphocytes [CD8T cells mainly]
- PAUL BUNNELL 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<sub>4</sub> count → <50
- DOC → GANCICLOVIR

## LAB DIAGNOSIS

- ① CULTURE on Human fibroblast cell line [WI-38]
- ② PCR for CMV
- ③ ELISA for CMV

## EBV

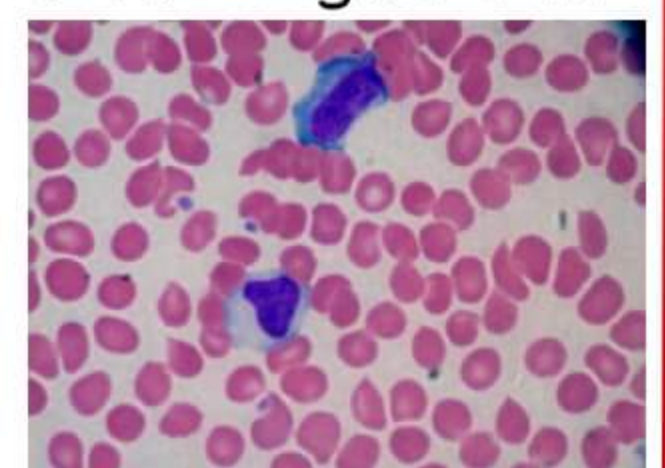
- affects B cells → polyclonal proliferat<sup>n</sup> of B cells

### → 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<sub>4</sub> : CD<sub>8</sub> ratio reversed for some times
  - atypical lymphocyte cells +nt
  - Paul Bunnell 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

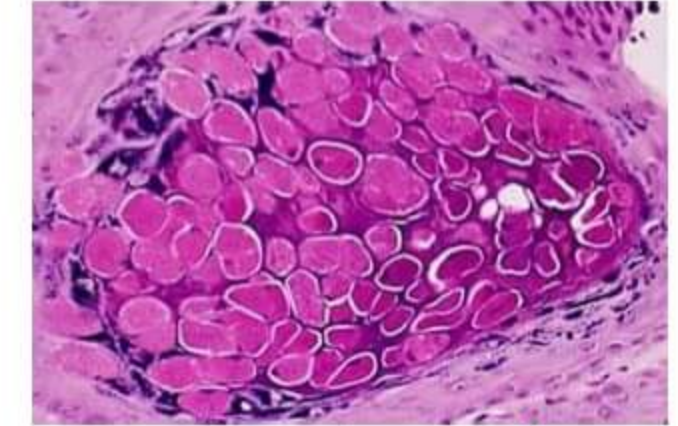
## HHV -8 [KAPOSI SARCOMA CAUSING VIRUS]

- 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 umbilicated nodule over the skin / genital & + 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

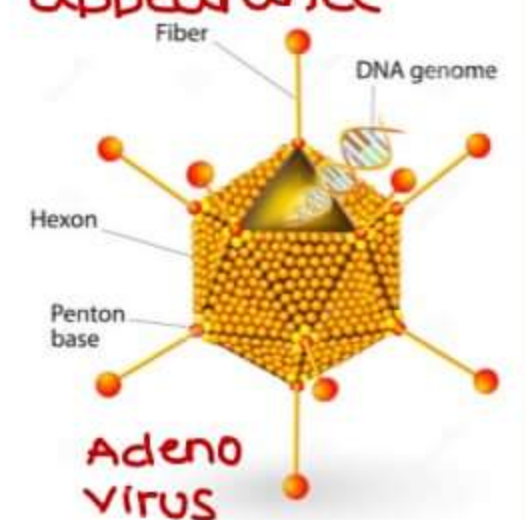
- SS DNA VIRUS
- PARVO B 19 → important
  - affects erythroid precursor cells → APLASTIC CRISIS in sickle cell anemic patients
- ASSOCIATED WITH
  - NIHF [Non Immune Hydrops fetalis]
  - Polyarthralgia syndrome
    - pain is more in upper limb joints than lower limb joints
  - Erythema infectiosum / 5th Disease
    - SLAPPED CHEEK APPEARANCE + nt



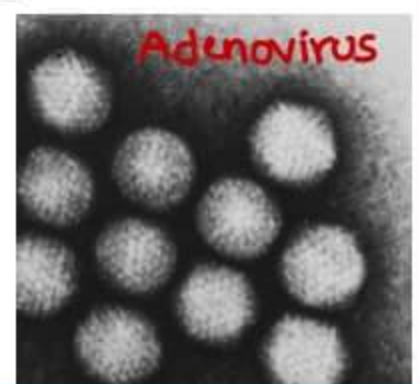
Slapped cheek appearance

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



Adeno virus



Adenovirus



## 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 [ 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 VACUOLATING -40]

- causes Progressive multifocal leukoencephalopathy in Rhesus monkeys
- cytopathic effect → cytoplasmic vacuolation

### merkel cell carcinoma virus

### KI virus [karolinska institute]

### WU virus [washington university virus]



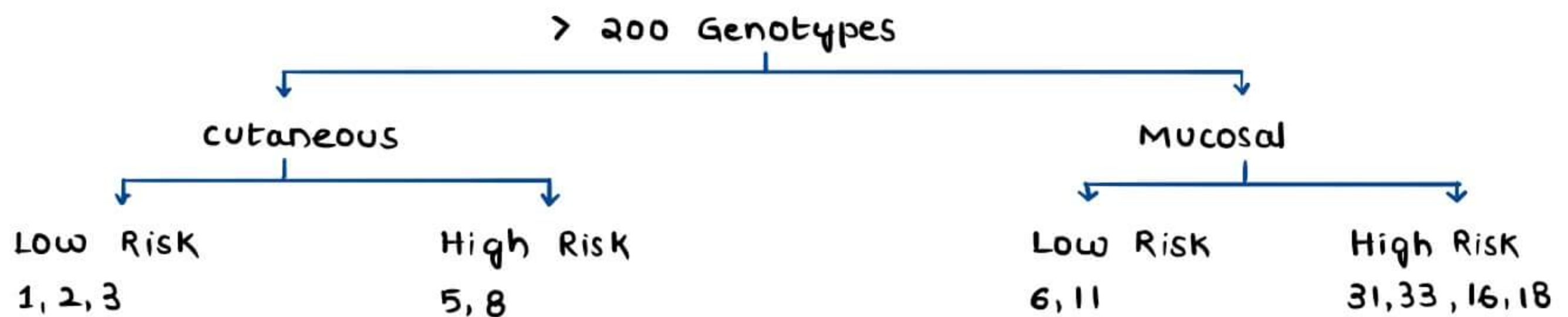
## GENERAL PROPERTIES OF PAPILLOMAVIRIDAE

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 } caused by HPV 6, 11
- laryngeal warts }

### 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 Enterovirus
- ④ ECHO [Enterocytotropic Human Orphan virus] → Aseptic meningitis
- ⑤ Rhinovirus → 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]
    - D antigen [Dense antigen]
- } Type specific

### COXSACKIE VIRUS

#### COXSACKIE VIRUS A

- **causes**
  - Flaccid Paralysis in mice
  - Herpangina [Acute vesicular pharyngitis]
  - Acute haemorrhagic conjunctivitis [Cox A24 + 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<sub>4</sub>]  
 HFMD  
 Aseptic meningitis

→ inoculation into mice cause Spastic paralysis

→ **CULTURE** on Newborn suckling mice [<48hrs]  
 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 [Togaviridae family, Arbovirus]  
 RSV  
 metapneumo virus  
 Nipah virus [Henipa virus]

**ORTHOMYXOVIRIDAE****INFLUENZA A, B, C**

→ SS RNA  
 → negative sense  
     ↓ RNA Polymerase  
 +ive sense

→ have segmented genome

A → 8 Segments  
 B → 8 Segments  
 C → 7 Segments

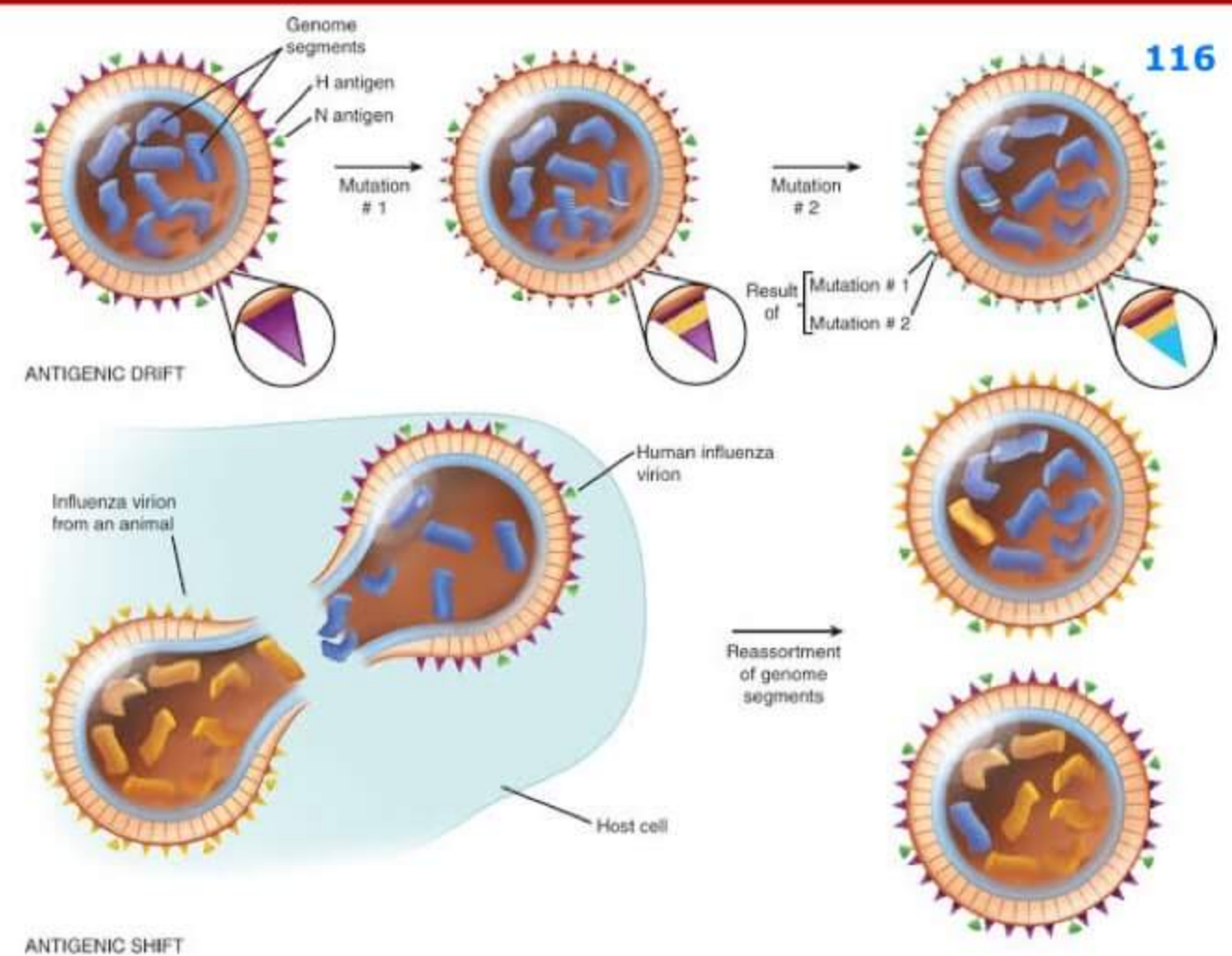
→ Glycoprotein H [Hemagglutinin] → adheres to cell  
 Glycoprotein N [neuraminidase] → Receptor Destroying Enzyme  
 - process is known as  
 ELUTION

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



## ANTIGENIC SHIFT

- abrupt, drastic & discontinuous process
- dit Reassortments
- causes Pandemic
- $H_3N_2 \rightarrow H_1N_1$



## ANTIGENIC DRIFT

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

## H5N1 [BIRD FLU]

- infected birds transmit to humans
- human to human transmission not possible
- PBIF<sub>2</sub> gene → targets the mitochondria of cell & damages it

## H<sub>1</sub>N<sub>1</sub>

- combination of Human influenza strain, Swine strain, avian flu strain

### LAB DIAGNOSIS

#### 1 NASOPHARYNGEAL SWAB

- taken w proper precautions
- Extract RNA [w 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

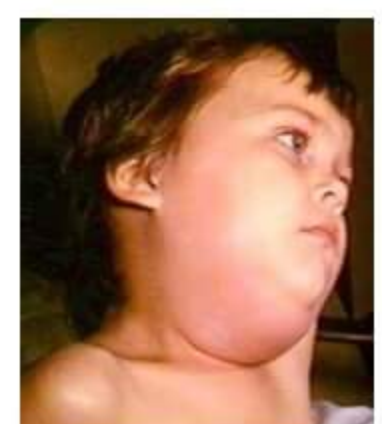
- Parainfluenza type 1 & 2 causes CROUP [Acute laryngotracheobronchitis]
- Parainfluenza type 3 causes LRTI [Lower respiratory tract infection]

## MUMPS

- mc presentation of mumps → B/L Parotitis

### → COMPLICATIONS

- oIL 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 precedes 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) complicat<sup>n</sup> → SSPE [sub sclerosing 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 Dx → 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]

- LAB DIAGNOSIS → PCR/ ELISA for measles virus



Congenital Rubella cataract



**RSV Respiratory Syncytial 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**

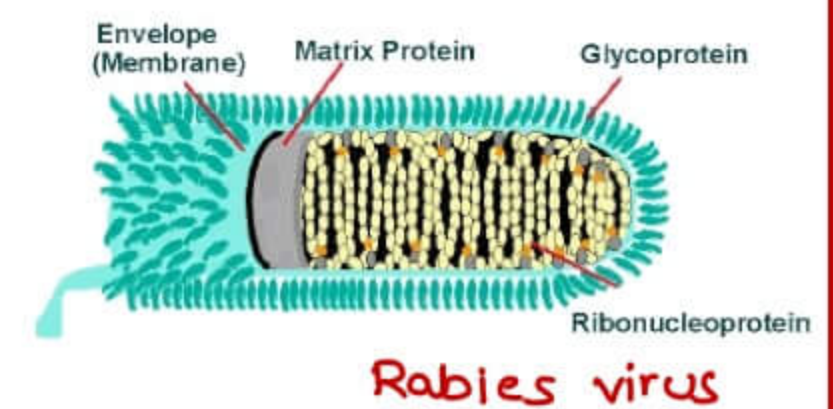
- Age group → 6 months to 12 months
- causes Acute Bronchiolitis
- DOC → RIBAVIRIN
- LAB DIAGNOSIS → PCR for Metapneumo virus

**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
- SS RNA virus  
Negative sense  $\xrightarrow[\text{Polymerase}]{\text{RNA}}$  Positive sense
- Speed of Rabies virus → 3mm/hr
- ↓ Ach at various sites & induce neural cell apoptosis



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

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

## ANTE MORTEM DIAGNOSIS

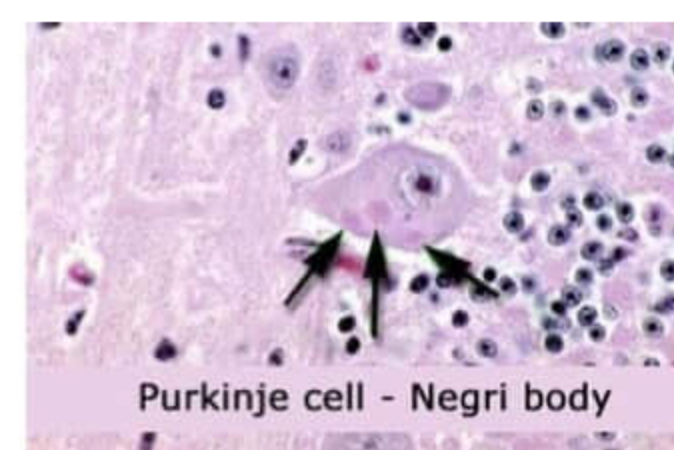
- done in corneal smear  
Nape of neck hair follicle
- Rabies antigen demonstration by DFAT  
DFAT → Direct fluorescent Antigen testing
- by Animal inoculation  
→ Now a days PCR is done

## 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]



HEP A	Hep B	Hep C	Hep D	Hep E
Picornaviridae	Hepadnaviridae only DNA virus	Flaviviridae	Dependent over B	Caliciviridae
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%		

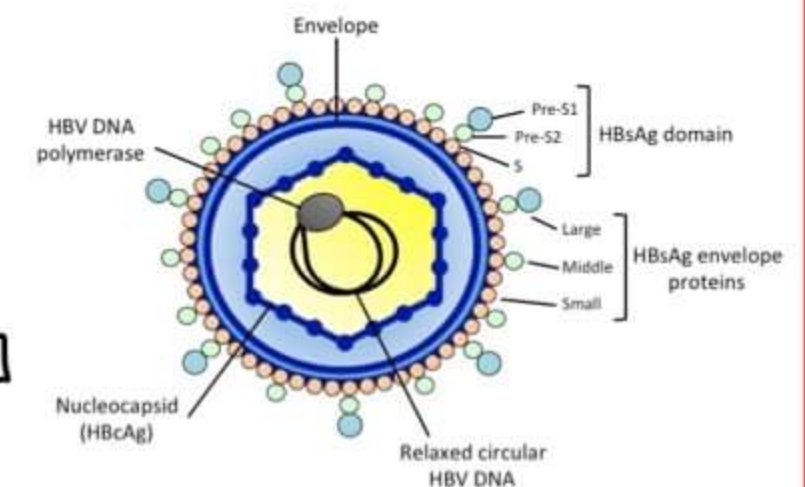
**HEPATITIS A**

- only hepatitis virus that can be cultured → has good vaccine
- LAB DIAGNOSIS → ELISA
  - IgM anti HAV demonstration
    - stool examination
    - serum examination [more significant]

**HEPATITIS B**

## → FORMS

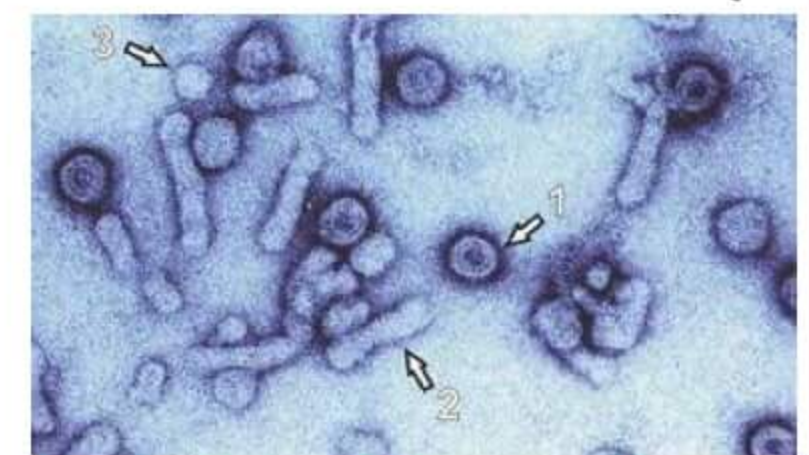
- Spherical → most common
- Tubular
- Dane particle → complete virus genome [42 nm]



## → ACUTE HEP B

- HBs Ag + IgM anti HBc → markers for Acute Hepatitis B
- IgM anti HBc [c-core Ag] → better marker
- HBe Ag +ve → Active Replicating phase  
maximum liver damage occurs
- Anti HBe +ve → Active replicative phase → ↓ing

- CHRONIC HEP B → > 6 months HBs Ag +ve
- 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 → ⓑ<sup>D</sup> [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



# < MICROBIOLOGY

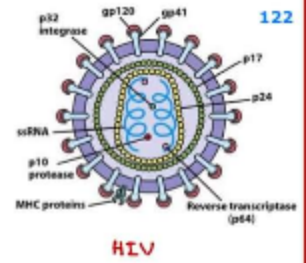
## HIV

→ Family → Retroviridae  
 → Sub Family → Lenti virinae

→ Prevalence in India

→ HIV 1 >>> HIV 2

→ in HIV 1 → group 'M' → subtype 'C'



### → STRUCTURAL GENES OF HIV

gag → codes for capsid, matrix protein, p24, p17, 18  
 env → codes for Gp120 / Gp41  
 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



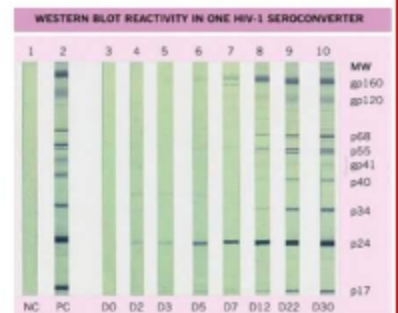
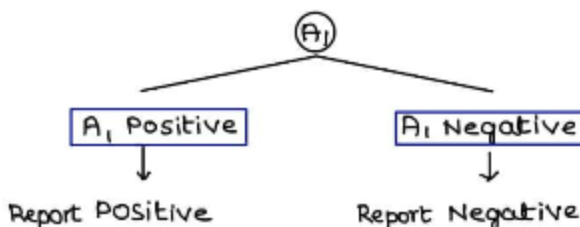
## NACO STRATEGIES

- 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

Imarsmf@hi2.in  
 +14386004539

### STRATEGY I / ALGORITHM 1

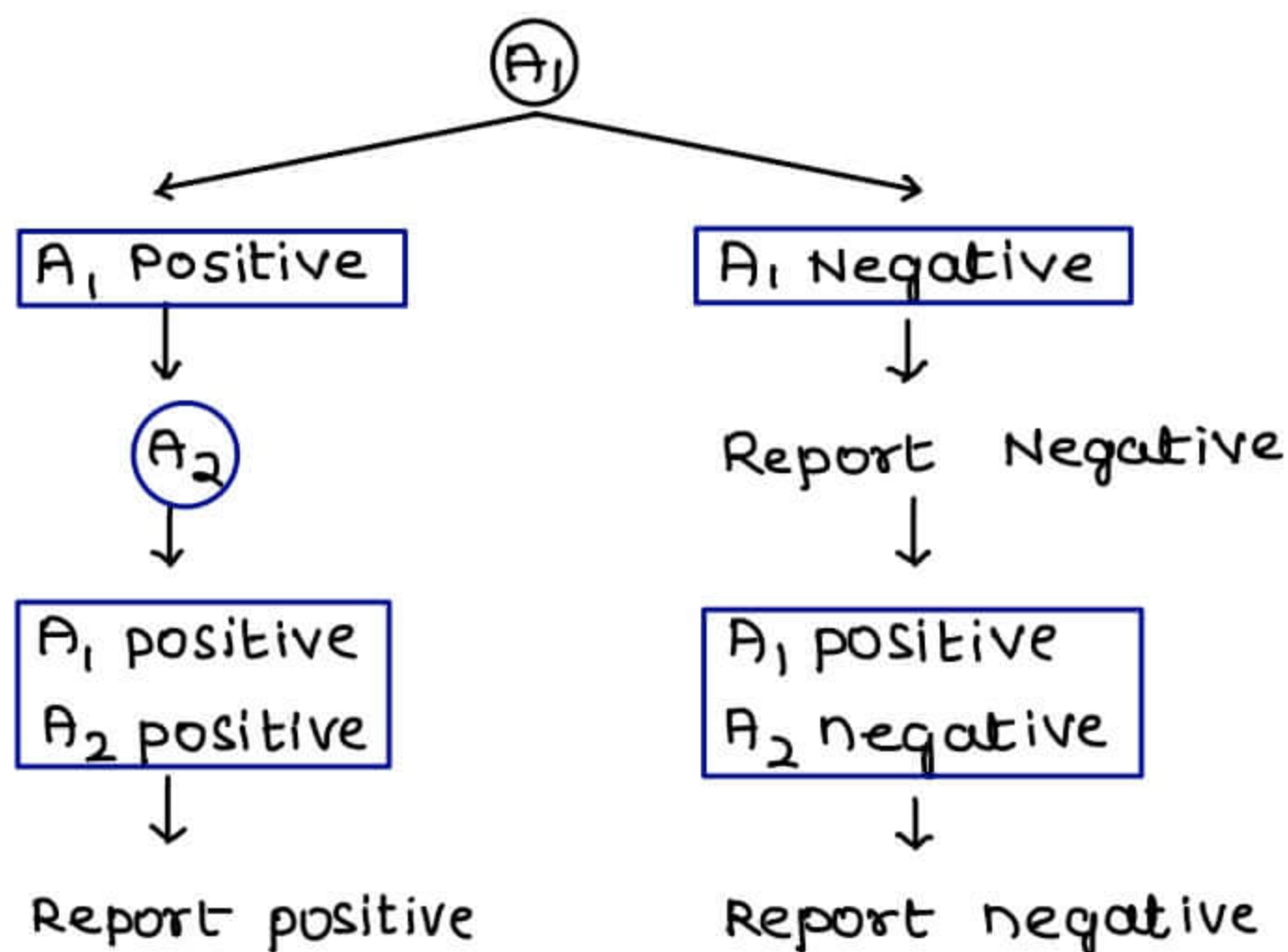
- done for transfusion & transplantation safety
- one test kit is required





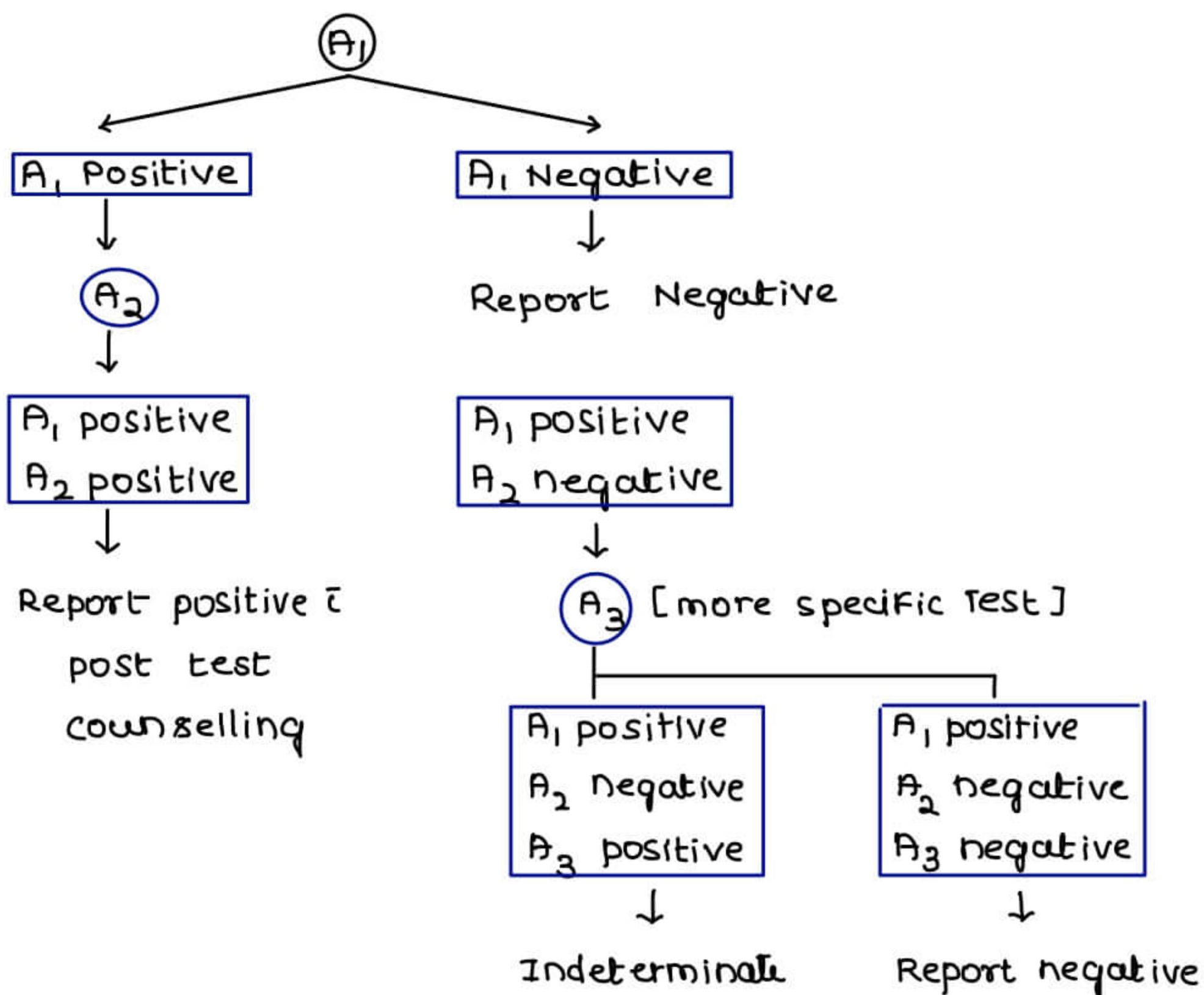
STRATEGY / ALGORITHM II

- done for surveillance by PSM department
- 2 test kits are required



STRATEGY / ALGORITHM IIB

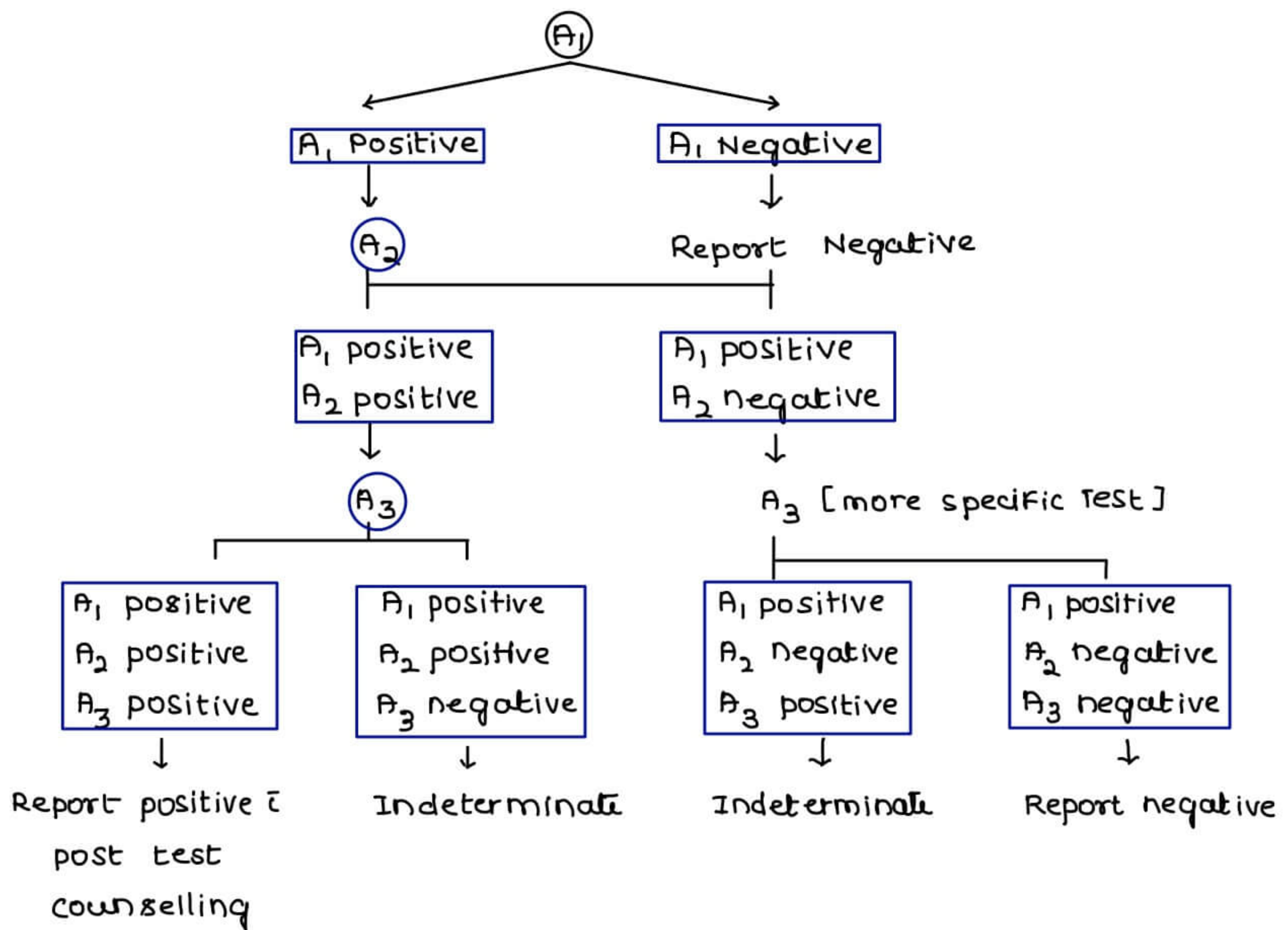
- done in symptomatic individuals
- 3 test kits required





## STRATEGY / ALGORITHM 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 replicat<sup>n</sup> 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<sub>4</sub> 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-contiguous 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 <sub>4</sub> T cell count	Opportunistic infections
400 - 200	Mycobacterium TB Candida (Oral Thrush) Herpes zoster Kaposi sarcoma Hairy leukoplakia Herpes Simplex
Below 200	P → Pneumocystis carinii C → Cryptococcus neoformans Coccidioidomycosis Cryptosporidiosis T → Toxoplasma gondii
Below 50	MAC [Mycobacterium Avium Intracellulare] CMV retinitis CNS lymphoma

### ARBO VIRUSES

FAMILY	GENUS	Imp. SPECIES
TOGAVIRIDAE	ALPHA VIRIDAE	Chikungunya Eastern Equine Encephalitis virus Western 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 Congo Hemorrhagic virus Gangnam virus
	HANTA	Hantaan, Seoul, Puumala
REOVIRIDAE	ORBIVIRUS	Colorado Tick fever
RHABDO VIRIDAE	VESICULO VIRUS	Chandipura virus Vesicular stomatitis virus



- Hantaan → causes Hantaan Renal syndrome, Hantaan pulmonary syndrome<sup>126</sup>  
→ a/w Rodent faeces

### INCUBATION PERIODS

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

### JAPANESE ENCEPHALITIS

- Reservoir → Ardeid birds [white cranes]  
Transmitters → Culex tritaeniorhynchus  
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



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  
→ TOURNIQUET TEST POSITIVE → > 20 petechial lesions / Sq. inch seen  
→ Thrombocytopenia → < 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
- transmitted 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 [ $<48$ hrs]



## FILOVIRIDAE

### IMPORTANT SPECIES

- MARBURG  
EBOLA VIRUS
- Filamentous in Shape
- MOT → contact & 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 chorioid meningitis virus
  - Lassa virus
  - Junin virus
  - Machupo virus

## REOVIRIDAE

- DS RNA virus
- Important viruses
  - ortho reo virus
  - orbi virus
  - Rota virus
  - colti virus

## ROTA VIRUS

- On Electron Microscopy → Wheel with spoke appearance
- DS RNA virus
- Segmented genome +nt
- 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 Q/W GASTROENTERITIS

- Enteric Adenovirus Type 40, 41 → infantile diarrhoea
- Norwalk virus
- calci virus
- Astrovirus



## 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 → PrP<sup>Sc</sup>]

→ HUMAN DISEASES

K → Kuru

F → Familial Fatal insomnia

C → Creutzfeldt Jacob disease

gaye → Gerstman Straussler Schienker syndrome



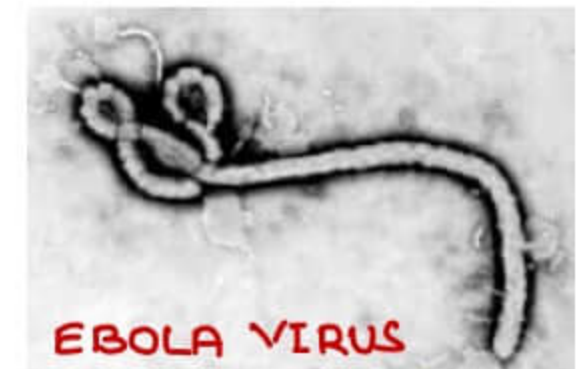
## ANIMAL DISEASES

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

## MUST KNOW

### EBOLA VIRUS

- discovered in 1976
- Family → Filoviridae
- 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]



### 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 with renal syndrome [Hanta virus]
- Crimean - Congo haemorrhagic fever virus from genus Nairovirus, reported from Gujarat
- Gariissa virus and Ilgha virus from Orthobunya virus
- Rift valley fever virus from genus Phlebovirus

#### ③ Family FILOVIRIDAE

- Ebola virus
- Marburg virus

#### ④ Family FLAVIVIRIDAE

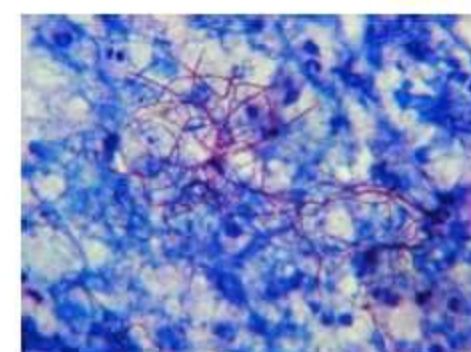
- benue & yellow fever and
- Tick borne encephalitis group → Omsk Hemorrhagic fever virus  
Kyasanur forest Disease Virus

#### ⑤ Family RHABDOVIRIDAE

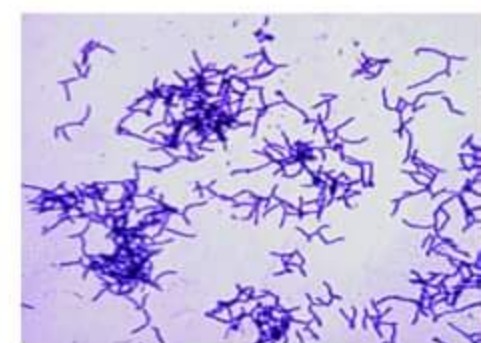


**NOCARDIA**

- 1%. Acid Fast
- associates w̄ actinomycetoma
- Pulmonary involvement is +nt mainly
- cultured by Paraffin bait Technique

**ACTINOMYCETES**

- Gram positive bacilli w̄ '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]
- w̄ interact<sup>n</sup> of Pentameric B subunit of toxin
- w̄ GM<sub>1</sub> ganglioside receptor on enterocyte

↓  
Endocytosis of toxin

- A/B cholera toxin undergo cleavage of A<sub>1</sub> domain from A<sub>2</sub> domain & A<sub>1</sub> becomes active enzyme

- In the cytoplasm, active A<sub>1</sub> activates the G protein G<sub>sα</sub> through an ADP ribosylation reaction that locks G protein in its GTP-bound form & continuously 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<sub>1</sub> OF CHOLERA

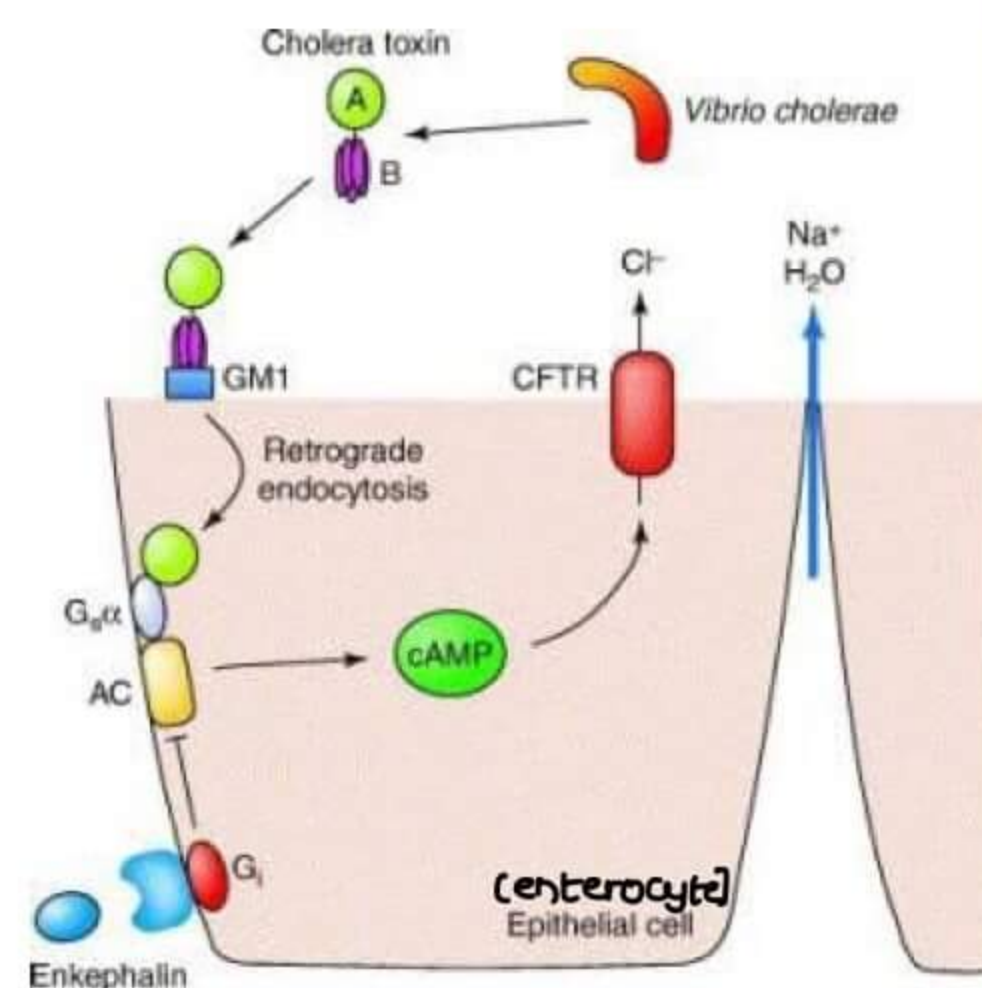
→ by direct action on enterocytes

→ MOA

binds to Opioid receptors on enterocytes



inhibit stimulat<sup>n</sup> of cAMP through G proteins





## GAMMA DELTA T CELLS [ $\gamma\delta$ T cells]

- have distinctive T cell Receptor
  - most T cells composed of  $\alpha$  &  $\beta$  glycoprotein chains
  - $\gamma\delta$  T cells composed of one  $\gamma$  & one  $\delta$  glycoprotein chains
- Less common than  $\alpha\beta$  T cells  
But highest in the gut mucosa,  $\tau$  in INTRA EPITHELIAL LYMPHOCYTES
- do not require antigen processing & MHC presentat<sup>n</sup> of epitopes

## GAS PLASMA STERILIZATION

- **GAS PLASMA**
  - Fourth state of matter [after solid, liquid, gas]
  - generated in an enclosed chamber under deep vacuum 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 vacuum

## CORONA VIRUS

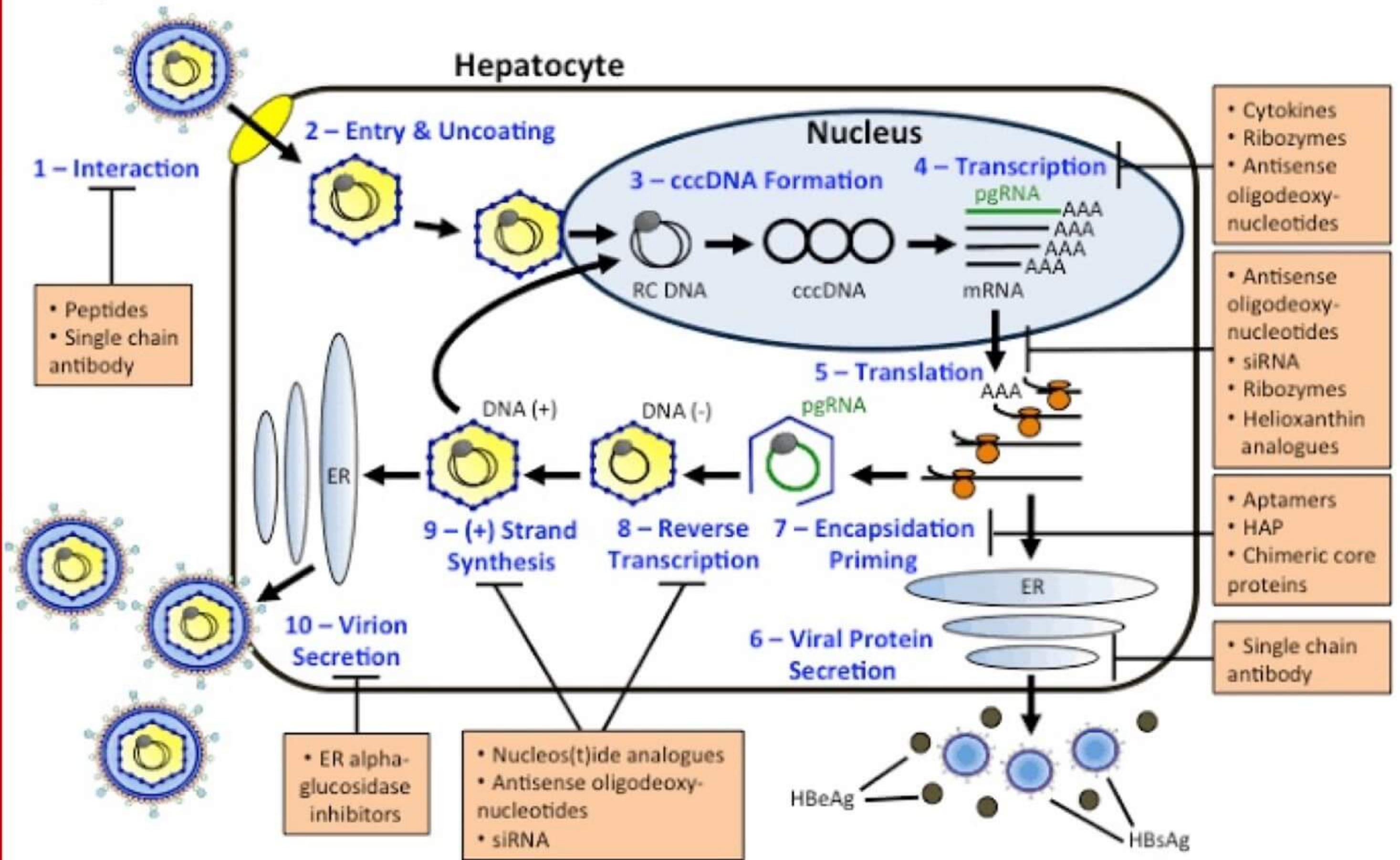
- Family → corona viridae  
Subfamily → corona virinal
- causes **SARS** [Severe Acute Respiratory Syndrome]
  - causes both upper & lower respiratory tract infections
  - presents  $\tau$  muscle pain, headache & fever followed in 2-14 days by onset of respiratory symptoms
- 6th new type identified in 2012
  - Initial name → NOVEL CORONA VIRUS
  - New name → MIDDLE EAST RESPIRATORY SYNDROME CORONA VIRUS [MERS-CoV]

## CRYOGLOBULINEMIA

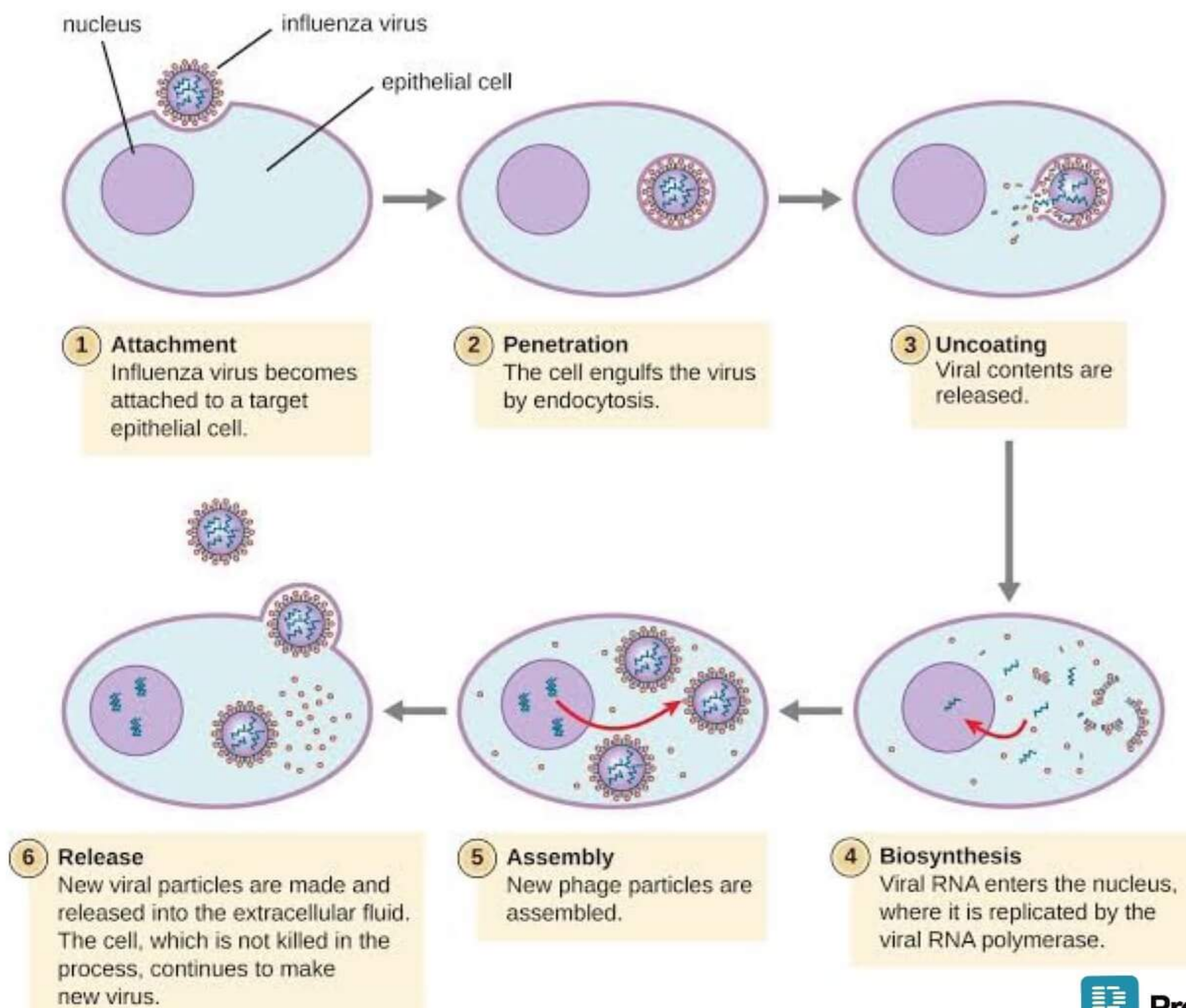
- characterized by clonal expansion of rheumatoid factor expressing B cells in liver, LN & peripheral blood, resulting in presence of cryoglobulins in circulat<sup>n</sup>.
- **CRYOGLOBULINS**
  - cold insoluble immune complexes  
contains Rheumatoid factor, polyclonal Ig G, HCV RNA
  - they precipitate & deposit on vascular endothelium, causing VASCULITIS in skin, kidneys & peripheral nerves



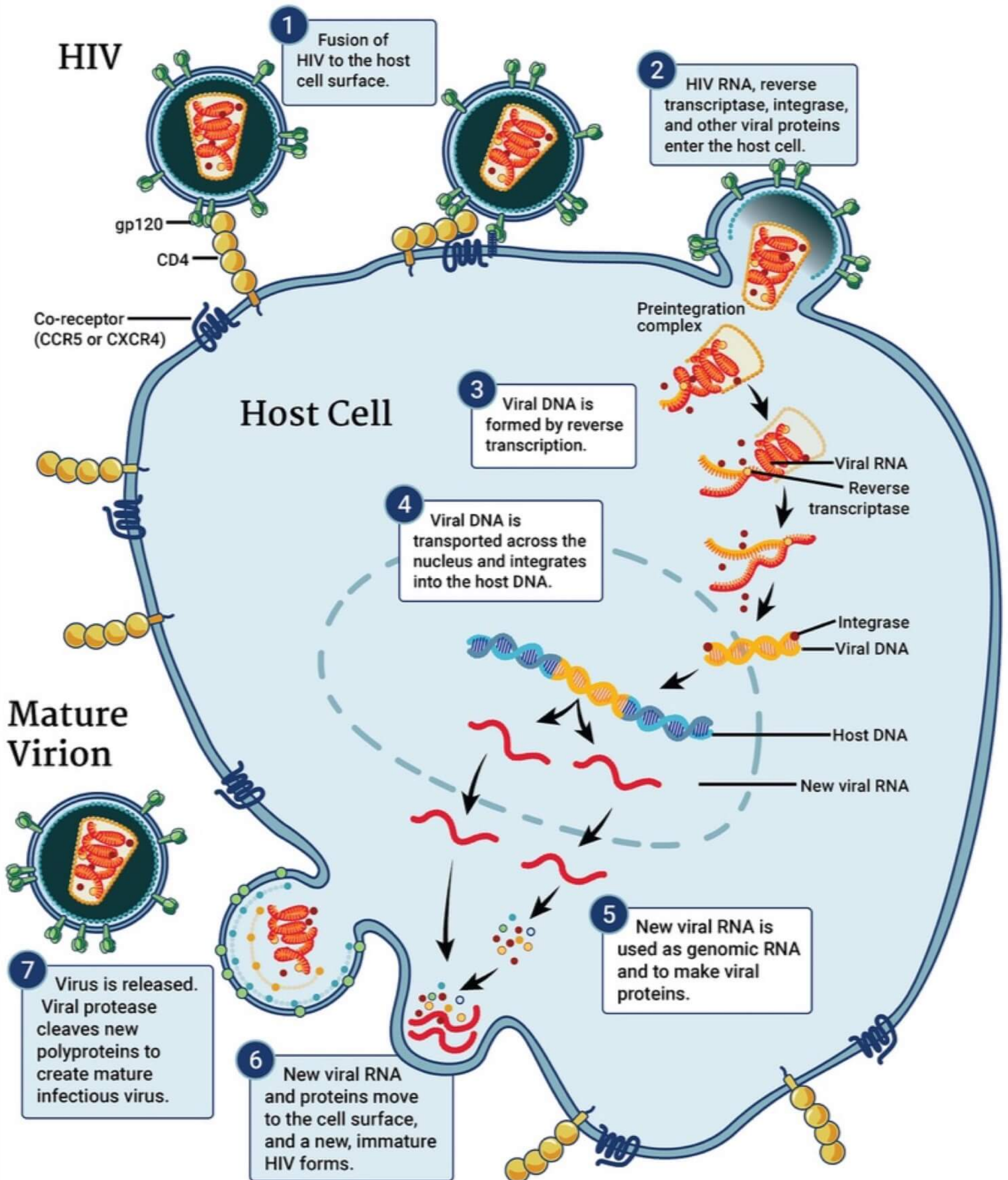
Hepatitis B Virion



INFLUENZA VIRUS PATHOGENESIS







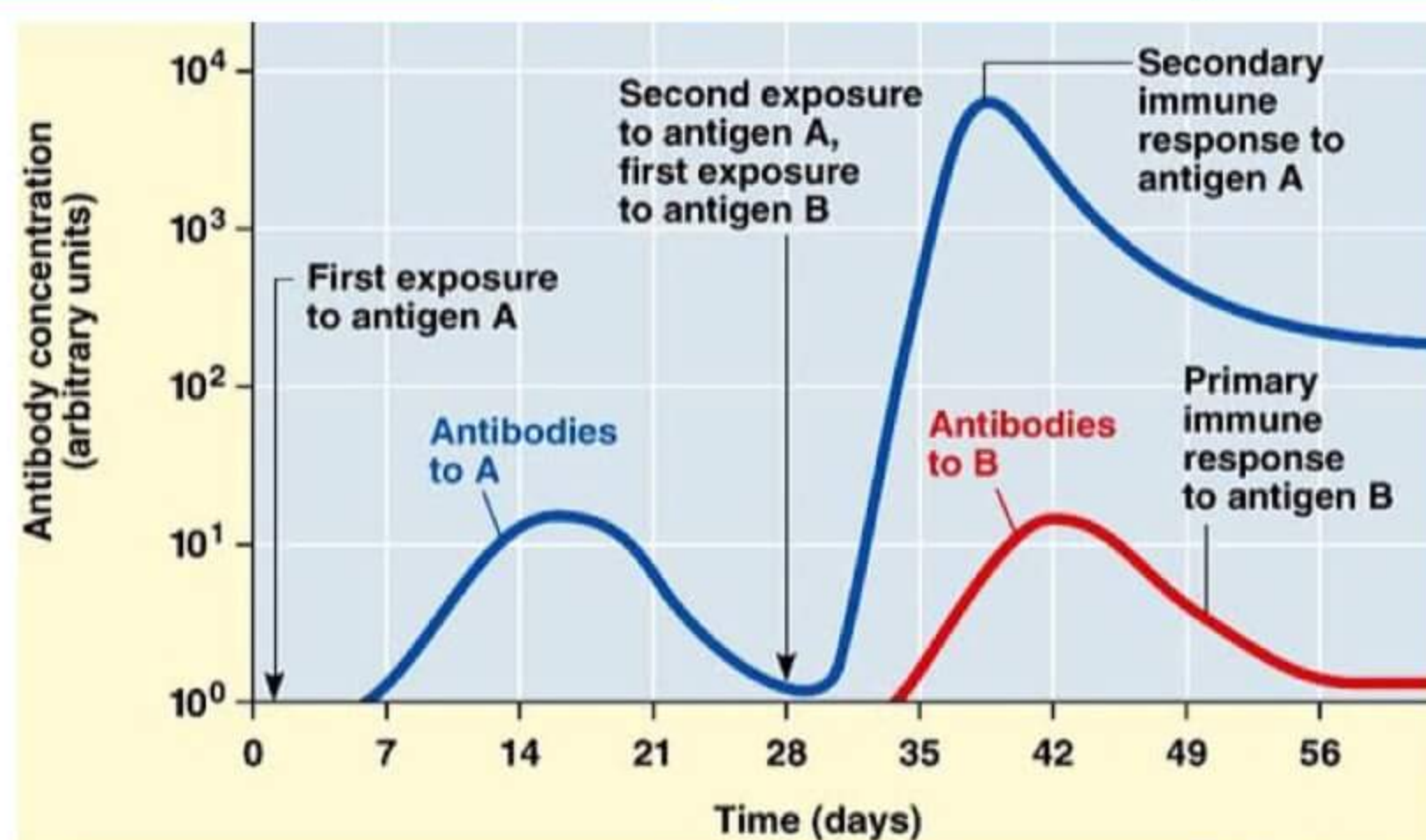


IMMUNITY → Resistance offered by the host against offenders

INNATE IMMUNITY	ACQUIRED IMMUNITY
Resistance to the infection from birth by its genetic or constitutional make up	Resistance to infection acquires during life time
<p><u>Depends on</u></p> <p><b>S</b> Species specific  <b>I</b> Individual specific  <b>R</b> Race specific</p>	<p><u>Different forms</u></p> <p>Natural Active → infections                      Natural Passive → mother to child                      Artificial Active → vaccines                      Artificial Passive → Immunoglobulins</p>
<p><u>COMPONENTS</u></p> <p>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 &amp; mucosal barrier</p>	<p><u>COMPONENTS</u></p> <p>B cell                      T cell                      classical complement pathway                      Antigen Presenting cells [APC]</p>
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 due change in the constant portion of heavy chain





**ANTIGEN**

- Any foreign 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 Foreignness ∝ immunogenicity  
∝ Hypersensitivity

**NATURE OF SUBSTANCE**

Proteins > carbohydrates > Lipids > Nucleic Acids

**SUPER ANTIGENS**

- non MHC restricted
- attached to TCR on lateral side on  $V\beta$  (variable  $\beta$ ) domain
  - ↓
  - induces > 20% T lymphocytes activation
  - ↓
  - Leads to massive lymphokine release
  - ↓
  - SHOCK

→ **Examples**① **STAPHYLOCOCCAL TOXINS**

- |                      |   |   |
|----------------------|---|---|
| Enterotoxin A        | → | Food Poisoning                              |
| Exfoliate Toxin A, B | → | SSSS [Staphylococcal skin scalded syndrome] |
| Enterotoxin F        | → | Toxic shock syndrome toxin                  |

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

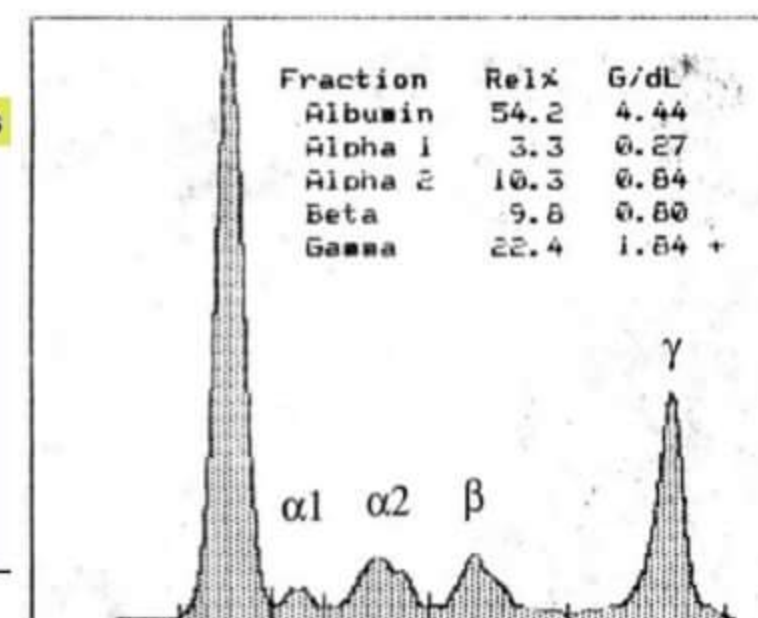
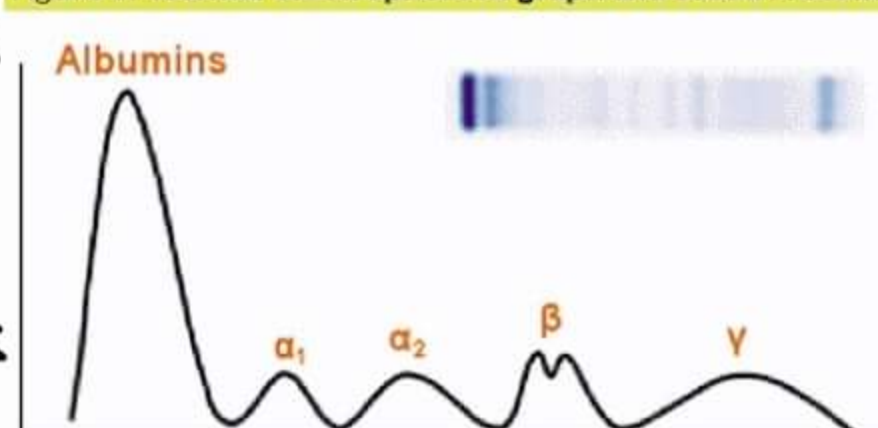


T INDEPENDENT ANTIGEN	T DEPENDENT ANTIGEN
→ Simple → LPS, capsule, flagella	→ Complex
→ Dose dependent	→ Immunogenic over wide range of dose
→ IgM & IgG3	→ All Ab rise
→ NO memory	→ memory + nt
→ NO macrophage processing	→ macrophage processing needed
→ Rapidly metabolized	→ rapidly metabolized
→ Directly acts on B cells & causes polyclonal B cell activation	

## ANTIBODIES

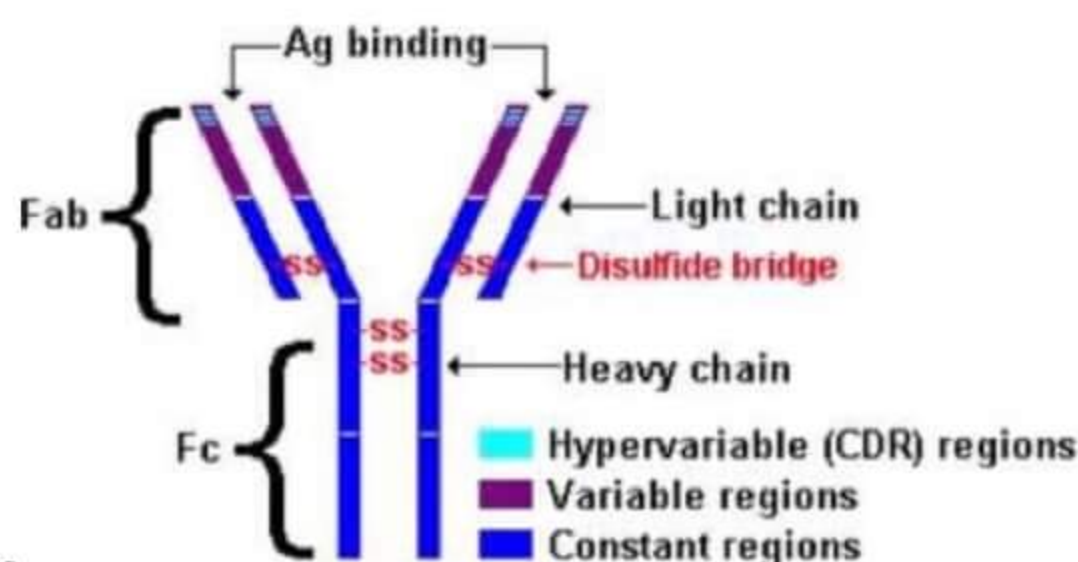
- Antibodies are immunoglobulins [γ globulins]
- KABAT & TISELIUS found them by doing electrophoresis

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

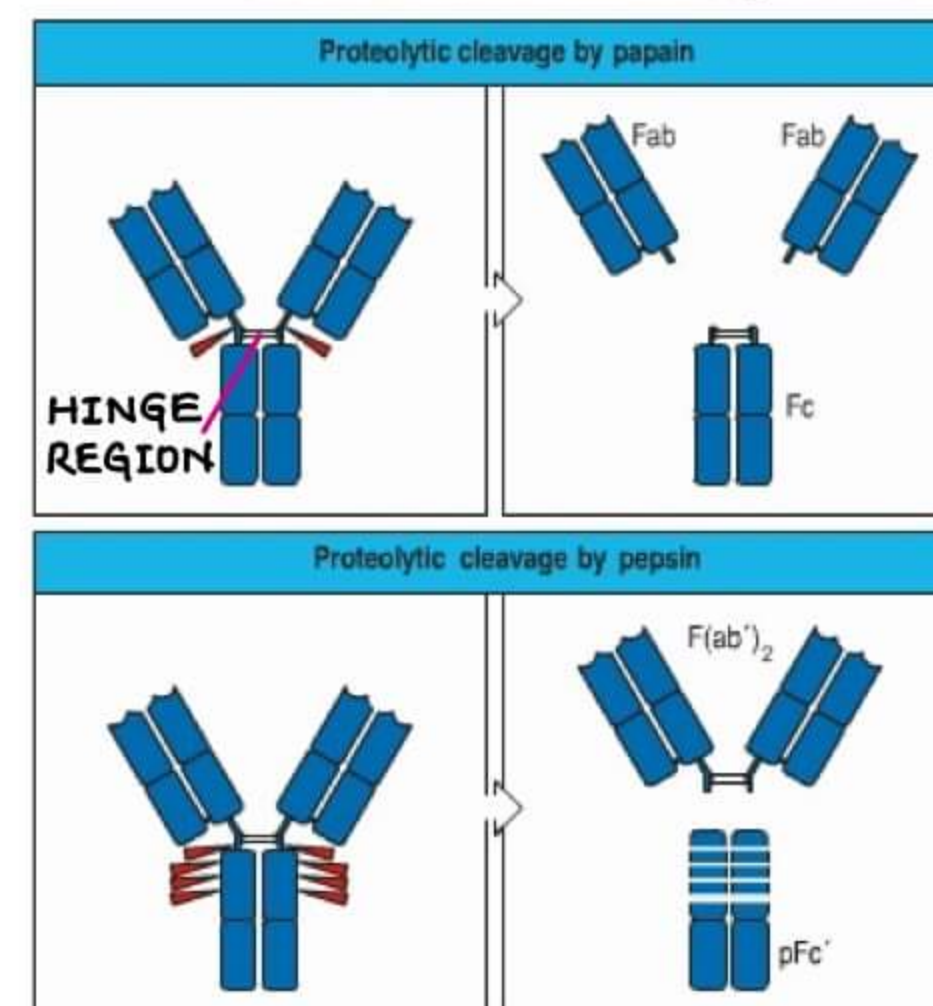


Gerald M. Edelman Rodney R. Porter

- 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 to each other by Disulfide bond  
 Two heavy chains binds to 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')<sub>2</sub> + Fc'] released



Ig G

- maximum in concentrat<sup>n</sup>
- maximum  $t_{1/2}$  → 23 Days
- Sub groups → Ig G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>  
G<sub>1</sub> → mc
- Fc portion of Ig G involves in Opsonisation [enhancement of phagocytosis]
- Only Ig which pass through placenta  
Except<sup>n</sup> → Ig G<sub>2</sub> doesn't cross placenta
- Ig G<sub>4</sub> → doesn't takes place in complement pathway
- Ig G<sub>3</sub> → doesn't involve in coagglutin<sup>n</sup>
- Role of IgG is more in precipitation [Precipitat<sup>n</sup> > Agglutin<sup>n</sup>]

Ig A

- Ig A<sub>1</sub> → present in serum
- Ig A<sub>2</sub> → present in mucosal surface & body secretions
- Dimer form bounded by joining chains
- mc selective Ig deficiency → IgA

Ig M

- maximum molecular weight
- aka MILLIONAIRE MOLECULE
- has maximum sedimentat<sup>n</sup> co-efficient
- valency →  $2 \times 5 = 10$   
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 concentrat<sup>n</sup>




- shortest  $t_{1/2}$  → < 2 Days
- plays a role in HOMOCYTOTROPISM (movement towards mast cells)  
- occurs in Type I Hypersensitivity
- Only heat labile antibody
- aka REAGINIC ANTIBODY

LIGHT CHAINS

→ Kappa :  $\lambda$  → 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 $\cong$ Ag Ab Interactions

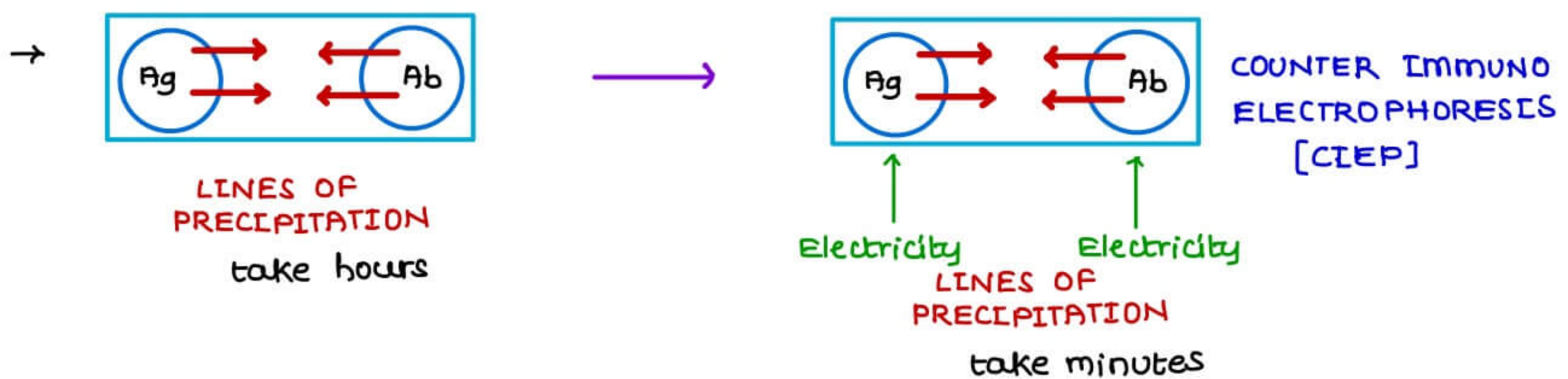
- AFFINITY** → INTENSITY OF ATTRACTION between the antigen & antibody  
**AVIDITY** → STRENGTH OF BOND after the format<sup>n</sup> of antigen & antibody complex

## COMPARITIVE EFFICIENCY OF Iq IN DIFFERENT SEROLOGICAL REACTIONS

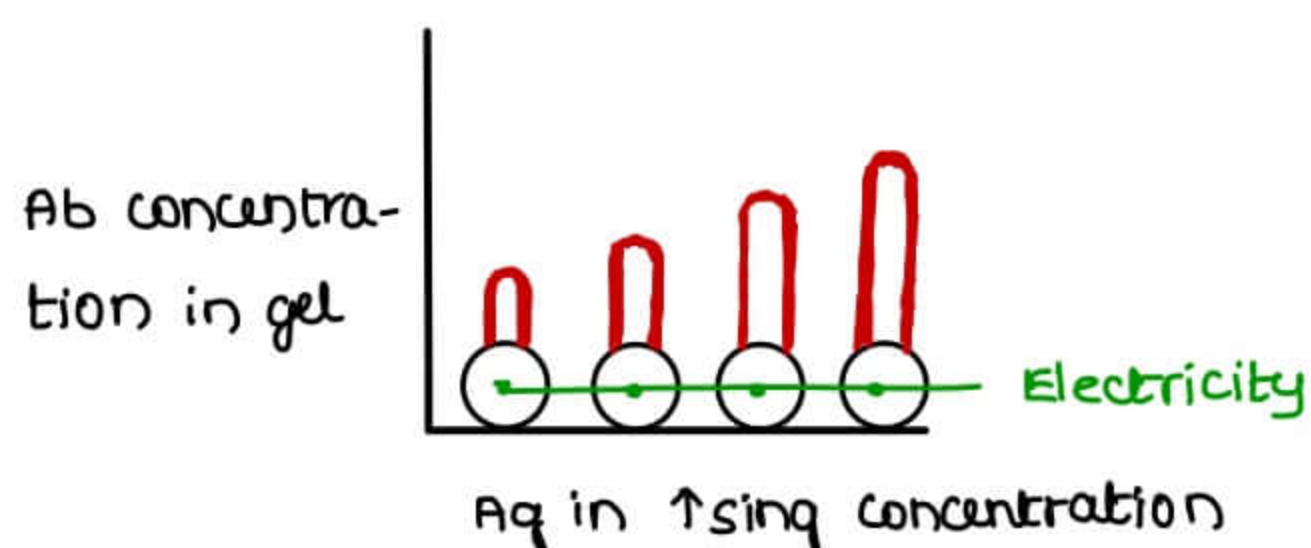
REACTION	IgG	IgM	IgA
Precipitation	Strong	weak	variable
Agglutination	weak	Strong	moderate
complement fixation	Strong	weak	negative
Lysis	Weak	Strong	negative

## PRECIPITATION

- Soluble antigen + Antibody → Insoluble precipitate  
 Eg → ASCOLIS THERMO PPT. TEST → Done for Anthrax antigen  
 → **FLOCCULATION** → modificat<sup>n</sup> of precipitat<sup>n</sup>  
 Eg → VDRL (Best test to assess prognosis)



## → ROCKET ELECTROPHORESIS





## ① SINGLE DIFFUSION IN ONE DIRECTION [OUDIN PROCEDURE]

Antibody incorporated in agar gel in a test tube

↓  
Antigen solut<sup>n</sup> 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 [OAKLEY - 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]

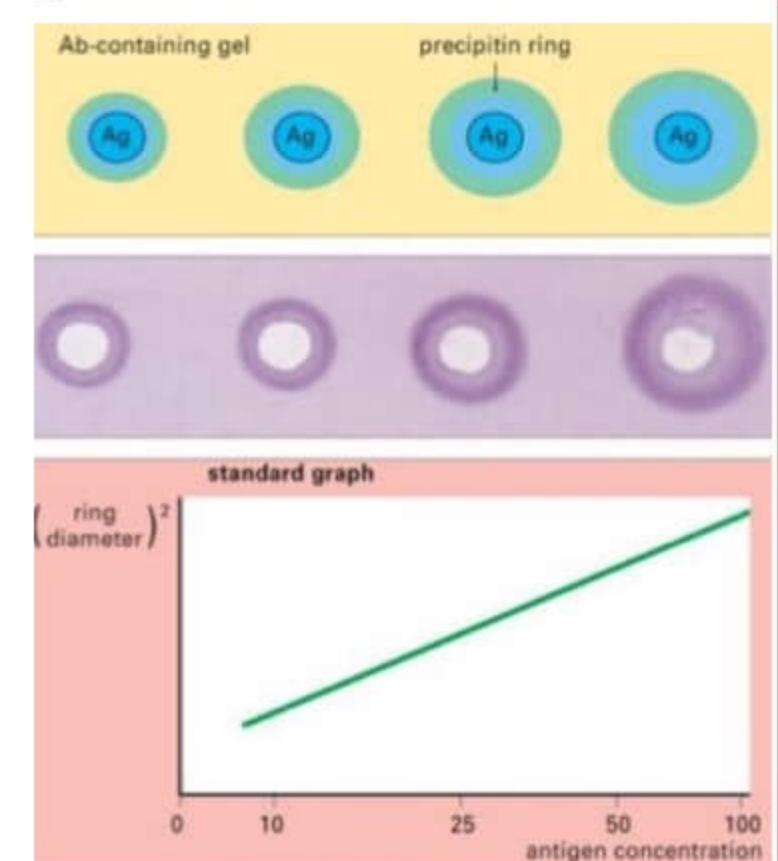
Antiserum incorporated in agar gel poured on a flat surface

↓  
Antigen is added to the wells

↓  
Ag diffuses radially & forms rings shaped bands of precipitat<sup>n</sup> → HALO  
Diameter of halo → concentrat<sup>n</sup> of antigen

## USES

- ① Estimati<sup>n</sup> of Ig in sera
- ② Screening of antibodies to influenza viruses



RADIAL IMMUNODIFFUSION



#### ④ DOUBLE DIFFUSION IN TWO DIMENSIONS [OUCHTERLONY PROCEDURE]

Agar gel poured on a slide



Wells are cut



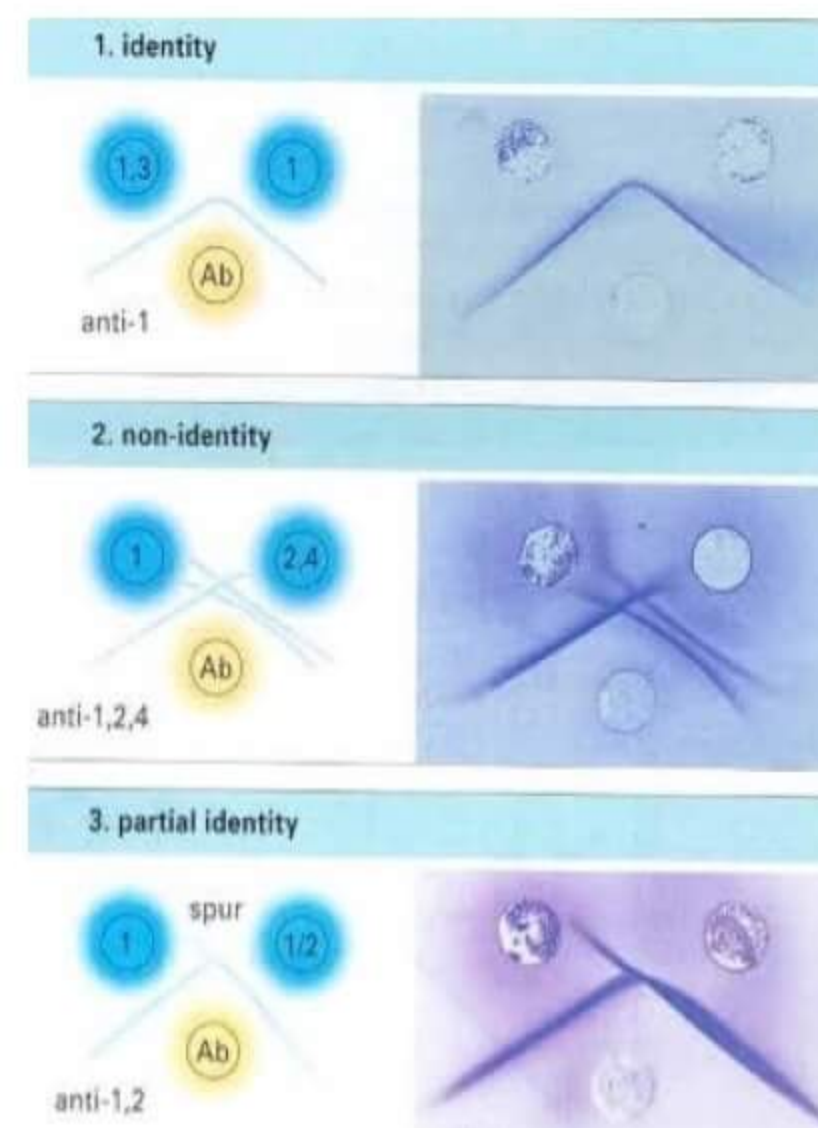
Antiserum placed in the central well  
Antigens in the surrounding wells



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<sup>+</sup> father + Rh<sup>-</sup> mother → Rh<sup>+</sup> child

Do incomplete IgG Antibodies testing

If Free incomplete IgG Antibodies in maternal serum

+

O Rh<sup>+</sup> 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* [ $\alpha$  lecithinase]

## COMPLEMENT FIXATION TEST

① WASSERMAN REACTION → done for Syphilis

② SABIN-FELDMAN DYE TEST → done for Toxoplasmosis

→ Antigen + Antibody + RBC system + Complement

↓

if Ab present

↓

Ag + Ab complex

↓

activate complement  $C_1$

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

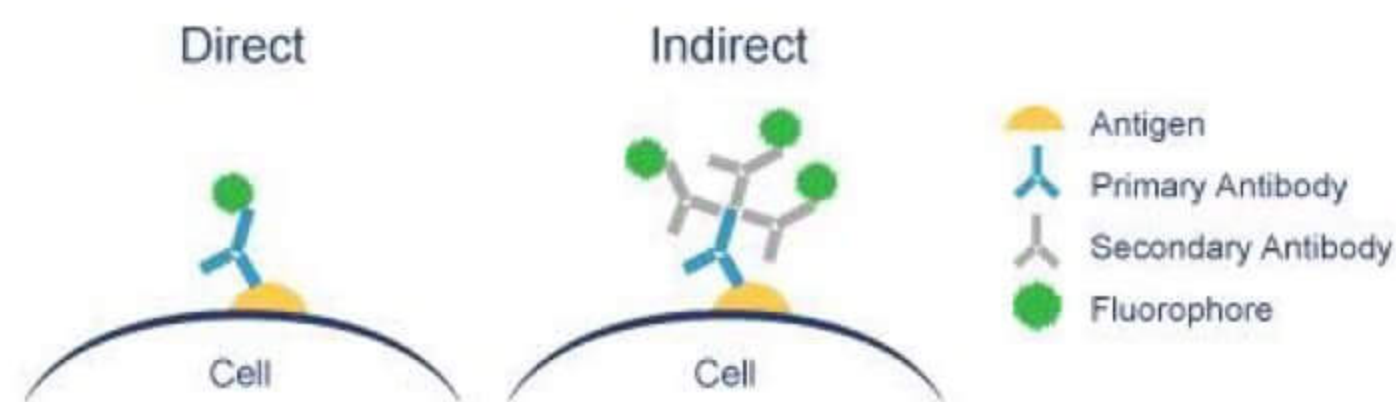
Direct

Indirect



## → Fluorescent dyes

Fluorescein isothiocyanate  
Lissamine rhodamine  
Rhodamine - Auramine etc



## → DIRECT IMMUNOFLUORESCENCE

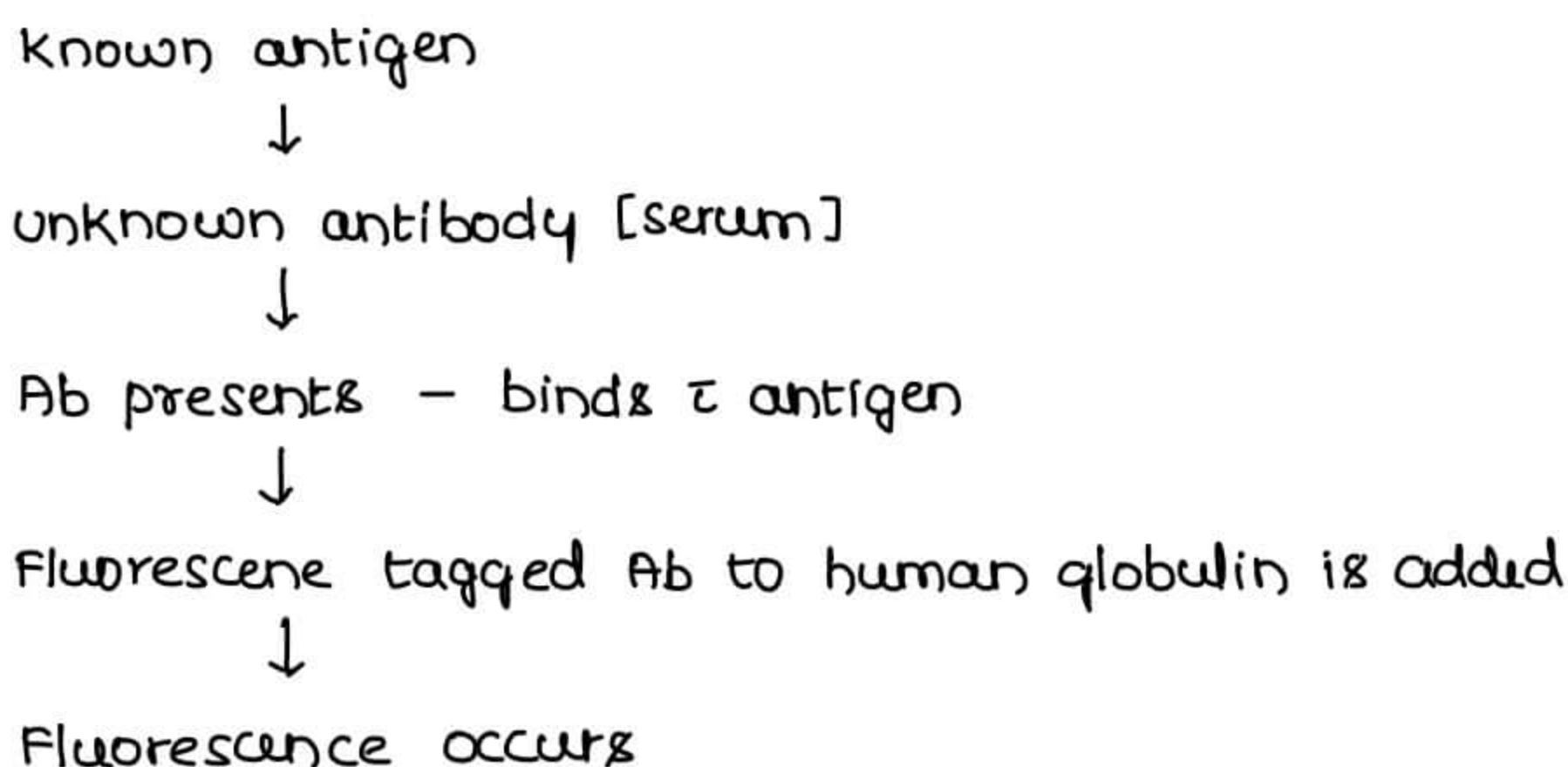
- Detect antigens
- Principle → Antibodies tagged with fluorescent dyes  
↓  
Detect unknown antigen

### - Uses

Detect<sup>n</sup> 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



- Advantage → single antihuman globulin fluorescent Ab to any Ag

## ELISA [ENZYME LINKED IMMUNO SORBENT ASSAY]

### TYPES

- ① Direct ELISA
- ② Indirect ELISA
- ③ Competitive 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 Phosphatase [AP]

→ There is no significant difference b/w test sensitivities

## Ⓐ 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 → Amino 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

→ I Aq + Ab\* E + S → Read  
W W

→ Stage (i) → passive absorpt<sup>n</sup> of antigen to plate by incubat<sup>n</sup> 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 w HIV Aq taken

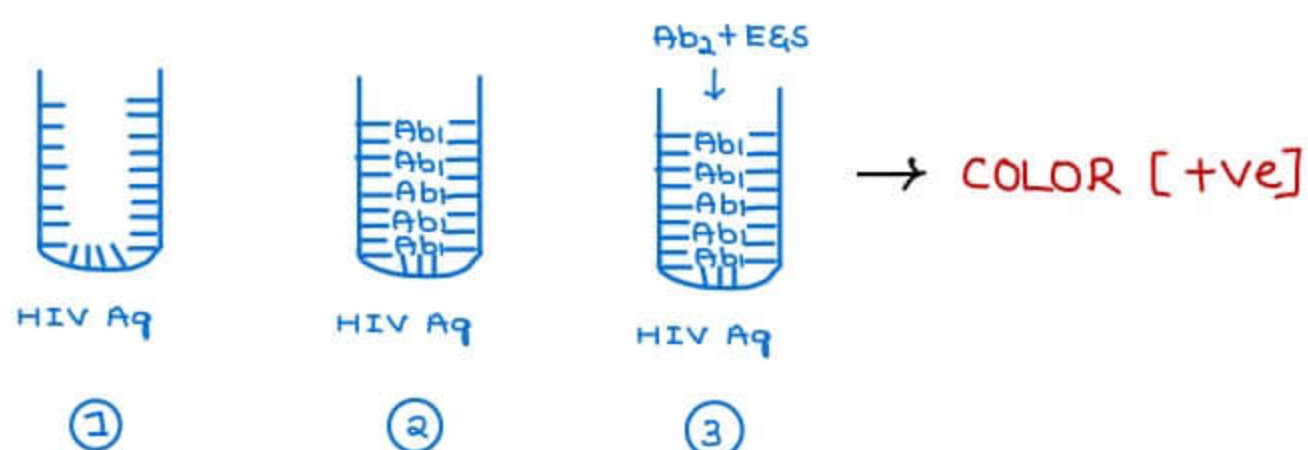
→ Patient serum w Ab<sub>1</sub> added to it

→ Ab<sub>1</sub>  $\xrightarrow{\text{injected to}}$  Goat  $\xrightarrow{\text{produces}}$  Ab<sub>2</sub>

Ab<sub>2</sub> + Enzyme + substrate added

→ IF COLOUR appears → +ive

→ can measure OD values [Optical Density values]

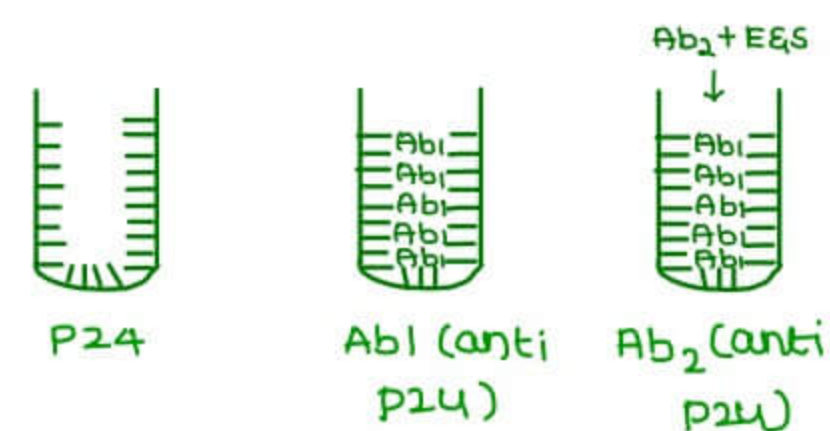




## COMPETITIVE ELISA

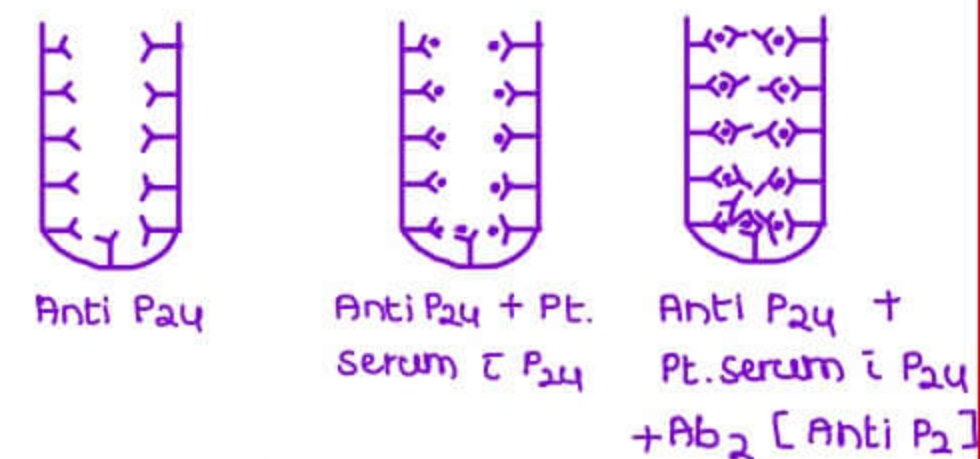
- A well tagged  $\bar{\tau}$  P<sub>24</sub> Ag taken
- Patient serum  $\bar{\tau}$  Ab<sub>1</sub> (anti P<sub>24</sub>) added to it
- P<sub>24</sub>  $\xrightarrow{\text{injected to}}$  Goat  $\xrightarrow{\text{produces}}$  Ab<sub>2</sub> [anti P<sub>24</sub>]

Ab<sub>2</sub> + Enzyme + substrate added  
NO COLOUR → POSITIVE



## SANDWICH ELISA

- A well tagged  $\bar{\tau}$  anti P<sub>24</sub> Ab taken
- Patient serum  $\bar{\tau}$  P<sub>24</sub> added to it
- Ab<sub>2</sub> [anti P<sub>24</sub>] + Enzyme + substrated added
- IF colour appears → +ive
- can measure OD (Optical density  $\bar{\tau}$  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

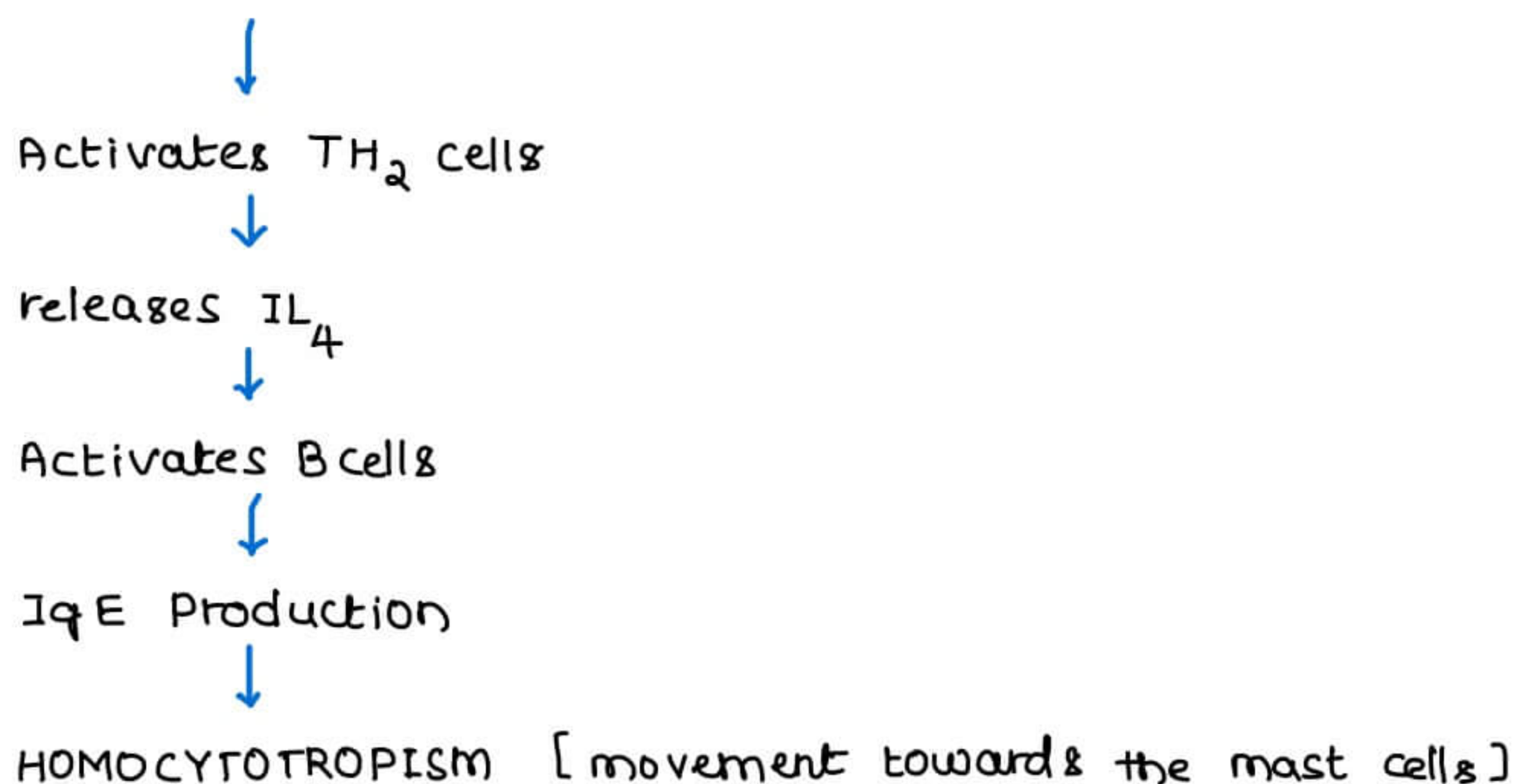
- |     |  |            |
|-----|--|------------|
| → A | → Anaphylactic hypersensitivity            | → TYPE I   |
| → C | → Cytotoxic hypersensitivity               | → TYPE II  |
| → I | → Immune complex mediated hypersensitivity | → TYPE III |
| → D | → Delayed hypersensitivity                 |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

## TYPE I

- Occurs in 2 phases
  - 1 sensitization phase
  - 2 Effector phase

### ① sensitization phase

- Allergen → Processed by APC  $\bar{\tau}$  Peptides of it presented to CD<sub>4</sub> T cells





Shocking Dose



Mast cell Degranulation



PRIMARY MEDIATORS

P → Protease

E → Eosinophil chemotactic factor

N → Neutrophil chemotactic factor

Hai → Histamine } causes vasodilation &  
Serotonin } Bronchoconstriction

SECONDARY MEDIATORS

B → Bradykinins

P → Prostaglandins

L → Leukotrienes

P → PAF [Platelet Activating factor]

C → Cytokines [IL<sub>1</sub>, TNF $\alpha$ ]

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 cytotoxicity
  - complement dependent inflammation
  - complement mediated Phagocytosis
- } OCCURS



- COMPLEMENT MEDIATED CYTOLYSIS → involves MAC (C5-C9)
- COMPLEMENT DEPENDENT INFLAMMATION → involves C3a, C5a (chemo attractants)
- COMPLEMENT MEDIATED PHAGOCYTOSIS → involves C3b/C4b (opsonins)  
Best opsonin → C3b

### 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]

- IgG Ab attach to target cell antigens through Fab region  
Fc portion of IgG in turn 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

Eq. Grave's Disease

- Activation of receptors takes place
- Auto Ab [Long Acting Thyroid stimulators LATS] produced
- Stimulate Thyroid to produce hormones



## ② INHIBITORY AD CD

Eq. 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 localize near the site of antigen administration



Localized Reaction [ARTHUS REACTION]

Localized area of necrosis d/t vasculitis

Aq Excess [monovalent Antigens]



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



Generalised React<sup>n</sup> [SERUM SICKNESS]



occurs following serum therapy [mostly z 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  $\bar{c}$  MHC<sub>2</sub> to TH<sub>1</sub> cells



TH<sub>2</sub> cells differentiate to form T Delayed type Hypersensitivity [T<sub>DTH</sub>] cells

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

CD<sub>8</sub> T cells

CD<sub>4</sub> TH<sub>17</sub> cells

- EFFECTOR PHASE

T<sub>DTH</sub> cells on subsequent contact  $\bar{c}$  antigen



release cytokines



attract & recruit inflammatory cells at the sites

### CYTOKINES

- I → Interferon  $\gamma$  [most important]
  - I → IL<sub>2</sub>
  - M → MCAF [Monocyte chemo Attractant factor]
  - to → TNF $\beta$  [Tumor Necrosis factor  $\beta$ ]
  - Medical → MIF [macrophage Inhibition factor]
  - Institute → IL<sub>3</sub>
  - Gujarat → GM-CSF [Granulocyte monocyte colony stimulating factor]
- 
- IFN  $\gamma$  → activates resting Macrophages
  - IL<sub>2</sub> → acts in autocrine manner & helps in proliferat<sup>n</sup> of T<sub>DTH</sub> cells

### PATHOLOGY

- continuous T<sub>DTH</sub> 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

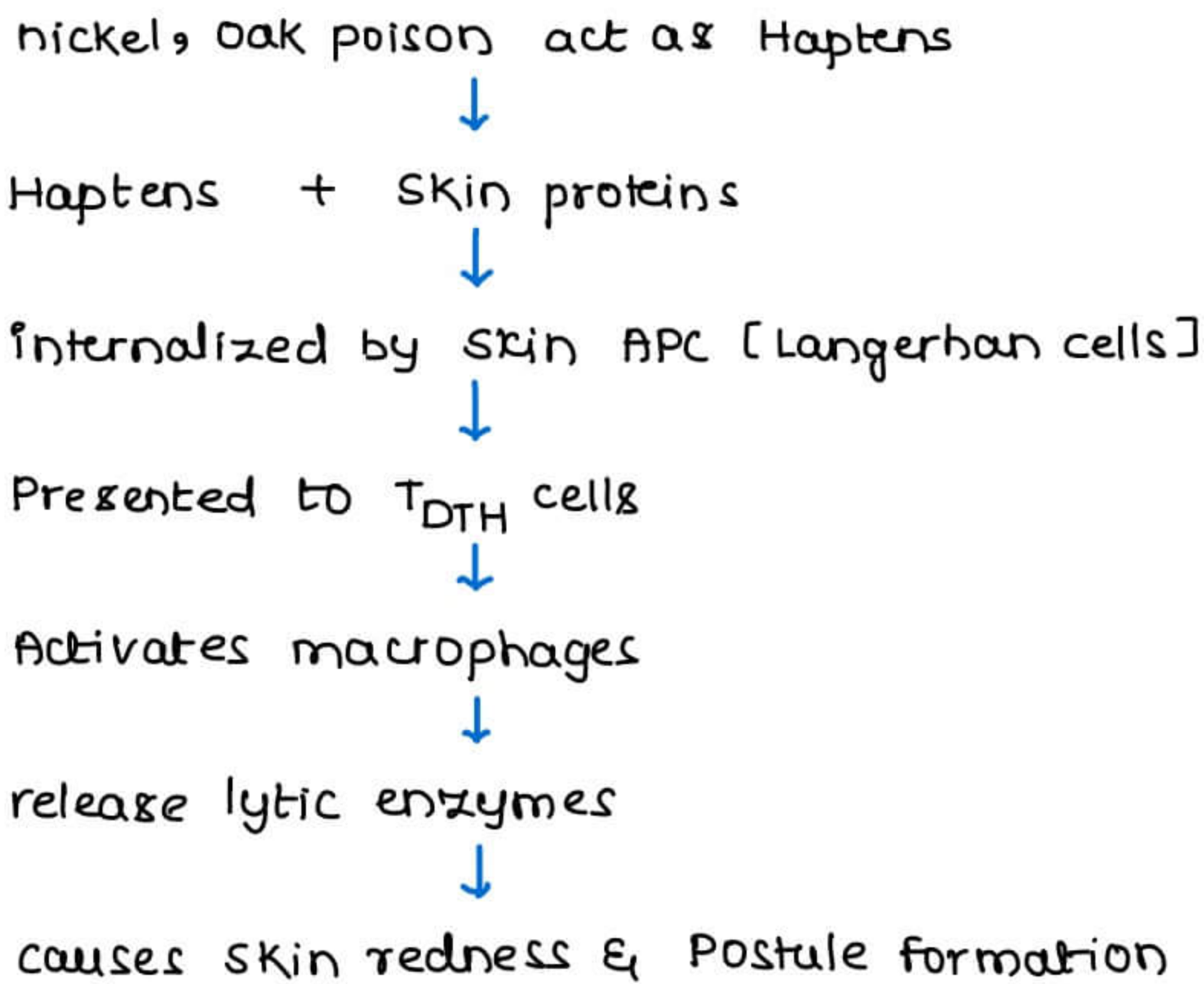
- ① Large, flat; eosinophilic cell [Epitheloid cell]
- ② Epitheloid cells fuse [ $\bar{c}$  the help of IFN $\gamma$ ] to form multinucleated giant cells



## EXAMPLES

TUBERCULIN TEST → delayed type hypersensitivity

CONTACT DERMATITIS



## HYBRIDOMA TECHNOLOGY

→ done for mass production of monoclonal antibodies

→ Principle → B cells fused up w myeloma cells on HAT media  
[Hypoxanthine Aminopterin Thymine media]

- mouse is injected w 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 monoclonal antibodies



## COMPLEMENT SYSTEM

- Non specific proteins
- constitute 5% of serum proteins
- Heat labile ( $56^{\circ}\text{C} \times 30 \text{ min}$ )
- can bind to  $\text{F}_c$  part<sup>n</sup> of Ig

## SITES OF SYNTHESIS

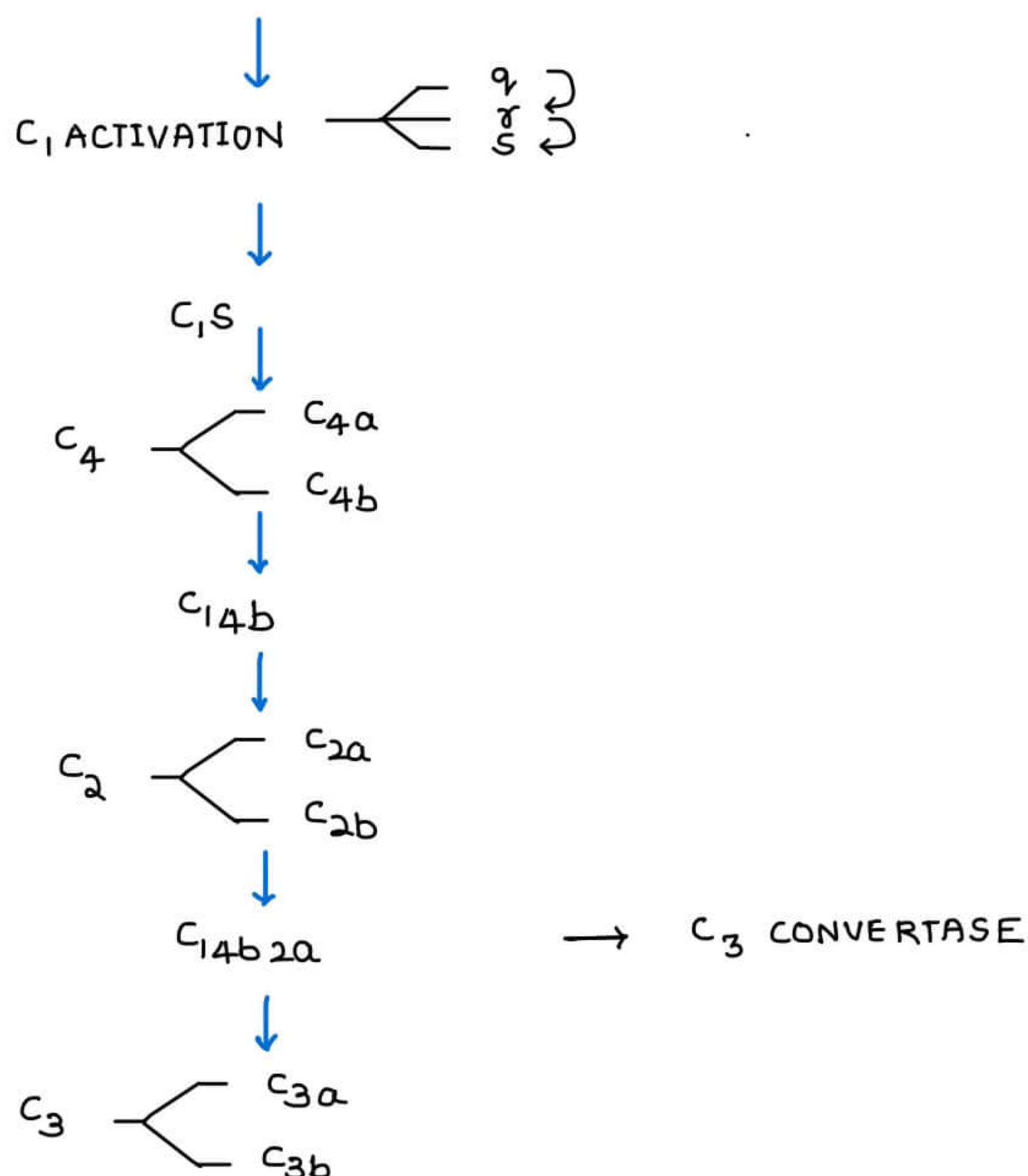
- GIT →  $\text{C}_1$
- Macrophage →  $\text{C}_2, \text{C}_4$
- Spleen →  $\text{C}_5, \text{C}_8$

## TYPES

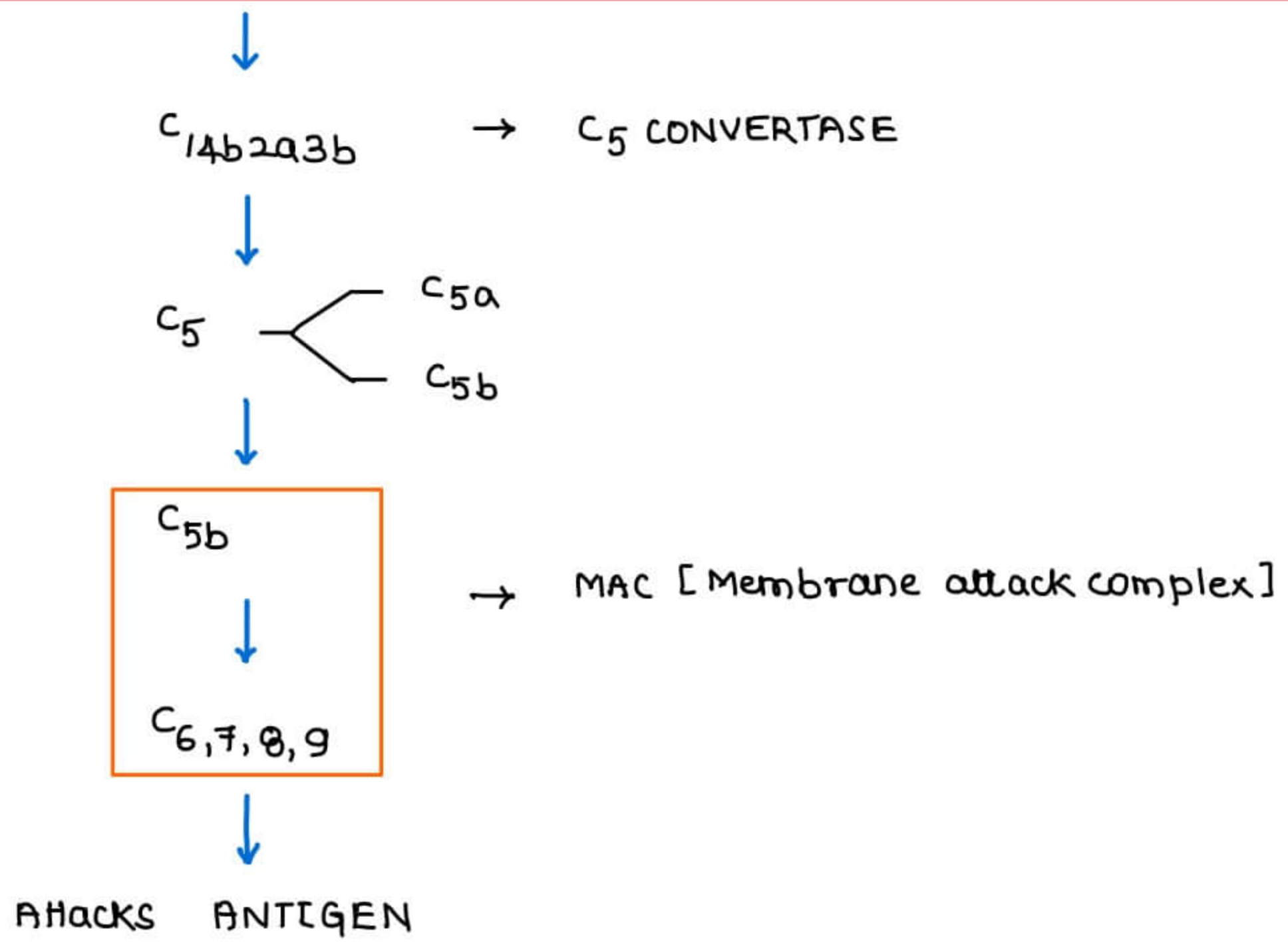
	CLASSICAL	ALTERNATIVE	LECTIN
ACTIVATORS	Ag + Ab complex	Zymogen Endotoxin Nephritic factor Properidin	mannose binding Lectin
1st ACTIVATED	$\text{C}_1$	$\text{C}_3$	$\text{C}_4$
PART OF	Acquired immunity	Innate immunity	Innate immunity

## CLASSICAL COMPLEMENT PATHWAY

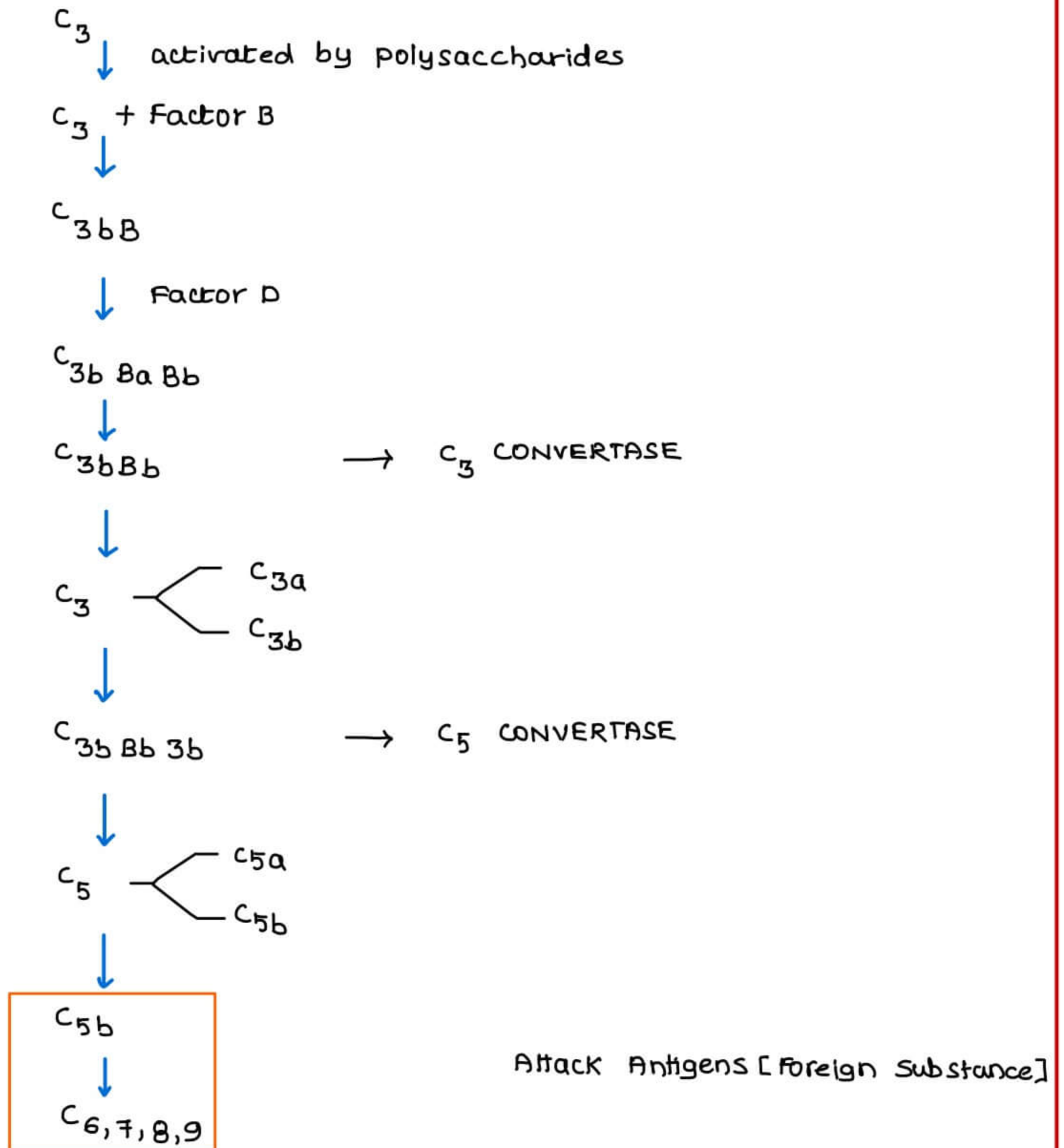
Antigen + Antibody [IgG, IgM]







ALTERNATE COMPLEMENT PATHWAY



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



## LECTIN PATHWAY

- similar to complement pathway, but starts w/  $C_4$
- $C_1$  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 → Hereditary 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 $\alpha$
F	→ Fibroblasts	→ IFN $\beta$
T	→ T-lymphocytes	→ IFN $\gamma$

→ 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
- maturat<sup>n</sup> 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 periaarteriolar lymphoid sheath
- B dependent area in a LN → Germinal follicle [marginal zone]

### CD MARKERS

T cells → CD<sub>1</sub> to CD<sub>8</sub>  
B cells → CD<sub>10</sub>, CD<sub>19</sub> to CD<sub>23</sub>



## B LYMPHOCYTES

- constitute 10-20% of total lymphocytes
- also acts as APC [Antigen Present cells] [z surface IgM]
- CD<sub>21</sub> → EBV receptor for B cells
- CD<sub>2</sub> → 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<sub>1</sub> → secretes IL-2  
IFN γ

TH<sub>2</sub> → secretes IL-4, 5, 6, 10

- The earliest identifiable T cell precursor in bone marrow are CD34 + 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 Presenting 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
- glycoprotein present on APC
- provides exogenous peptide fragments to CD4 T cells.
- play central role in transplantat<sup>n</sup> antigens immune response

### CLASS III MHC GENES

- a/w complements → C<sub>2</sub>, C<sub>4</sub>, properdin factors, C<sub>3</sub> convertase
- TNF, HSP (Heat shock protein)

### MHC RESTRICTION

Exogenous antigen



Peptide fragments  $\bar{i}$  MHC II



CD4 T cells

Endogenous antigens



Peptide fragments  $\bar{i}$  MHC I



CD8 T cell

- only above process will occur

### 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
  - Phagolysosomal Fusion
  - lysosomal degranulat<sup>n</sup>
  - O<sub>2</sub> 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 inflamm <sup>n</sup> , fever
IL 2	TH 1 cells	LAK cells from NK cells
IL 3 [GM-CSF]	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
IL 12	Macrophages	promotes TH1 induction & inhibits TH 2

## CHEMOKINES

### CXC CHEMOKINES

- act on neutrophils
- IL 8, IL 1, TNF

### CC CHEMOKINES

- monocyte chemo attractant protein 1
- Eotaxin; RANTES

### C CHEMOKINES

- specific for lymphocytes & lymphotactin

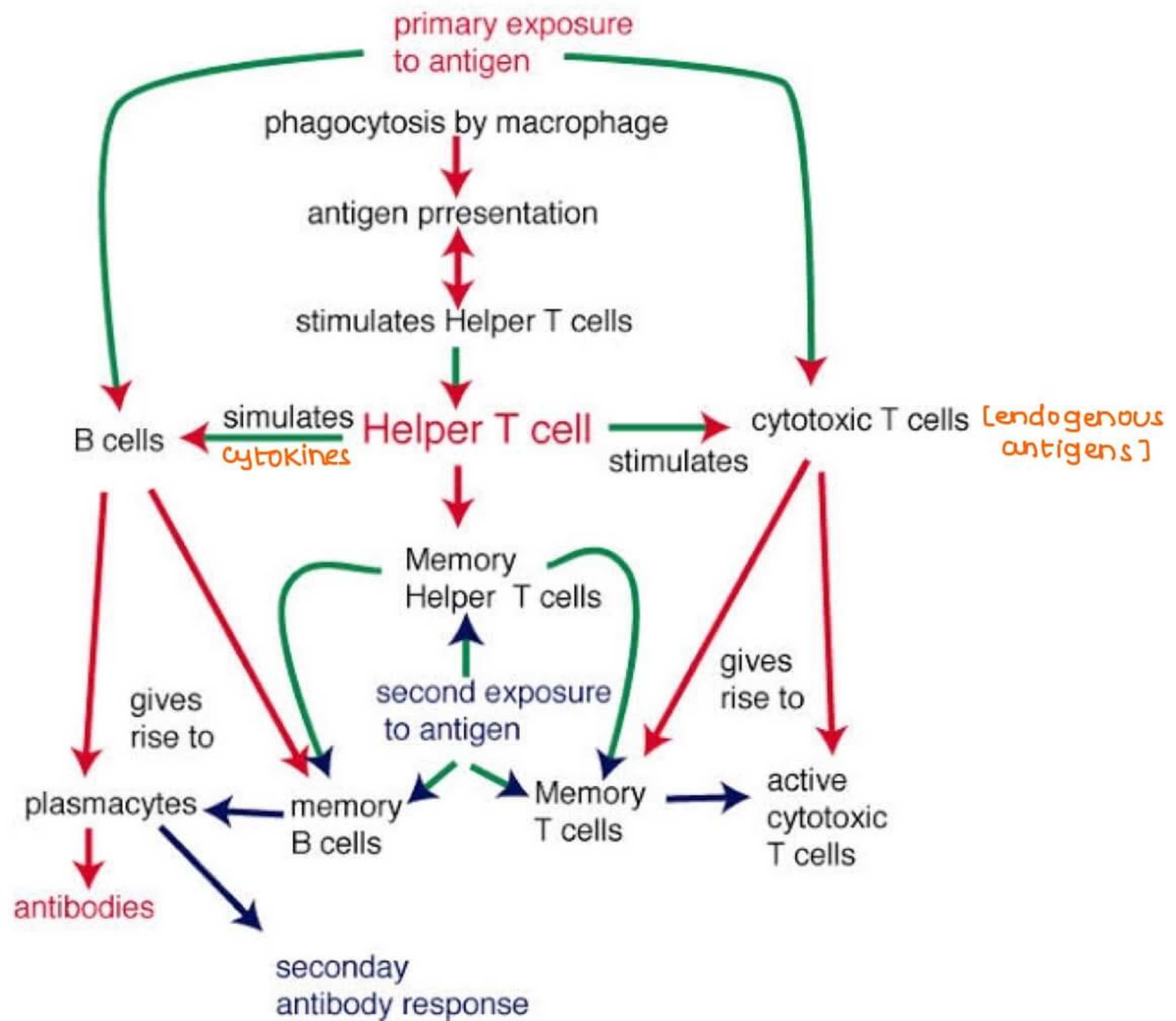
### CX<sub>3</sub>C CHEMOKINES [FRACTALKINE]



## FUNCTIONS

T cells → cell mediated Immunity

B cells → Humoral Immunity



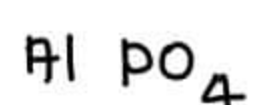
## CELL MEDIATED IMMUNITY ROLES

- 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

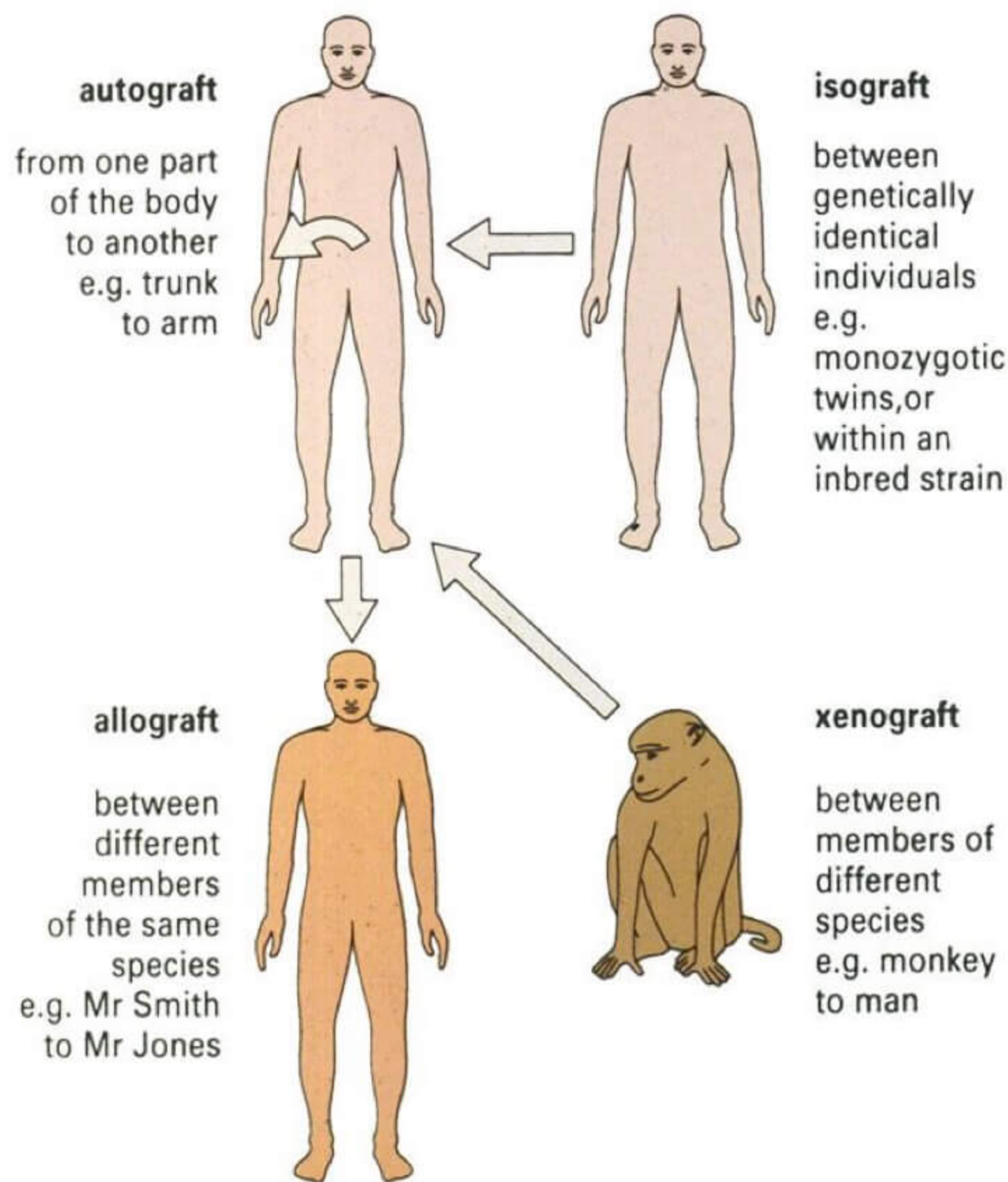
→ Examples



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 donor → rejected in an accelerated fashion
  - vascularisation commences
  - 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

### BILLINGHAM'S CRITERIA

- ① Graft contains immunocompetent 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

- Microcytotoxicity
- Mixed leucocyte reactions

#### ② DNA PCR TYPING [Best]

## AUTOIMMUNITY

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

### MECHANISMS

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

### SEQUESTERED ANTIGENS

- Lens protein } considered as foreign
- Sperm protein }

### FORMATION OF NEO ANTIGENS

- Biological exposure } Neo antigens
- Chemical exposure }

### IMMUNOLOGIC REACTION BY CROSS REACTIVITY

- Carbohydrate 'C' cross reacts i cardiac valves
- Carbohydrate 'M' cross reacts i myocardium

### BREAKDOWN OF IMMUNOLOGICAL HOMEOSTASIS

- NO coordination b/w cell mediated & humoral immunity

FORBIDDEN CLONES are later exposed



## EXAMPLES

- ① AIHA [Auto Immune Hemolytic Anaemia]
- ② Myasthenia Gravis
- ③ SLE [COB is due to kidney lesions mainly]
- ④ Rheumatoid Arthritis
  - IgM against Fc portion of IgG
  - IgM + IgG complex deposited in synovium of joints & activate complement
- ⑤ Hashimoto's Thyroiditis
  - age group → middle aged women
  - infiltrat<sup>n</sup> of gland by sensitized lymphocytes leads to progressive destruction of thyroid follicles
  - presents  $\bar{c}$  goitre

## MECHANISM OF AUTO IMMUNITY IN A NUTSHELL

- ① Exposure of sequestered antigens
- ② formation of neo antigens
- ③ cross reacting antigens
- ④ Polyclonal activation of B cell
- ⑤ Inappropriate expression of MHC class II 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  $\beta$  chains of integrins
  - leads to recurrent infection
- ③ CHEDIAK HIGASHI SYNDROME
  - Defective phagolysosomal fusion
  - ↓↓ in melanocytes
- ④ JOB SYNDROME / HYPER IgE SYNDROME
  - deficiency of TH17
  - Leads to recurrent staph. cold abscess
  - ↑ IgE



### 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  $\xrightarrow{\text{BTK gene defect}}$  PRE B CELLS

- 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 CD40L 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  $\xrightarrow{\text{X}}$  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
- Ig M → ↓ ed
- Ig E & Ig A → Normal
- Ig E → ↑ 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 IL4, 7, 9
- 2nd most common defect → Adenosine deaminase deficiency
- Rag1 & Rag2 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

- 22q11 defect
- absence thymus & para thyroid glands
  - presents  $\bar{c}$ 
    - hypocalcemic tetany
    - congenital heart defects

### ② CHRONIC MUCOCUTANEOUS CANDIDIASIS

- also a T cell Disorder
- causes recurrent candidiasis



## 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



LOUIS PASTEUR

## 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** i disease must be present i causative organism
- **isolation** in a pure culture media should be possible
- culture growths **inoculated** in animal should 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



ROBERT KOCH



- 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 [ $\mu\text{m}$ ]	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]	80s [60s + 40s]	80s [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 separate
    - Human eye → 0.2 mm
    - Light microscope → 0.2  $\mu\text{m}$
    - Electron microscope → 0.2 nm

contrast can ↑ed by staining

## LIGHT MICROSCOPE

- White light is used as source
- used for
- motility [hanging drop method] demonstrat<sup>n</sup>
  - Gram staining
  - Acid fast staining
- internal details can not be demonstrated
- Highest magnificat<sup>n</sup> 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 demonstrat<sup>n</sup>

### 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
  - calcofluor 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
Respirat <sup>n</sup>	Mesosomes	Mitochondria
cytoskeleton	absent	present
Ribosomes	70S	80S
mRNA	no introns	Introns & Exons + nt



## SHAPES

cocci in clusters	→	staphylococcus
cocci in chains	→	streptococcus
cocci in pair [diplococci]	→	Pneumococcus
Kidney shaped	→	Gonococci
lens shaped	→	Meningococci
cocci in Tetrads	→	Micrococci
cocci in octate	→	Sarcina
spectacle like appearance	→	Enterococcus

## BACILLI

Bamboo stick appearance	→	Bacillus anthracis	
Diplo bacilli	→	Klebsiella pneumoniae	
Branching	→	Actinomyces	
curved GNB	→	Campylobacter	→ Gull wing shape
		Helicobacter	
Spiral	→	Spirillum	
		Spirochaetes	
Pleomorphic	→	Haemophilus	
		Brucella	

All cocci are Gram positive **Except**

meningococci  
Gonococci  
Veillonella  
Moraxella

All Bacilli are Gram negative **Except**

M → Mycobacterium species  
A → Anthracis Bacillus  
C → Clostridium species  
D → Diphtheriae Corynebacterium  
O  
N → Nocardia  
A → Actinomyces  
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 to a tetrapeptide side chain	Tetra peptide are directly
	cross linked by penta peptide crossbridge	linked to each other

- NAG → N acetyl Glucosamine  
 N acetyl Muramic Acid
- AA → Amino Acids

### CELL WALL DEMONSTRATION

- R → Reaction to specific antibody
- M → micro dissection, Mechanical Rupture of cell
- P → Plasmolysis
- Doctor → Differential staining

### INCLUSIONS

#### STORAGE GRANULES

- corynebacterium diphtheriae
  - Polymetaphosphate
  - 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
  - McFadyen's Reaction

### FLAGELLA

- used for motility
- 0.02  $\mu$  thick
- 3-20  $\mu$  long
- Antigenic
- **PARTS**
  - Filament
  - Hook
  - Basal Body → attached to cytoplasmic membrane by rings
- **T** → **T**umbling → *Listeria*
- **D** → **D**arting → *Vibrio*
- **S** → **S**warming → *Proteus*
- Falling leaf like → *Giardia lamblia* [dit 4 pairs of flagella]
- WIDAL TEST → measures antibodies against 'O' & 'H' [more immunogenic]

### FIMBRIA

- Fine hair like structure
- 0.5  $\mu$  long
- helps in adhesion
  - conjugation [sex pili]
- Antigenic

### SPORES

- Highly resistant resting forms
- **Spore forming Bacteria**
  - B** → *Bacillus anthracis*
  - S** → *Sporosarcina*
  - C** → *Clostridium*
    - drum stick appearance seen in *C. tetani*
    - most have sub terminal spores [most of clostridia]
    - central spore seen in *C. bifermentans*
      - *Bacillus cereus* also produces central spores

**PROTOPLAST** → seen in Gram positive

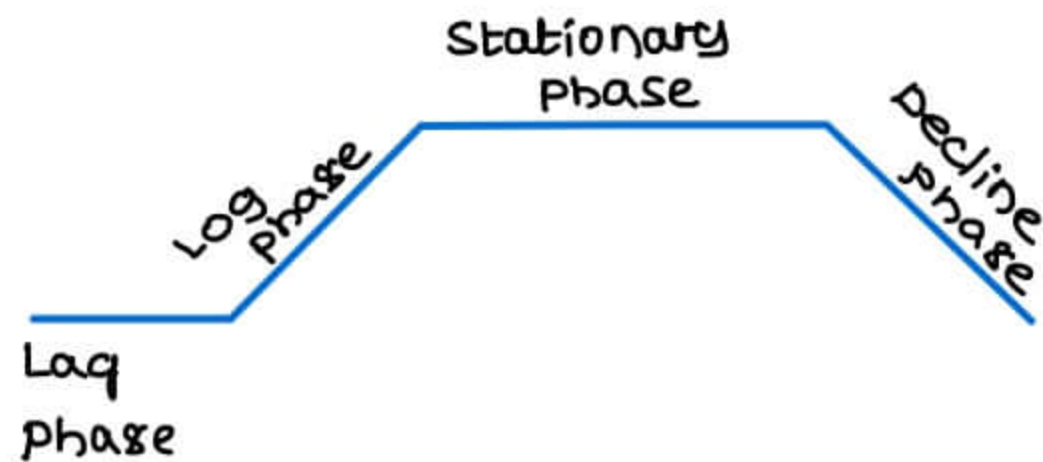
**SPHEROPLAST** → seen in Gram negative [still some cell wall is present]  
 [out cell wall] Forms [loses cell wall in hypertonic solution]



## 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 accumulati<sup>n</sup> of toxic metabolites
- involution forms formed



## STERILIZATION

→ 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 microorganisms

## 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 > fungi > 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

Autoclave

### 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**

1. SUN LIGHT
2. DRYING
3. DRY HEAT

**PRINCIPLE**

Active microbicidal activity of Light UV LIGHT

70-80% weight of bacterial cell is water

- 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 with 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 STERILIZATION**

- coagulation & denaturation of proteins
- < 100°C → pasteurization, inspissation
- at 100°C → Boiling, Tyndallisation
- > 100°C → Autoclave

**PASTEURIZATION OF MILK****Flash method**

- 72°C for 15-20 sec → bring down to 13°C
- kills *Coxiella burnetii* spores also
- Better method than Holder's

**Holder's method**

- 60°C for 30 min



**WATERBATH**

- Disinfection of serum, Body fluids & vaccines can be sterilized
- Bacterial vaccines → disinfected at  $60^{\circ}\text{C} \times 1 \text{ hr}$
- Serum or heat labile body fluids → disinfected at  $56^{\circ}\text{C} \times 1 \text{ hr}$

**INSPISSATION**

- $80 - 85^{\circ}\text{C}$  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  $100^{\circ}\text{C}$** **BOILING**

- $100^{\circ}\text{C}$  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 [ $100^{\circ}\text{C}$ ] at atmospheric pressure for 90 min.

**TYNDALLIZATION**

- $100^{\circ}\text{C} \times 20-30 \text{ min} \times 3 \text{ Days}$
- Done for sugar & Gelatin containing substances

**AT  $> 100^{\circ}\text{C}$** **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  $100^{\circ}\text{C}$ .

**3 PARTS OF AUTOCLAVE**

- pressure chamber
- Lid
- Electrical heater

**TEMPERATURES OF AUTOCLAVE**

- $121^{\circ}\text{C} \times 15 \text{ min} \times 15 \text{ psi}$  [Pounds square inch]
- $126^{\circ}\text{C} \times 10 \text{ min} \times 20 \text{ psi}$
- $133^{\circ}\text{C} \times 3 \text{ min} \times 30 \text{ psi}$

**Done For**

- Surgical instruments
- All culture medias except LJ & LSS | 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 (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 microorganisms
- 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 marcescens*



## RADIATION

### IONIZING RADIATION / COLD STERILIZATION

- Gamma rays / X Rays
- breaks upon DNA  $\bar{c}$  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 & ISOPROPANOL [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  $\bar{c}$  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 cytosopes & Endoscopes

### Advantages

- does not require activation
- low vapour property
- Better odour
- more stable
- ↑ mycobactericidal activity

## PHENOLIC COMPOUNDS

### PHENOL [CARBOLIC ACID]

- 1st 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 [chlorhexidine + cetrizide]

**CHLOROXYLENOL** → component of dettol

## HALOGENS

- Iodine & chlorine have antimicrobial activity
- Exist in free state & form salt like compounds i sodium & most other metals

## IODINE

- skin antiseptic
- oxidising cell constituents & iodinating cell proteins
- at higher concentration, it may kill spores

### TINCTURE OF IODINE [2% IODINE]

- in water Ethanol solution of Potassium Iodide

## IODOPHOR

- complexing iodine i 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         | } | Produce HClO [Hypochlorous Acid] |
| 2. SODIUM HYPOCHLORITE  |   |                                  |
| 3. CALCIUM HYPOCHLORITE |   |                                  |
- ↓  
oxidation of cellular materials &  
destruction of vegetative bacteria & fungi

→ Organic matter interferes in 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 vacuum 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 w bacterial cell proteins, often w 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 b/w 2 liquids or b/w 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 an 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  $\bar{c}$  synthesis of peptidoglycan in cell walls

**ACRIDINE DYES**

ACRIFLAVINE	} affected very little by organic waste
EUFLAVINE	
PROFLAVINE	
AMINACRINE	

- more active against Gram positive than Gram negative
- interferes in synthesis of Nucleic Acid & proteins in bacterial cells

**GASEOUS STERILIZATION**

- Ethylene oxide [ETO]
- Low temperature steam formaldehyde
- $\beta$  propiolactone

**ETO**

- most widely used gaseous chemical sterilant
- Both microbicidal & sporicidal
- acts by combining  $\bar{c}$  cell proteins
- high penetrating power
- used for prepacked syringes
- highly inflammable, irritant & explosive
- mixed  $\bar{c}$  CO<sub>2</sub> 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 sterilization is achieved [5-8 hrs]

CONTROL → Bacillus globigii Spores used



**USES**

- to sterilize
  - disposable plastic petri dishes
  - Syringes
  - 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 NAEKER 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 =

Highest dilution of test disinfectant that kills S. typhi in given time

Highest dilution of phenol that kills S. typhi in given time

- it does not assess in presence of organic waste

**CHICK MARTIN TEST**

- modification of RIDEAL WALKER COEFFICIENT 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  
 Peracetic 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 with 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 with 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 → Thymine & 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 Enterotoxin 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 $\beta$ galactosidase	Examples ① Mutations ② Transfer of genes by - conjugation - Transformation - Transduction

## MUTATION

- Random, undirected, heritable variation
- Alterat<sup>n</sup> 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 live at  $35^{\circ}\text{C}$
    - but dies at  $39^{\circ}\text{C}$  [Restrictive temperature]
- TYPES
  - ① Point mutation
    - Base pair substitution
    - frame shift mutat<sup>n</sup>
  - ② Multisite Mutations
    - Gain, Loss or Duplicat<sup>n</sup> can occur



TRANSITION	TRANSVERSION
mc type purine is replaced by purine pyrimidine is replaced by pyrimidine	purine can be replaced by pyrimidine & VICE VERSA

## FRAME SHIFT STUFF

Normal → AAT AGT GCC (serine)  
 Deletion → AAT GTG CCA (valine)

Normal → AAT AGT GCC (serine)  
 Insertion → AAT CAGT GCC (Glutamine)

## MULTI SITE MUTATIONS

## TYPES

- ① Addition / Gain
- ② Deletion / Loss
- ③ Duplication
- ④ Inversion

## MISSENSE MUTATIONS

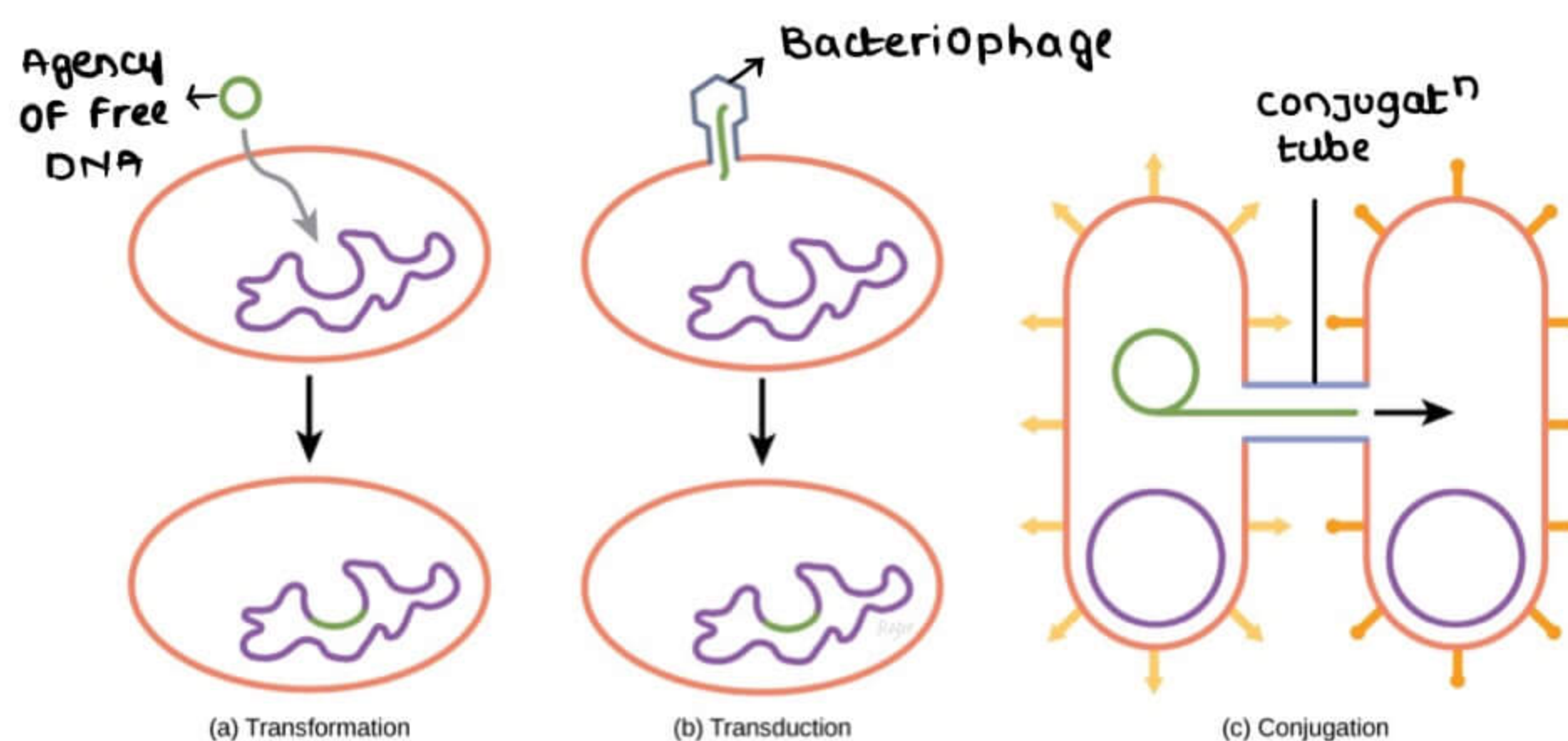
→ Triplet code is altered, different amino acid produced from that normally located at that particular position

## NONSENSE MUTATION

→ premature polypeptide chain terminat<sup>n</sup> by stop codon

## GENE TRANSFERS [sexual process]

- ① TRANSFORMATION<sup>N</sup> → naked DNA taken up from the environment by bacterial cells
- ② TRANSDUCTION → use of bacteriophages [bacterial virus] to transfer DNA b/w cells
- ③ CONJUGATION → direct transfer of DNA from one bacterial cell to another





## TRANSFORMATION

- Discovered by GRIFFITH
- Rediscovered by AVERY, MACLEOD & MCCARTY [1944]

### CASE A

Living non-encapsulated bacteria [Rough strains of Pneumococcus] when injected into mouse → Mouse dies

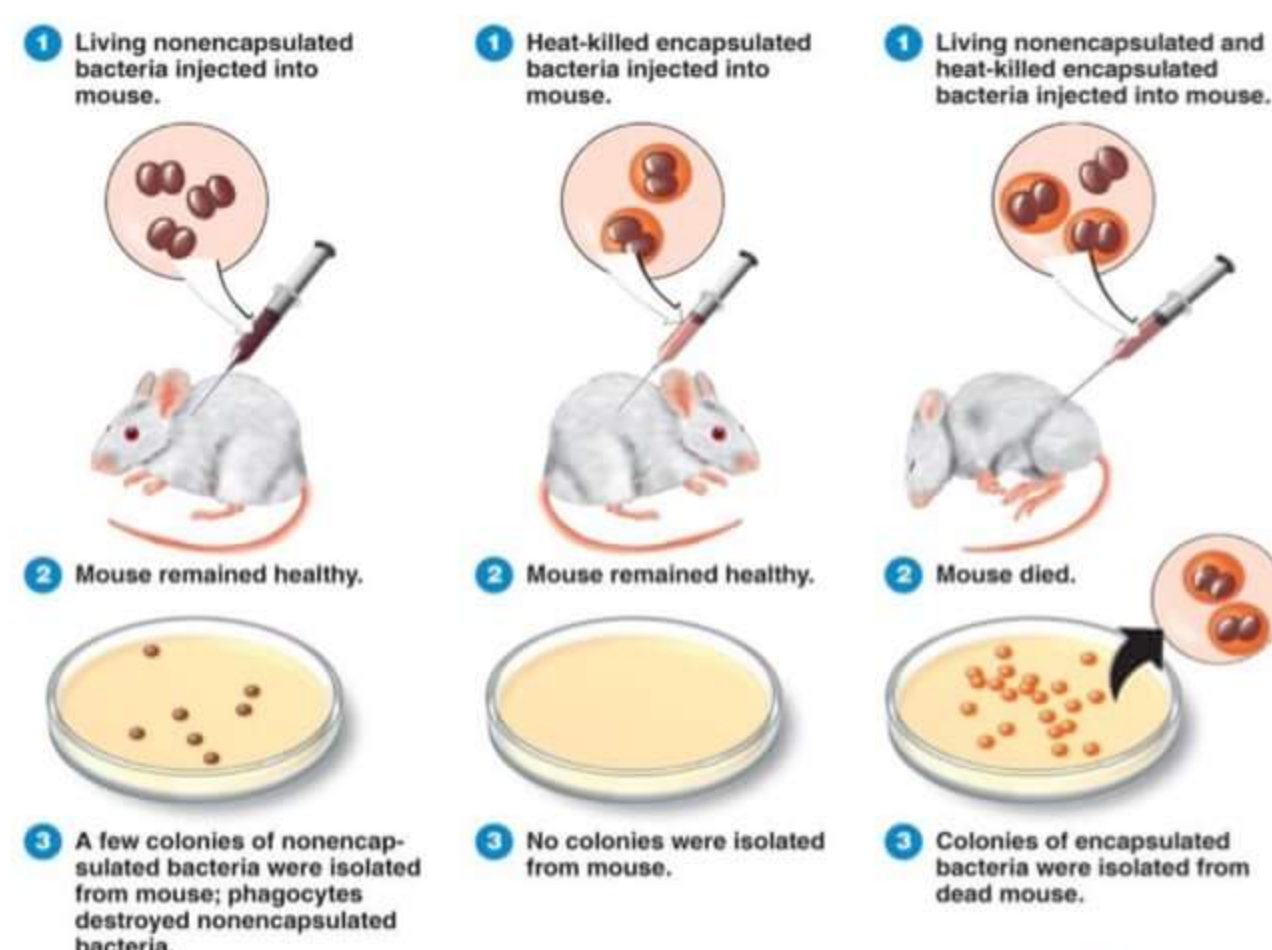
### CASE B

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]



(A) (B) (C)  
Demonstrat<sup>n</sup> 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 donor 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 i bacterial chromosome as prophage that multiplies synchronously i 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



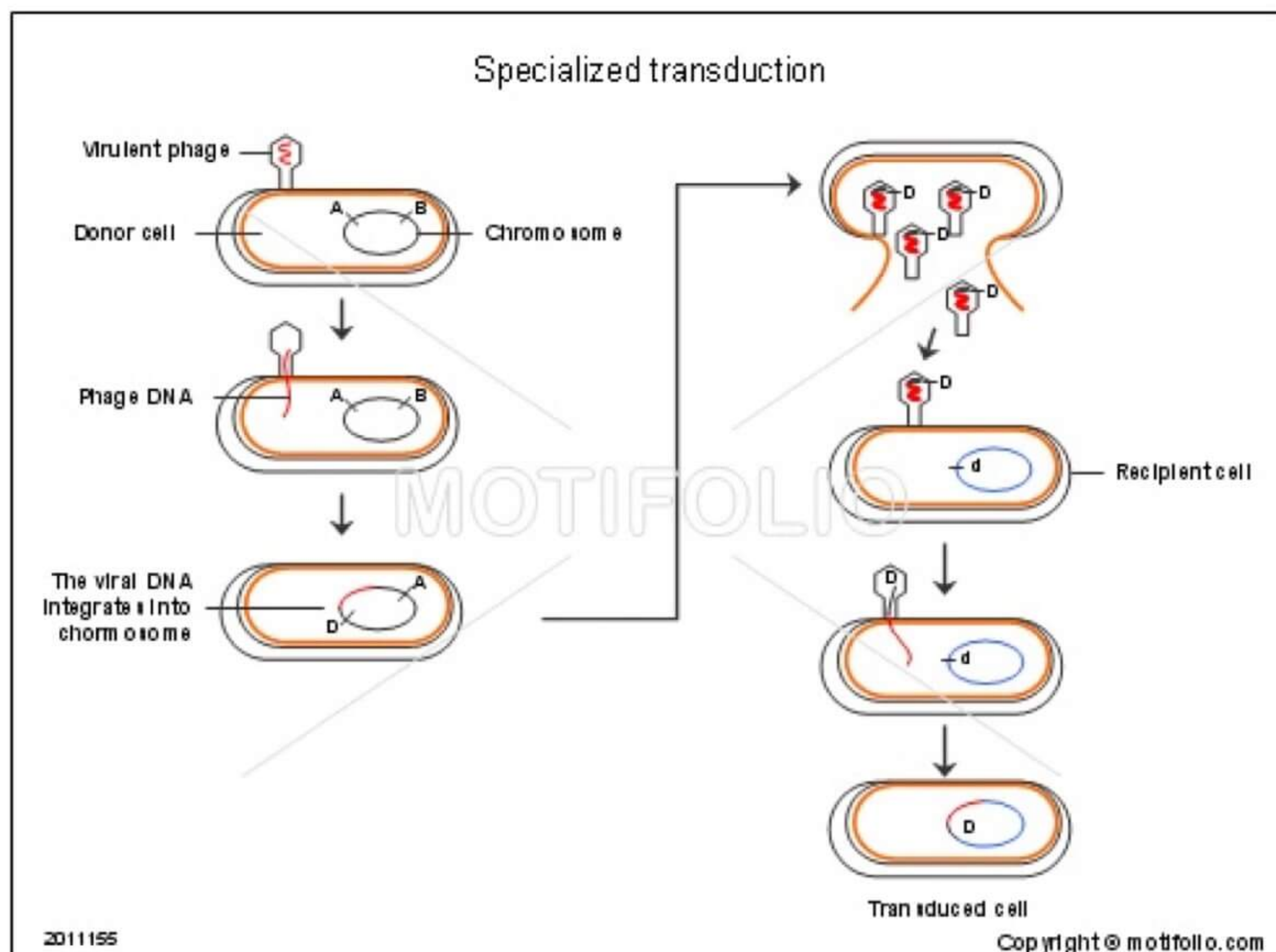
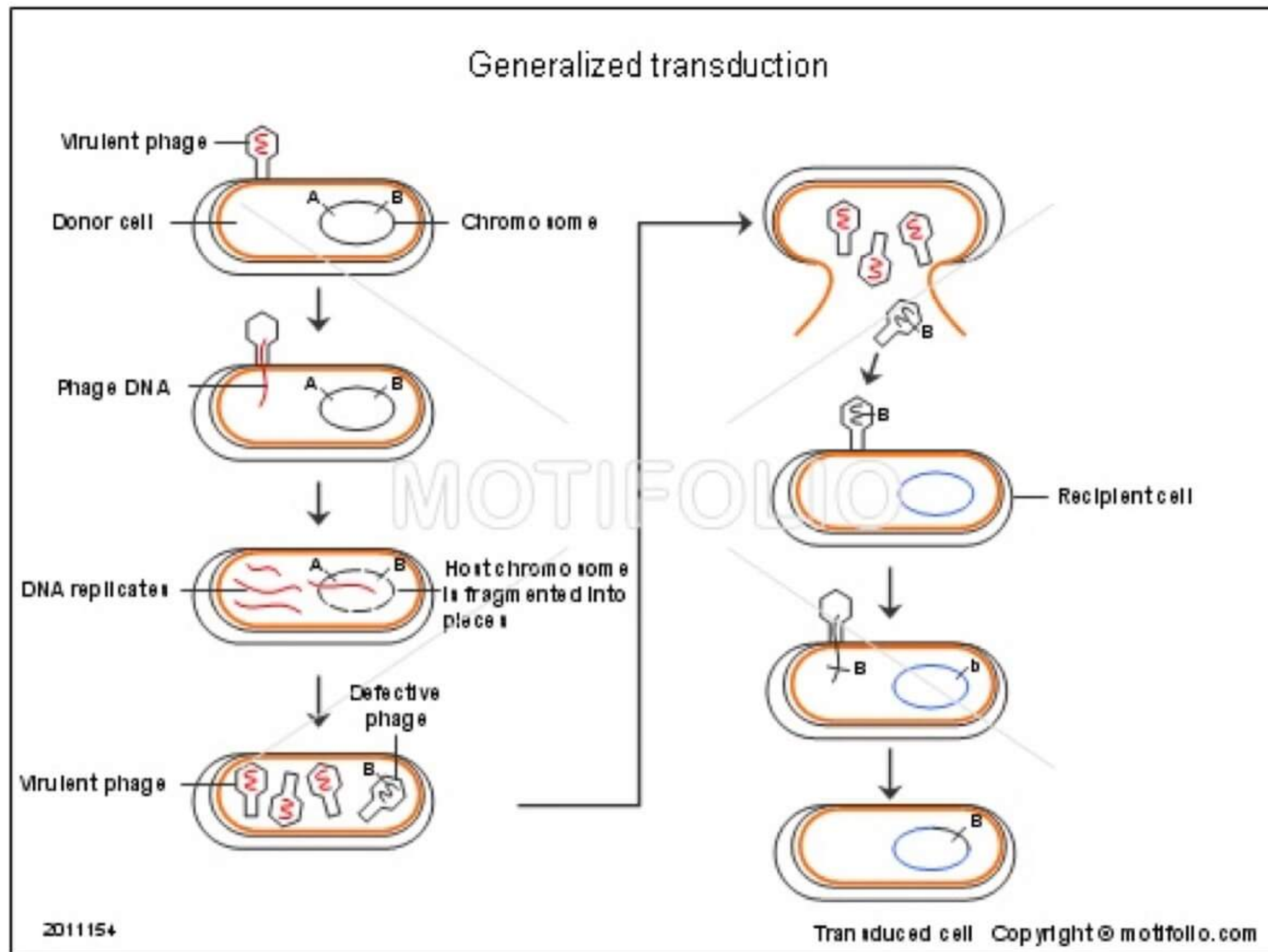
## TYPES OF TRANSDUCTION

### 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 in 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**

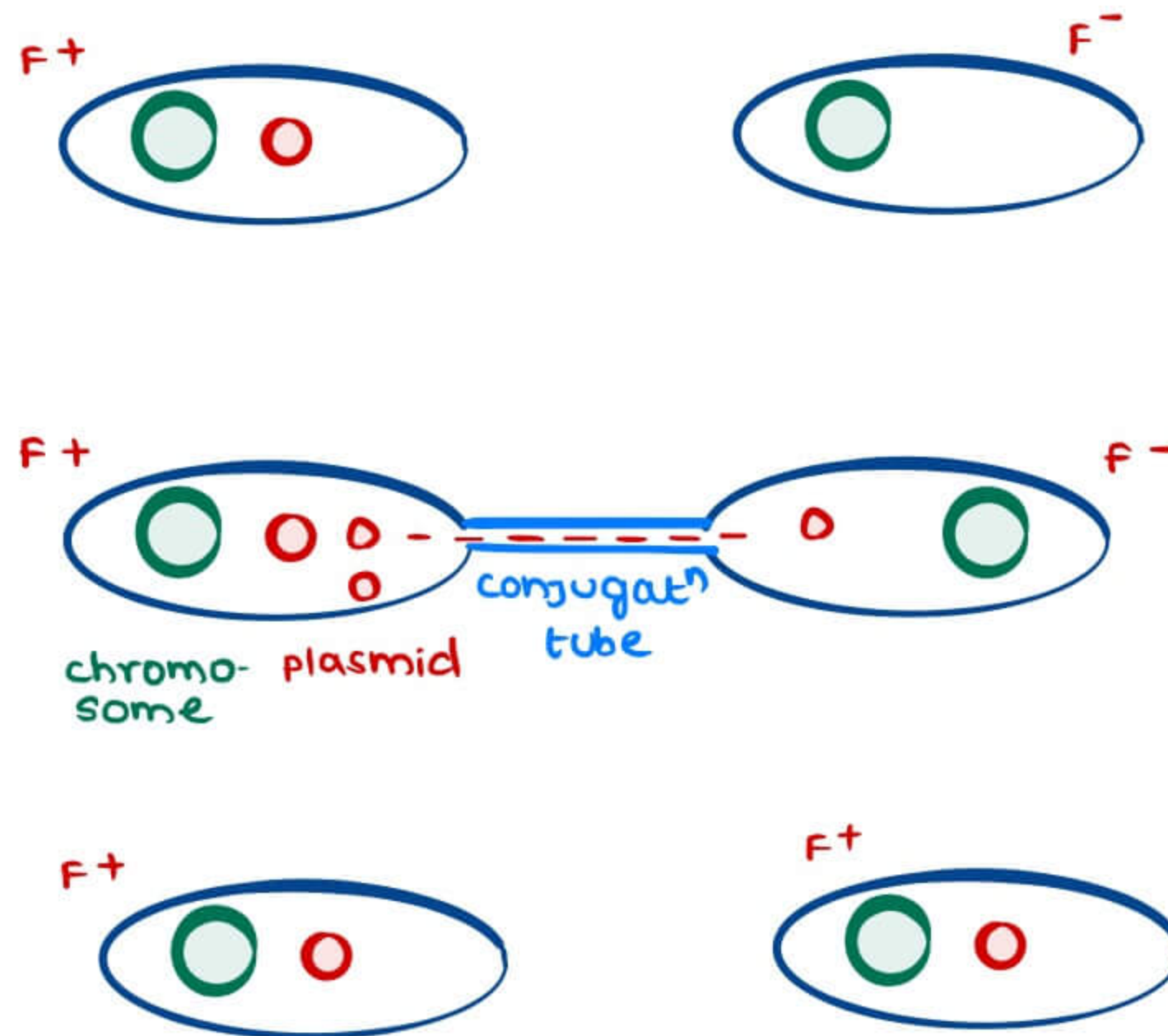
## BACTERIAL TOXINS THAT ARE CODED BY LYSOGENIC PHAGES

- A** → **A** & **C** pyrogenic toxins of *Strepto. pyogenes*
- B** → **B**otulinum toxin **C** & **D**
- C** → **C**holera toxin
- D** → **D**iphtheria toxin
- E** → **E**HEC [*Enterohemorrhagic E. coli*] [aka **V**ero**T**oxin]

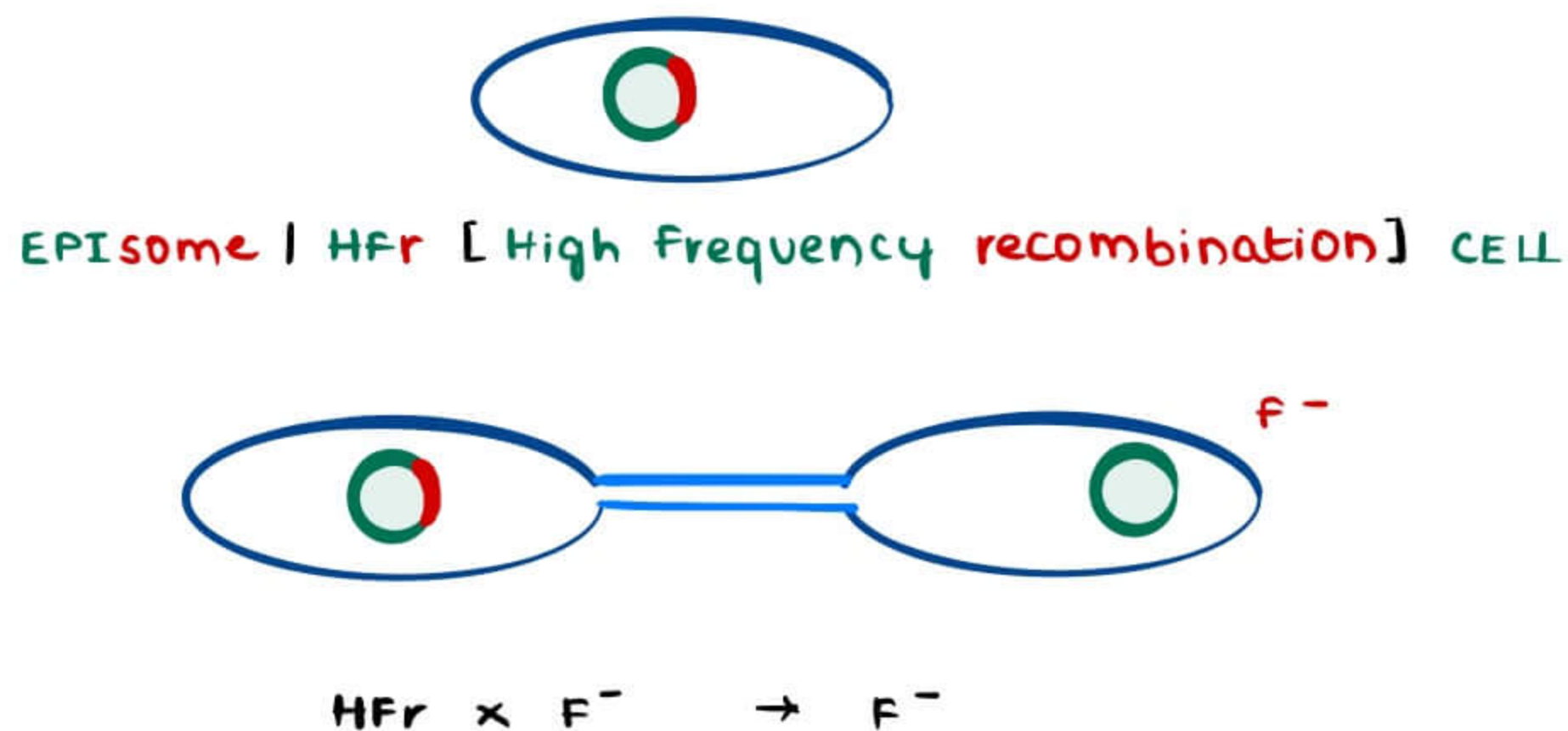
Panton valentine toxin of *Staph. aureus*

## CONJUGATION

- MC method of resistance transfer in lysogenic bacteria
- **POSSIBILITY 1**



## POSSIBILITY 2 / EPISOME / HFR [High Frequency Recombination] CELL







→ POSSIBILITIES

$F^+$	$\times$	$F^-$	$\Rightarrow$	$F^+$
HFr	$\times$	$F^-$	$\Rightarrow$	$F^-$
$F'$	$\times$	$F^-$	$\Rightarrow$	$F'$

TRANSFERABLE DRUG RESISTANCE

- 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<sub>y</sub>
- TRANSFERABLE DRUG RESISTANCE → MULTI DRUG RESISTANCE → DIFFICULT TO R<sub>y</sub>
  
- 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  $\tau$  transposons



CATEGORY A → carry highest risk

CATEGORY B

CATEGORY C → Emerging threats

CATEGORY A

Plague → Plague

VHF → 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<sub>157</sub>: H<sub>7</sub>]

Vill → Vibrio cholerae

take → Typhus fever

Questions for → Q fever

Medical → Melioidosis

P → Psittacosis

G → Glanders [non motile]

CATEGORY C

N → Nipah

H → Hanta virus

S → SARS