

# BACTERIAL VIRULENCE FACTORS

17/2/18

3

Responsible for pathogenesis

Vaccine production is also by virulence factors

Q. Smooth → virulent strain → so these are used for vaccines  
Rough → avirulent strain

Q. All of the following can be used as vaccine to prevent E. coli diarrhoea except

a) CS2

b) K88

c) CFA

d) P<sub>1</sub>

All are fimbriae

a, b, c show adhesion to intestinal epithelial cells

d shows adhesion to uroepithelial cells.

## FIB FIMBRIAE (PILI)

- They are glycoprotein

- Antigenic

- agglutinate RBC

- Nomenclature is based on the RBC agglutinated by RBC.

Function:-

1> Adhesion (Gram -ve)

In Gram +ve → adhesion is due to Teichoic Acid

2> Conjugation.

Mechanism of transfer of genetic material

They are Plasmid encoded.

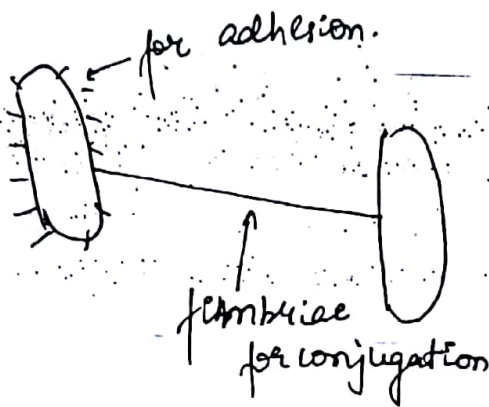
F<sup>-</sup> bacteria can't initiate conjugation

Enterococcus conjugates out fimbriae.

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Plasmid encoded fimbriae

- Mannose resistant fimbriae
- CFA (colonising factor antigen) [ETEC]
- Type 1 fimbriae



## FLAGELLA

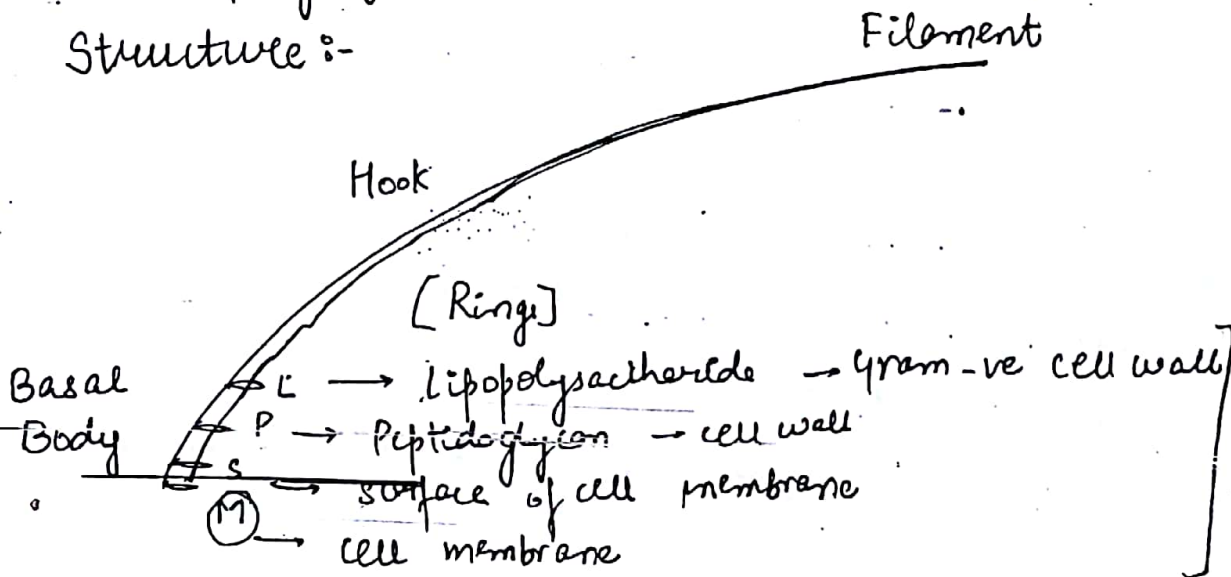
Protein

- antigenic 'H' Ag
- thin  $< 0.02 \mu\text{m}$

Function :-

- 1) Motility - due to rotation of M Ring
- 2) Antiphagocytosis

Structure :-





In Gram -ve → all King are found 5

In Gram +ve → only S.M. King

↓  
only cell membrane attachment

Motility helps in identification of bacteria

Flao

Resolution Power

naked eye → 200  $\mu$ m

Light microscope →  $\frac{200 \mu\text{m}}{1000} = 0.2 \mu\text{m}$   
= 200 nm.

Electron microscope =  $\frac{200 \text{nm}}{1000} = 0.2 \text{nm}$ .

Flagella can't be seen by light microscope.

Demonstration of Flagella/Motility :-

→ Electron microscope

→ Ryu stain  
silver based stain.  
not sensitive

→ Serology.  
H antigen.

→ Hanging Drop (for vibrio only)

→ Dark ground microscope (Spiriochaetes)

Best Method Utracult in semisolid agar (Motility medium)  
↓  
0.2 to 0.5% agar

# Peritrichous Flagella

flagella all round  
Enterobacteriaceae

Lophotrichous flagella

# Lophotrichous Flagella

tuft of flagella at one end.

Campylobacter  
Helicobacter

Single polar  
Vibrio

Pseudomonas

Swarming can be inhibited by all except --

a) 6% agar

b) phenol agar

c) blood agar

d) McConkey agar

## Culture Media

Liquid

Solid

Basal media -> Nutrient broth

- Peptone
- Meat extract
- NaCl } 0.85N
- H<sub>2</sub>O
- Buffers

agar 1-2%

solidifying temp 42°C

⊖ flagellin  
except

Proteus	} swarming
C. Tetani	
B. cereus	

Inhibition of swimming → 6% agar

Phenol

Boric Acid

Bile salts (not in McConkey)

Craig's tube contain semisolid media but ~~not~~ not used for motility.

But in case of solid, semisolid media, it can be preferred for motility.

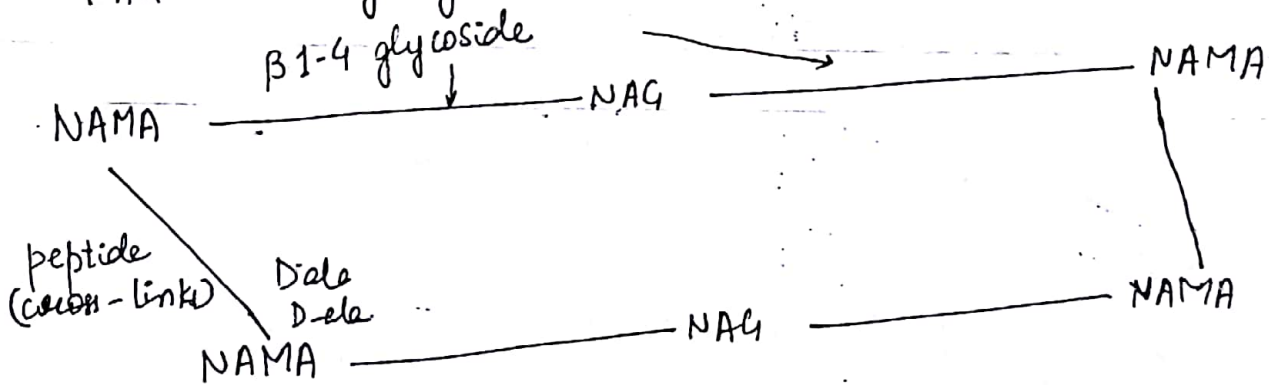
## CELL WALL

Peptidoglycan

Structure

NAMA → N-acetyl muramic acid

NAG → N-acetyl glucosamine



COO<sup>-</sup> terminal in NAMA has unsaturated ends, so form bonds while NAG doesn't have this.

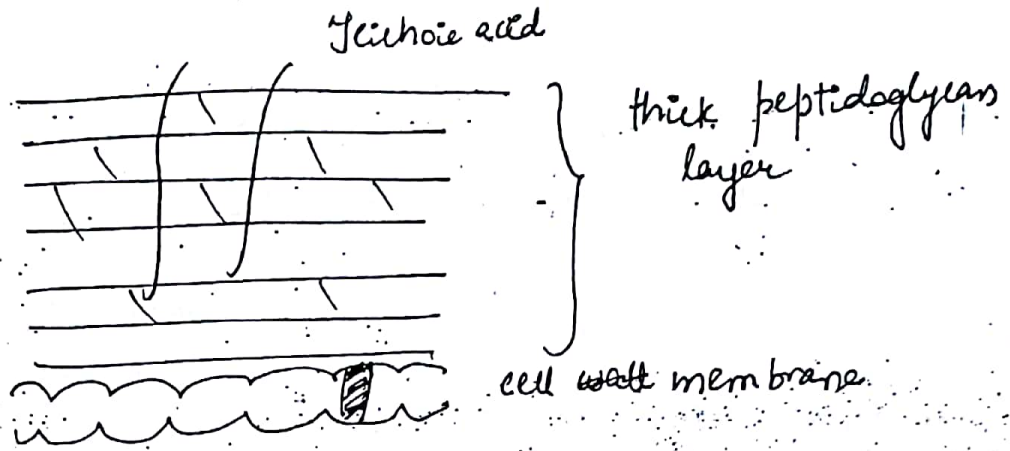
Synthesis:-

Peptidoglycan is synthesised by **PBP** (penicillin binding protein) located in cell membrane

cross-linking (4<sup>th</sup> Phase → last phase)

# Types of cell wall

Gram +ve

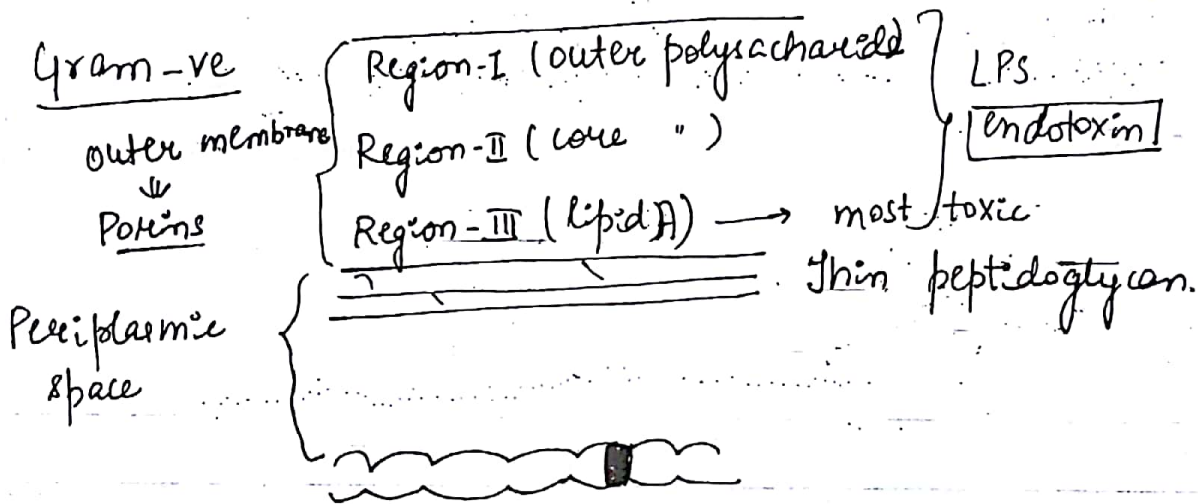


Teichoic acid

thick peptidoglycan layer

cell wall membrane

Gram -ve



outer membrane  
↓  
Proteins

Region-I (outer polysaccharide)

Region-II (core " )

Region-III (lipid A)

→ most toxic

thin peptidoglycan.

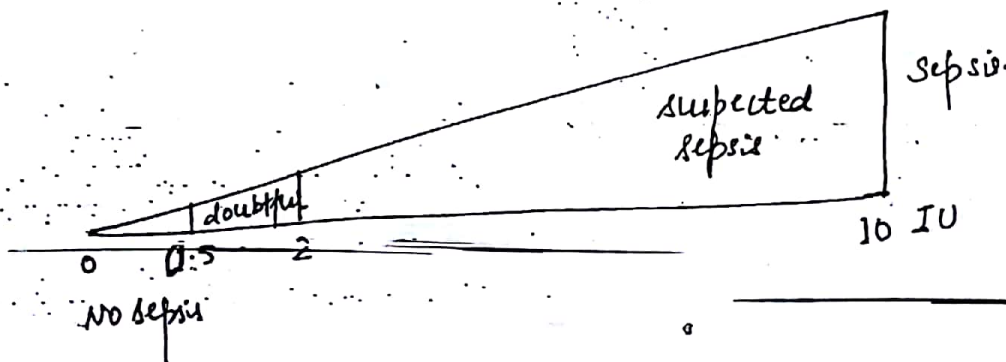
LPS

endotoxin

Periplasmic space

Lipid A → binds to Toll like Receptor 4 on macrophages.  
(most toxic)

Best marker of Sepsis = Pro calcitonin





LPS → Hydrophobic

Porens help in passive diffusion of hydrophilic agents

Region I (outer polysaccharide) is antigenic  
It is called as O antigen

## O Antigen

- useful for diagnosis (immunogen)
- Serological ⇒ Serotyping (variability of O antigen)  
↓  
epidemiology Q.

Cholera endotoxin doesn't cause virulence

Acid Fast cell wall :-

Gram +ve cell wall = Mycolic acid

Function:-

Rigidity (cell wall)

## CAPSULE

- Polysaccharide.

except B. anthracis → made up of polypeptide  
(D-Glutamic acid) Q.

- Polysaccharides are not stained. ~~are~~

But capsule of B. anthracis can be stained by

Polychromic Methylene Blue (McFadyean Reaction)

Function:

Antiphagocytosis Q.

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Demonstration of capsule

1) M/I  $\Rightarrow$  **Negative stain**  $\rightarrow$  India Ink  
or  
Nigrosin

2) Serology  $\rightarrow$  a) **Quellung Rx**  
swelling of capsule using capsular Ab.  
 $\downarrow$   
Microscope

b) capsular Ag detection by **Latex agglutination**

$\downarrow$   
Reverse passive agglutination

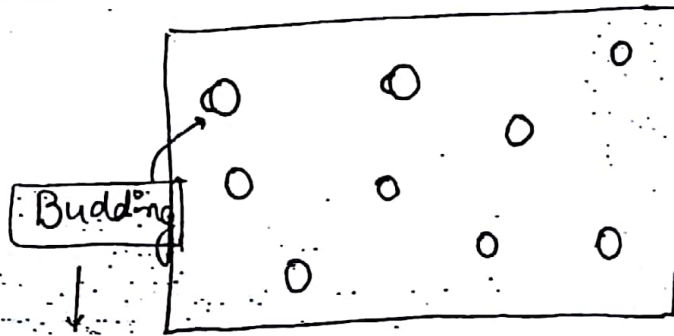
$\downarrow$   
when Ag is detected, then it is called reverse passive.

$\downarrow$   
when particles are used for Ab detection.  
eg. Latex  
RBCs

Q. A pt. presented  $\bar{c}$  headache, projectile vomiting along  $\bar{c}$  altered sensorium. Image of india ink.

$\Delta$

- a) Cryptococcus
- b) Pneumococci
- c) Histoplasma
- d) Coccidioides



seen in **Cryptococcus**.

pneumococcus show Lancelet shaped.

Diplococci  
Gram -ve → Neisseria

Gram +ve → Pneumococci.

Neisseria (Intracellular Diplococci)

Meningococci  
capsulated

Gonococci

uncapsulated

kidney shaped

serological assay on microscope slides are not done except VDR

Quellung Reac<sup>n</sup>

↓  
non-specific test

Polyvalent antepolysaccharide serum

Capsulated Organisms

Yes the PM cooks Very ~~like~~ Fine Chicken to keep his BBB healthy

- Yersinia
- Pneumococci
- Meningococci
- Cryptococci
- Vibrio parahaemolyticus
- Vibrio cholerae O139 Q

- Meth strains of E. coli, Staph, Strep, pseudomonas

↓  
Nosocomial (HAI)

- Clostridium perfringens × Butyrium

- Klebsiella

12

- B. anthracis

- Bordetella

- Bacteroid

- Burkholderia pseudomallei

- Haemophilus influenzae

\* Histoplasma capsulatum

✓ Non-capsulated.

✓ intracellular yeast & appear as capsulated

## ENZYMES & TOXINS

Secreted by Secretion System.

→ Seven Pathways - type I to VII

Type I & IV - Both in Gram +ve & Gram -ve

Type II, III, V, VI → only in Gram -ve

Type VII → M. TB.

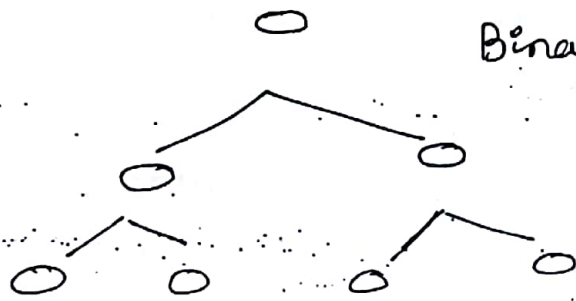
→ Proteins secreted by Type I, III, & VI pathways - traverse inner membrane & outer membrane in one step

(see independent - Do not involve amino terminal

processing of secreted protein)



# Growth Curve



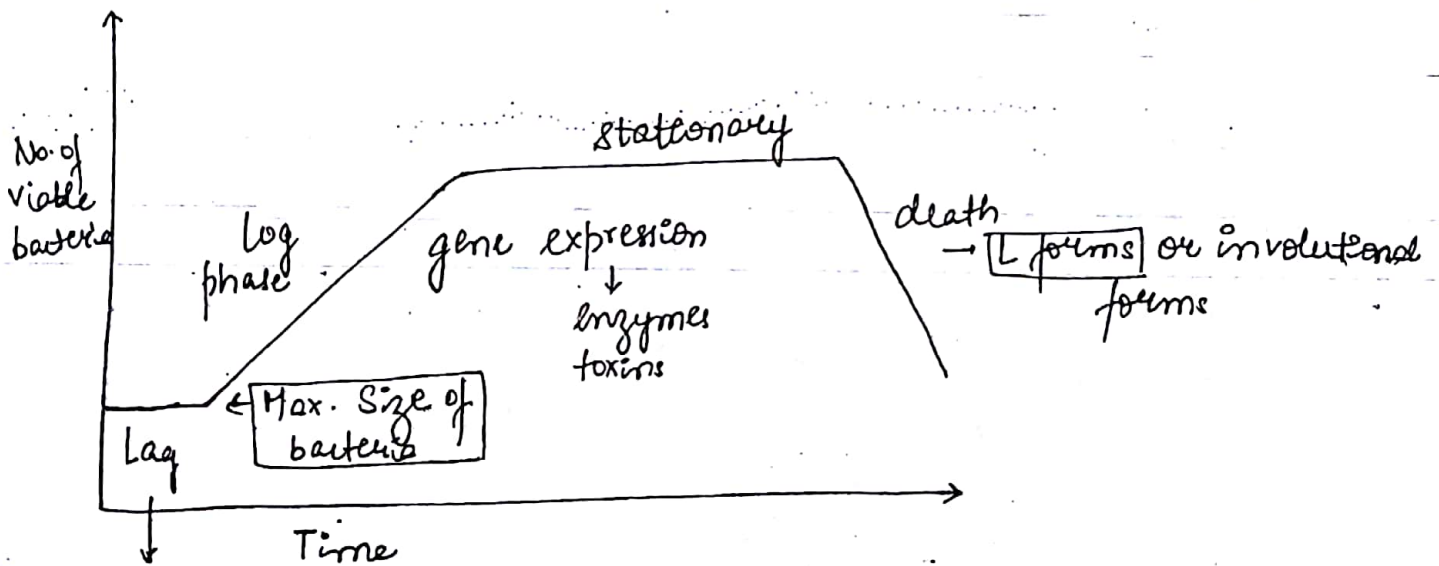
Binary fission (asexual)

Doubling Time is called Generation Time

Max generation Time seen in *M. Leprae* - 13-14 days

*M. Tb* → 16 hrs

other bacteria → 20 min. <sup>M/c</sup>



preparatory phase

↓  
enzymes are released

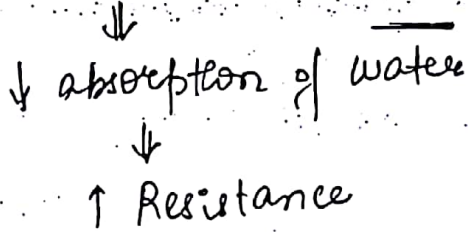
[Metabolic Phase]

Anti microbial susceptibility is done by Phenotype Method  
↓  
in lag phase

→ Stationary phase is due to toxic metabolic products  
→ bacterial colony form → exhaustion of nutrients  
on solid medium

\* Sporulation is seen in Stationary phase. & this is for  
survival.

They are highly resistant bcoz they have  
Ca dipicolinic acid synthetase



Δ - 0.5% H<sub>2</sub>SO<sub>4</sub> → Zn stain

L forms -

- No cell wall

- Mycoplasma

- Spheroplast → Gram -ve organisms  
incomplete destruc<sup>n</sup> of cell wall.

↓  
Reversible

- Protoplast → Gram +ve organism  
complete destruc<sup>n</sup> of cell wall

P → +ve

↓  
Irreversible

- L-forms are virulent

Q. A 25 year old lady presents  $\bar{c}$   $\uparrow$  frequency & dysuria  $\bar{c}$  from  $\ominus$  bacilli in urine?

After 2 weeks of penicillin t/t she comes back w/out resolution of her complaints?

$\bar{c}$  Gram -ve cocci.

a)  $\beta$  lactamase producing strain

b) Gonococci

c) Spheroplast

d) protoplast

Rx- Discontinue Rx for 24-48hr. + ~~let the drug~~  
start the drug  $\bar{c}$  proper dosage

# BACTERIAL GENETICS

## Prokaryotes

↳ No membrane bound organelle.

(no nu. mitochondria, no ER, golgi complex)

↓

Respiratory func<sup>n</sup> → mesosomes.

(invagination of cell membrane)

No histone proteins.

↓

packaging of DNA → supercoiling

Genes are located in chromosome

Plasmid,

Transposon.

Chromosome + Plasmid → ds DNA

Circular

self replicating

↓

DNA polymerase.

Plasmid are extra-chromosomal.

↓

No metabolic func<sup>n</sup>

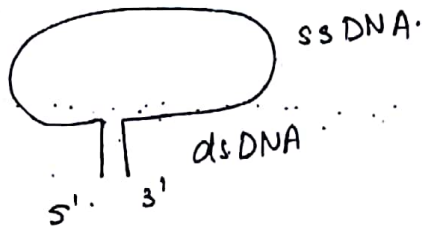
Mobile



# Mobile Genetic Transposon Elements

## ① Transposon

oligonucleotide stranded  $\bar{c}$  complementary ends.



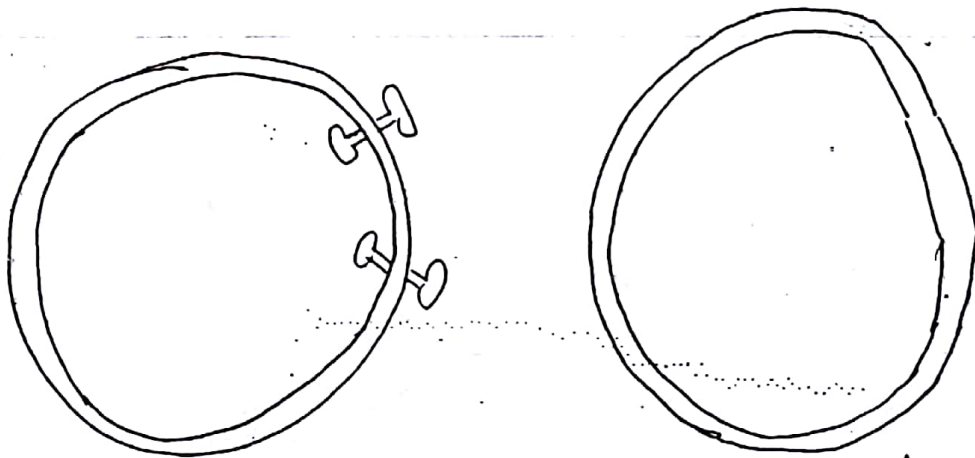
carry gene  $\rightarrow$  impart new character

Transposons move in cut & paste manner bind to tRNA

Jumping Gene

Insertion is  $\bar{c}$  out complementation.

insert directly between nucleotides  $\rightarrow$  Repeat sequence formation



Plasmid mediated drug resistance is due to insertion of Transposons.

## ② Insertion Sequence (IS)

Similar to transposon (smaller)

18

No genes (phenotypic silence)

↓  
Using this Genotyping can be done

Gold std for MTB for genotyping is IS 6110 typing  
This is not for M. Bovis

M. Bovis - Spoligotyping  
(spacer oligonucleotide) → we see here repeats

## TRANSFER OF GENE

seen in bacteria

✓ Transformation

✓ Transduction

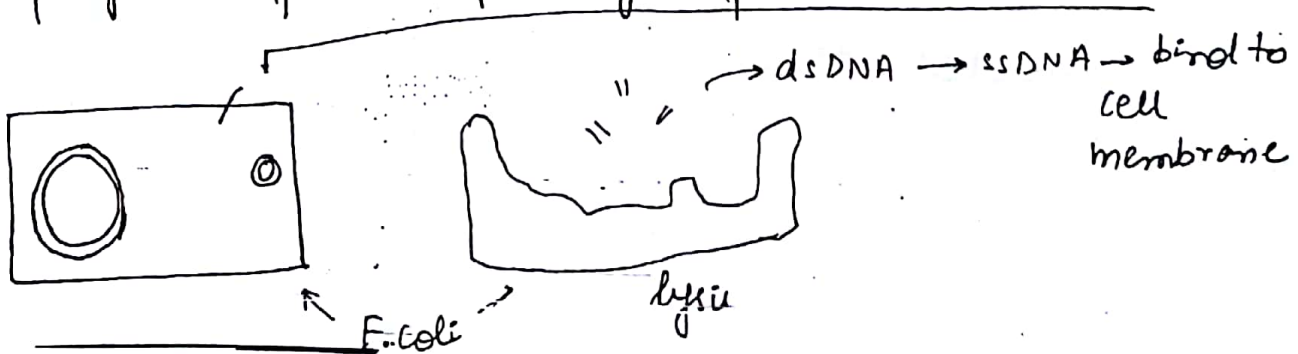
✓ Conjugation

## TRANSFORMATION

Transfer of genes from 1 bacteria to another by  
naked DNA

↓

fragment of DNA after lysis of bacteria



## Homologous Recombination

Replacement of a part of 1 strand in bacterial<sup>19</sup> genome by ss naked DNA fragment at similar genes or alleles

Griffith - Live type II non-capsulated Pneumococci (R)

↓  
injected into mice → no pathogenicity

(+)

Killed Type I capsulated Pneumococci (S)

↓  
injected into mice

↓  
no pathogenicity

When both were mixed together & injected into mice

↓  
Death

↓  
Bacteria is cultured

↓  
Live Type I capsulated pneumococci

He coined the term Transformation.

This was called his Pioneering experiment in genetics.

Transformation is used in Recombinant DNA Technology

- Antigens
- Vaccines

Drug resistance

Transformation may lead to ~~to~~ drug resistance.

## TRANSDUCTION

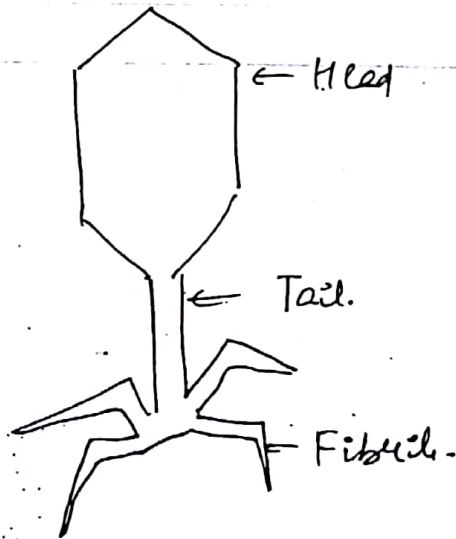
MC method of gene transfer

Transfer of genes from 1 bacteria to another by

Bacteriophage



virus infecting bacteria



RNA phage



filamentous

DNA phage can be used for bacteriophage typing

Q e phage do not carry out transduction?



a) Lytic

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b) Lysogenic

c) temperate

d) lambda

Q.  $\lambda$  phage shows lysogenic to lytic phase conversion?

Ans. ① Temperate phage i lambda

Q.  $\lambda$  phage do not show lysogenic to lytic phase conversion?

Lysogenic phage

Q. Genes associated with galactose metabolism is transferred by  $\lambda$  phage

Lambda phage

Q. Sex conversion in salmonella is

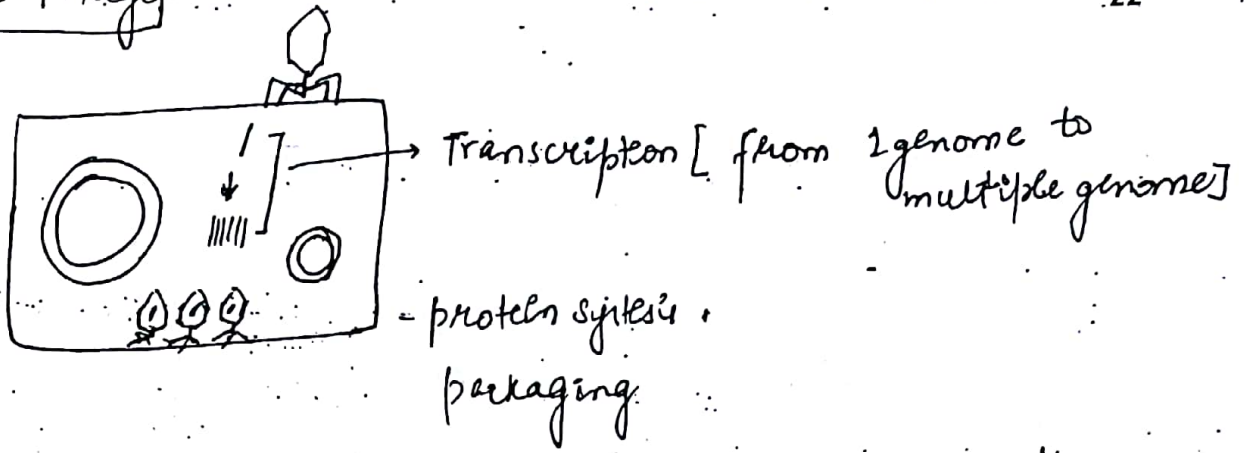
a) transformation

b) transduction

c) Lysogenic

d) conjugation

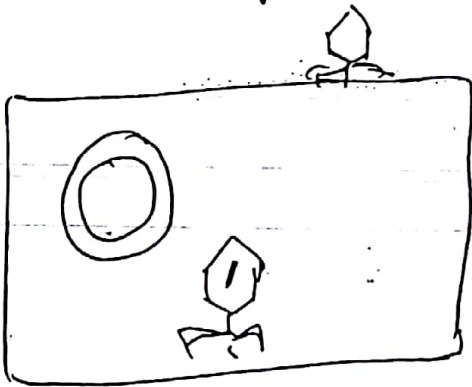
# Lytic Phage:



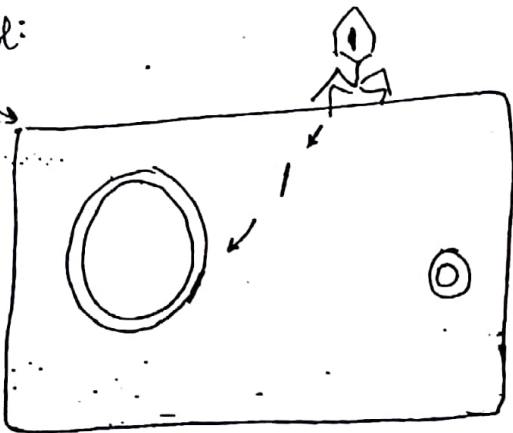
No transductions occur normally → if error is not there

In case of error while packaging, if bacterial genome enters ~~viral~~ viral —

↓  
It goes to other bacteria. → inserts the —  
transfer the gene to this bacteria.



E. Coli:



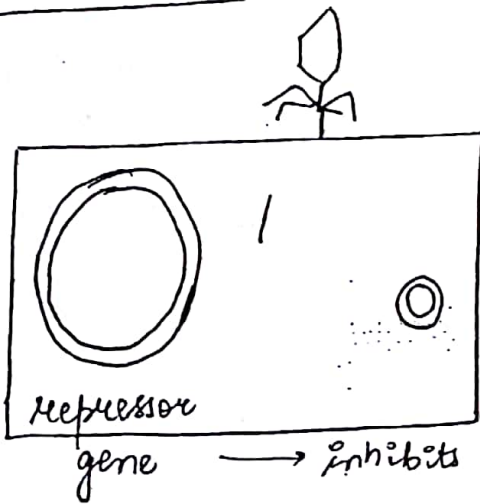
# Generalized Transduction :-

- lytic phage
- error in packaging
- Homologous recombination
- every ~~the~~ gene has equal chance of transfer.

**Lysogenic Phage** :- NO Transduction

- Incorporation of **phage DNA** ~~to~~ into bacterial genome by homologous recombination  
↓  
**Lysogeny** [Transfer from viral to bacteria]

- Rare phenomenon
- Seen in certain bacteria
- Imparts new character to the bacteria
- No lysogenic to lytic phase conversion.  
↓  
**NO transduction.**



inhibits lysogenic to lytic conversion

Repressor gene is stimulated by lysogenic

eg:-

### 1) Seroconversion in salmonella

S. anatum O<sub>3, 10, 12</sub> H<sub>6, e</sub>.

↓ Phage 15

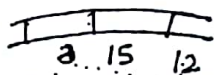
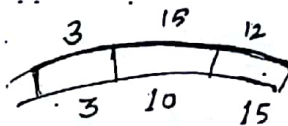
S. Newington

↑ Phage 34 O<sub>3, 15, 12</sub> H<sub>6, e</sub>

S. Minnesota

O<sub>3, 15, 34, 12</sub> H<sub>6, e</sub>

ANM



for serotyping → use monovalent 'O' antigens.

for polyvalent is used for identification of bacteria as a whole (eg. salmonella)

### 2) Corynebacterium Diphtheriae

β phage → tox gene

↓  
'toxin'

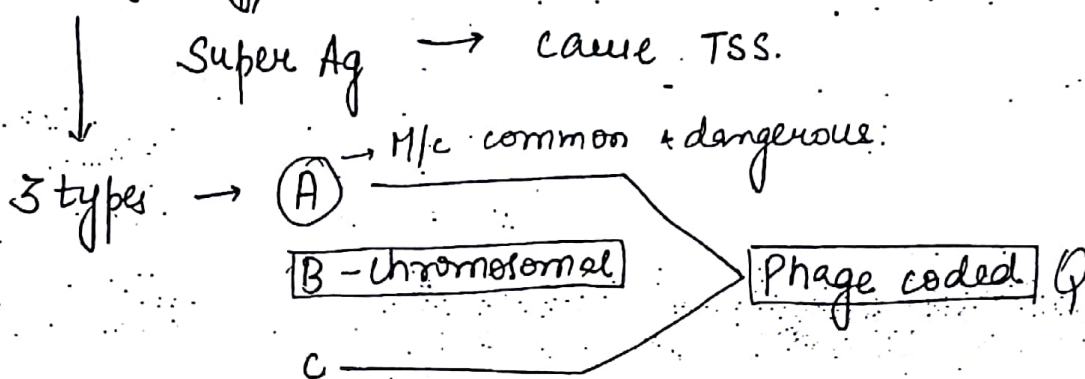
due to lysogenic phage, β phage imparts tox gene into corynebacterium leading to toxin production. then it gets released toxin, hence "pathogenic".

↓  
Hence we do toxigenicity test in Lab Δ of corynebacterium.



3) Gr. A Streptococcus (S. pyogenes)

Pyrogenic exotoxin (erythrogenic toxin)



Scarlet fever.

TSS in Group A streptococcus leads to scarlet fever.

if lysogeny occurs → ~~severe~~ severe scarlet fever

Scarlet fever w/out lysogeny → mild

4) V. cholerae

5) Shigella

6) C. botulinum

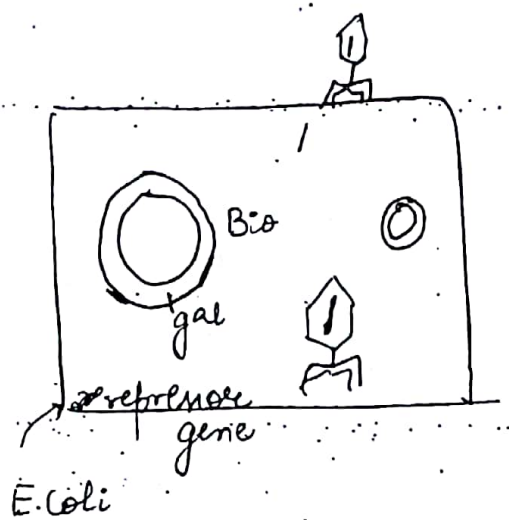
} exotoxins.

Temperate Phage ⇒ Transduction (+)

They show Lysogenic to Lytic conversion

eg. Lambda phage → insert phage DNA bet<sup>n</sup> gal + bio genes

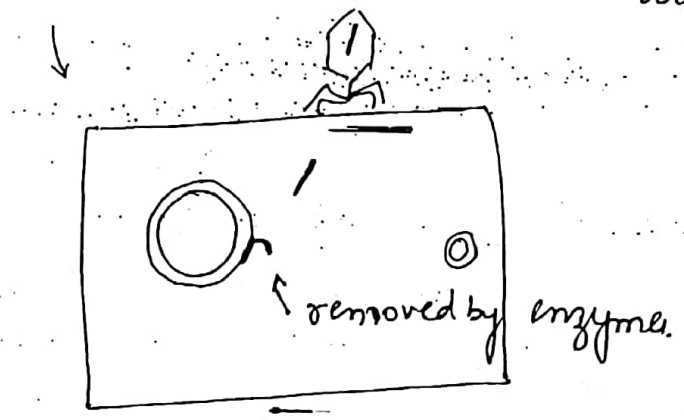
↓  
Galactose metabolism



When bacterie starts dying.  
(repressor gene stops acting)

↓  
Phage. pusten are formed,  
genetic material is taken out

↓  
due to error bacterial gene  
are also detached along  
viral genome (error in  
excision)



↓  
Phage infects other  
E. coli

Specialized or restrictive  
transduction

But due to more  
similarity to bacterial genome  
only bacterial genome is

inserted into this  
bacteria & viral  
genome is removed  
by enzymes

- Temperate phage
- error in excision
- Homologous recombination seen

- Specific genes transferred

Transduction

↓  
Lambda → genes associated  
galactose metabolism

Transduction also can lead to Drug Resistance<sup>27</sup>

## CONJUGATION

Transfer of genes from 1 bacteria to another by Plasmid

Q. Transfer of chromosomal genes by conjugation is seen in-

a)  $F^+$

b)  $F^-$

c)  $Hfr$

d)  $F'$

PGI -  $Hfr$ ,  $F'$

NBE -  $Hfr$ .

If 1  $Hfr$  (cell 1) having A, B, C & D genes after the plasmid conjugate  $\bar{c}$  100  $F^-$  bacteria. (cell 2) then predominant genotype would be

a) cell 1  $\bar{c}$  A, B, C, D

b) cell 1  $\bar{c}$  A

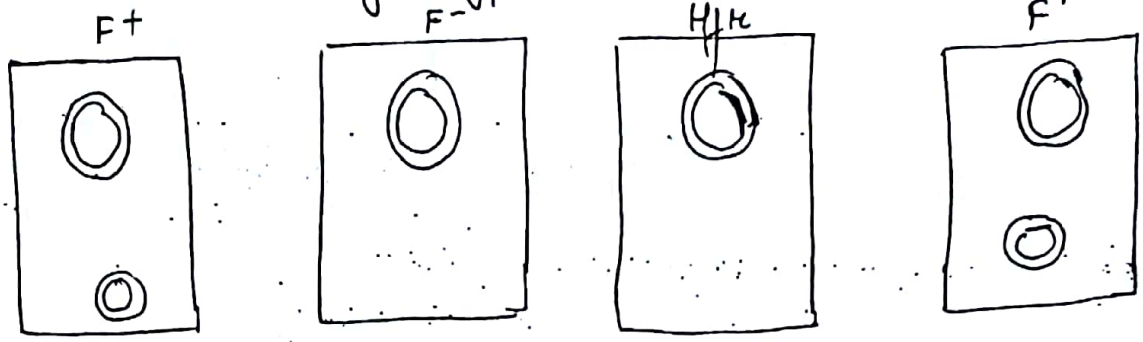
c) cell 2  $\bar{c}$  A, B, C, D

d) cell 2  $\bar{c}$  A

↳ since no change in genotype

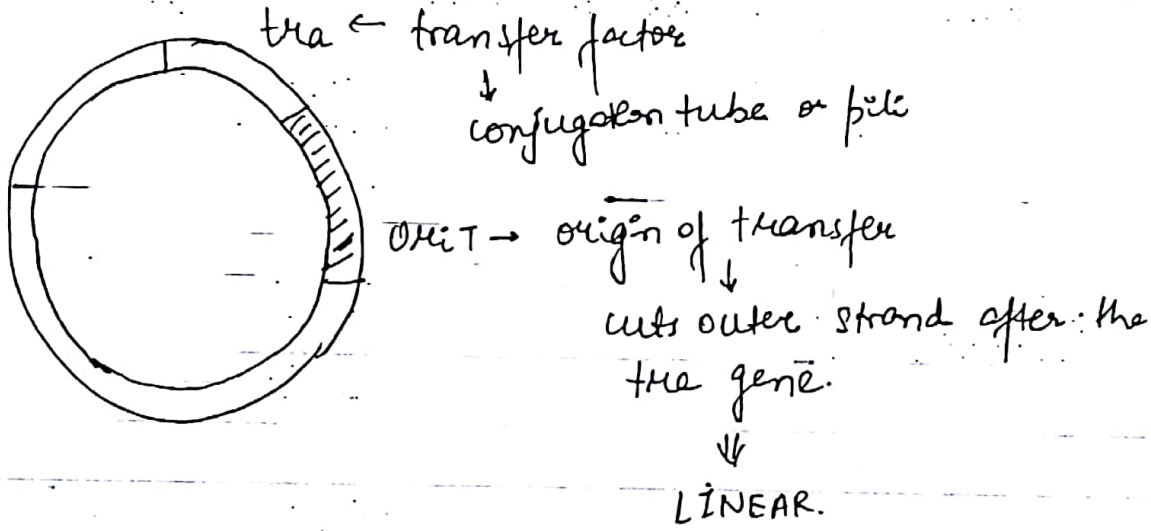
[ $F^-$  remain the same].

There are 4 genotype in bacteria

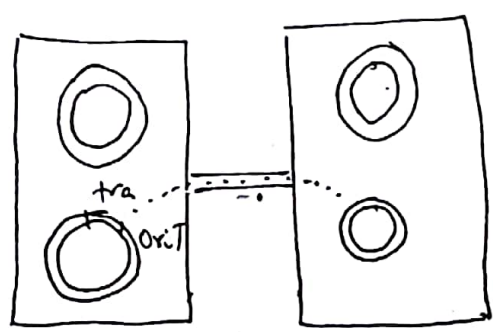


F plasmid

F' is the bacteria where plasmid contain chromosomal material



F<sup>+</sup> x F<sup>-</sup>



⇒ - change in genotype.  
 - No Homologous recombination is not seen. Q.

∴ No transfer of chromosomal genes

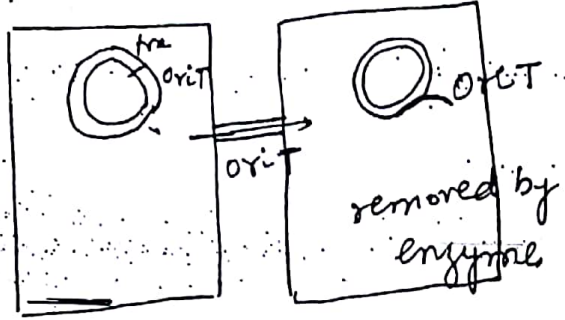
Plasmid is self replicating hence 1 strand from the other

- Horizontal Transfer
- Rapid



# Plasmid mediated Drug Resistance

$Hfr \times F^-$

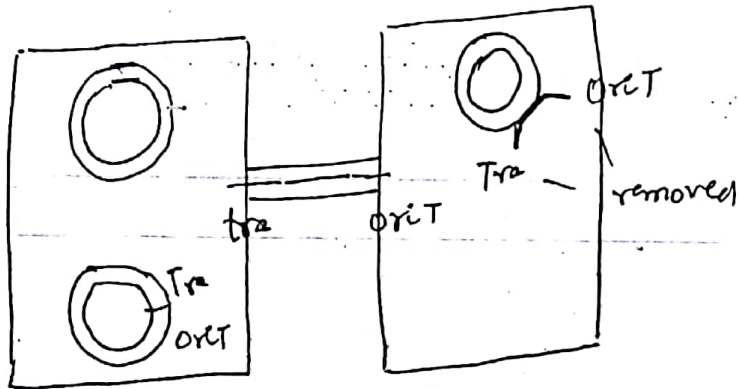


conjugation tube is stable  $\approx 10$  min

- $\Rightarrow$  No change in genotype
- $\Rightarrow$  Homologous recombination is seen
- $\Rightarrow$  Transfer of chr. genes
- $\Rightarrow$  Horizontal  $\rightarrow$  not rapid

$F' \times F^-$

- Sexduction.



- $\Rightarrow$  No change in genotype
- Homologous recombination is seen
- Transfer of chromosome genes
- $\downarrow$  frequent

$F^+ \times F^+$   
 $Hfr \times F^+$

conjugation (X)

# STERILIZATION & DISINFECTION

Killing of all organisms including spores.

↓  
Biological Indicators (BI)  
10<sup>6</sup> ~~species~~ spores of *Bacillus* sp. } Quality control.

## STEAM STERILISER (AUTOCLAVE)

- 121°C for 15 min at 15 lb pressure
- surgical equipments.
- dress material
- bandages
- culture media except L.J.

Loefer's serum slope } ↓

By Inspiration  
80°C for 1hr x 3 days

fractional  
sterilization.

## Tyndallisation

100°C for 1hr x 3 days.

Sugar media → Heat at 100°C

BI → *B. stearothermophilus*

once a week.

**BEST**

Bowie Dick Test or Vacuum leak Test

chemical indicator

→ every run.

measure the penetration of steam.

**HOT AIR OVEN**

160°c for 2hrs.

→ glassware

→ liquid

→ sharp equipments.

↳ autoclave (X)

→ chemical disinfectant can also be used for sharps

BI → **B. Subtilis subspecies NIGER**

↓

B. atrophaeus (new name)

**ETHYLENE OXIDE**

warm cycle → 50°c ± 5°c

cold cycle → 37°c ± 5°c

{ plastic ware (syringe, IV tube, catheters, urine bag)  
{ gloves

Gamma waves are preferred.

ETO → Heart Lung Machine

BI - **Bacillus Subtilis GLOBIGI**

# IONISING RADIATIONS (cold sterilization) 32

$\gamma$  rays - plastic  
glass  
catgut sutures

BI  $\rightarrow$  B. pumilio

UV rays  $\rightarrow$  Biosafety cabinets

$\downarrow$   
HEPA filter (Highly efficient particulate  
air  $(0.3 \mu\text{m})$ )

ULPA filter (ultra low particulate air  
 $(0.12 \mu\text{m})$ )

## FILTRATION

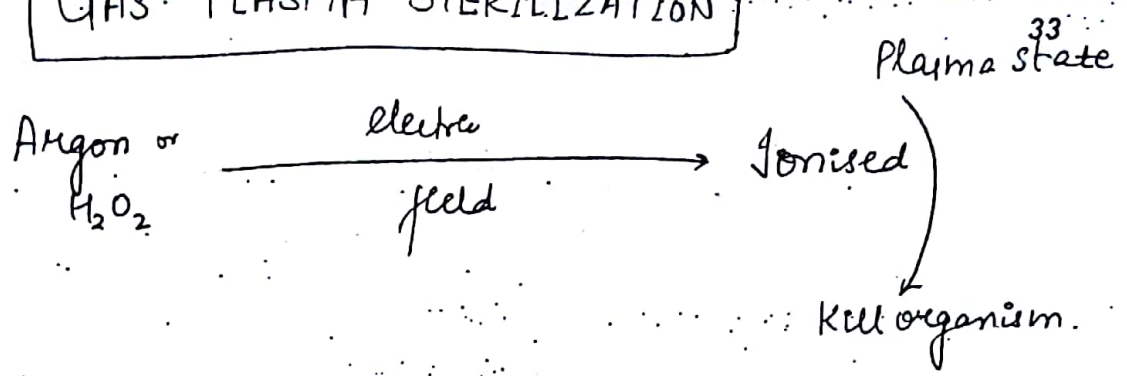
- Vaccines + anti-sera
- Membrane filters  $\rightarrow 0.22 \mu\text{m}$ .

BI  $\rightarrow$  Brevundimonas diminuta (pseudomonas)

Bubble point Testing.



# GIAS: PLASMA STERILIZATION



BI - B. atrophaeus

## DISINFECTION

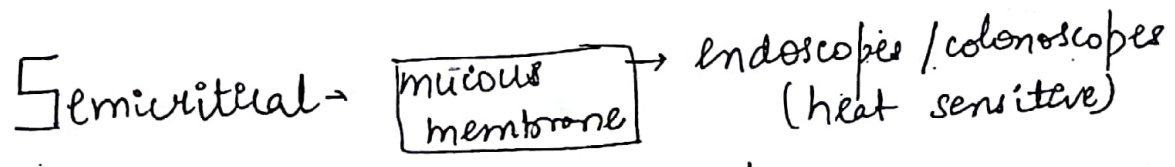
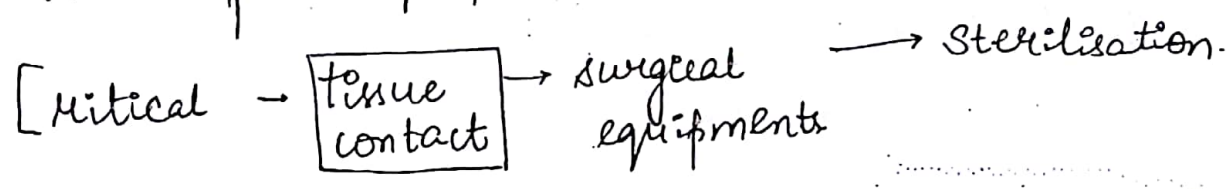
Reduction of no. of pathogens including spores

### ANTISEPTIC

Chlorhexidine

### SPAULDING CLASSIFICATION

It classifies equipments



$\downarrow$   
2% Glutaraldehyde for 20 min  
(CIDEX)

Laparoscope (critical)  $\rightarrow$  2% Glutaraldehyde for 2 hrs

Non-Critical → Skin → Thermometer

34

↓  
intermediate level disinfectant

Ethanol.

## CLASSIFICATION OF DISINFECTANTS

Level	Sporicidal	Virucidal Non-enve	enveloped	Others
High	+	+	+	+
Intermediate	-	+	+	+
Low	-	-	+	+

High → • 2% Glutaraldehyde

• formaldehyde

↓

fumigation ←

Peracetic acid + H<sub>2</sub>O<sub>2</sub>

Intermediate → alcohol

Phenol

Cl<sub>2</sub> releasing agent

Low → Quarternary Ammonia  
Lysol (cresol + soap)

## EFFICACY OF DISINFECTANT

- 1) Concentration
- 2) pH
- 3) Contact time
- 4) Organic compound → ↓ efficacy except phenol

Sputum } 5% phenol. 18 hrs  
Stool } ↓  
          } RNTCP

## TESTING OF EFFICACY

→ Phenol Co-efficient  
Reidel Walker  
Chick Martin (organic matter) } not used nowadays

→ In use Test (MIC)

↓  
→ Kelsey Sykes capacity test

↓  
In case of organic matter, this test can be done.

Endoscopes → 1st rinsed in H<sub>2</sub>O to remove organic matter  
↓  
disinfectant

Complication

↳ M. chelonae abscess

PRIONS → autoclave at 134°C for 5 hrs.

or  
✓ 2N NaOH.



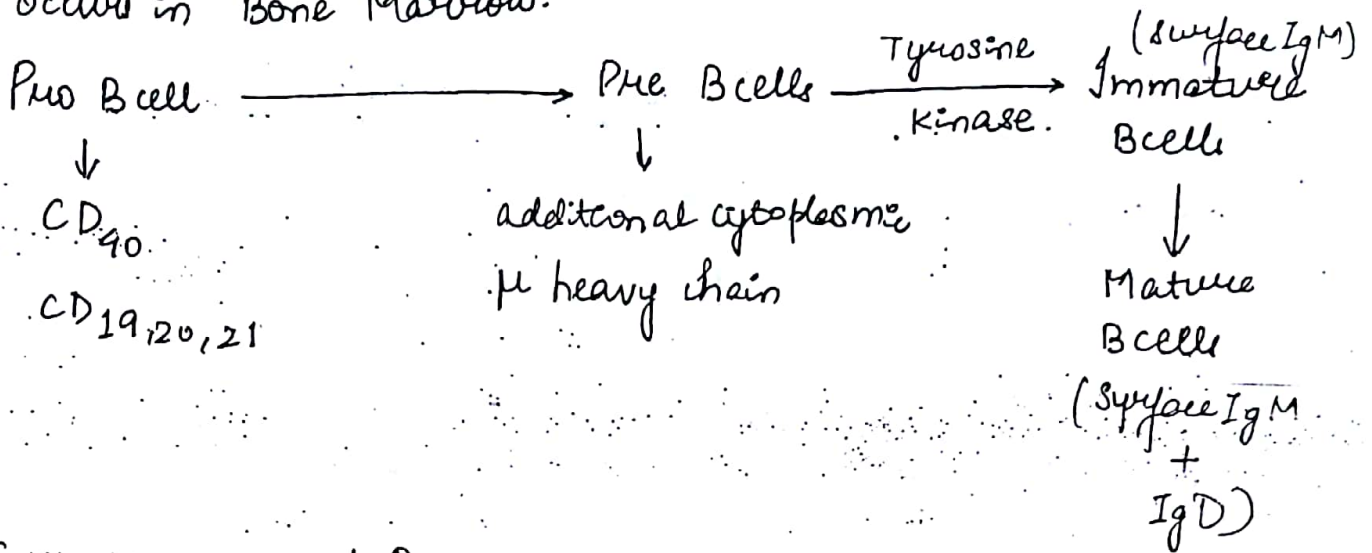
# IMMUNE SYSTEM

Attribute	Innate	Adaptive
① Response Time	min/hours	Days.
② Specificity	Low (for str. shared by group of microbes)	High (for specific Ag of microbes. <sup>every</sup> epitope)
③ Diversity	Limited.	High. $10^8$ to $10^{10}$ idio type
④ Memory	Low (only in NK cells)	High
⑤ Self/non self Discrimination	Yes	Yes
⑥ Anatomical physical barriers 1st barrier $\rightarrow$ low pH	Skin, mucosa, chemical (lysozyme, IFN $\alpha$ & $\beta$ , temp. & pH)	L.N., spleen, MALT
⑦ Blood proteins	Complement	Antibodies
⑧ Cells	Phagocytes, monocytes, macrophages, neutrophils, NK cells, other leukocytes, epithelial, endothelial cells	Lymphocytes other than NK cells



# B CELL MATURATION

occurs in Bone Marrow.



Follicular cell of Bone marrow

carries out +ve feedback selection, -ve feedback selection.

- NO MHC

- They check the maturation ⇒ +ve selection.

sent to 2° lymphoid organs.

Few B cells

↓  
autoAb.

killed by apoptosis

↓  
-ve selection

prevents autoimmunity

antigen exposure

Hence B cell, has both +ve x -ve selection.

Memory B cells (long lived) vs Plasma cells (No surface Ab, secrete Ab in blood)

- have Surface Ab

Monomer: IgM, IgG or IgA or IgE

Differential RNA splicing: IgM pentamer, IgD

[Not class-switching]

IgG or IgA or IgE (short lived)

[class-switching]

1 plasma cell  $\rightarrow$  2000 Ig/sec.

$\hookrightarrow$  short lived

Q. **One B cell**  
(mature)

$\rightarrow$  1.5 to 3 lakhs surface Ig

- IgM \* IgD

**1:1000**

$\rightarrow$  surface Ab are monomeric.

Peak Response Time in 1° Immune Response = 7-10 days

Secondary

3-4 days

## T CELL MATURATION

- Occur in Bone Marrow & Thymus

**Bone Marrow**

B cell  $\leftarrow$   $CD7^-$

Lymphoid cells.

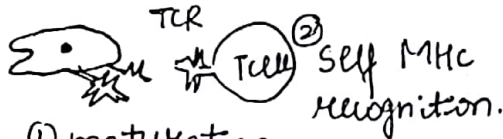
Notch1 gene  $\rightarrow$   $CD7^+$

cells expressing  $CD7^+$  go to thymus

$CD1^+, CD2^+, CD3^+, CD7^+, TCR^+, CD4^+, CD8^+$

**Cortex**

Positive selection  $\rightarrow$  ① maturation



$CD3^+, CD2^+, CD3^+, CD7^+, TR^+ \leftarrow CD4^+ CD8^- : TH$   
 $CD4^- CD8^+ Tc$

**Medulla**

95%  $\rightarrow$  Negative selection  $\rightarrow$  few T cells have TCR  $\bar{e}$   $\uparrow$  affinity for self MHC.

killed by apoptosis

**2° Lymphoid organs**

prevents autoimmunity



APC → MHC II & MHC I (nucleated cells)

Professional [ Dendritic cells ] → specialised



→ Macrophages

→ B cell

Non-Professional

→ Microglial cell (brain)

→ fibroblast on skin

→ pancreatic  $\beta$  cells

→ vascular endothelial cells

→ epithelial cells — N cells

Thymic epithelial etc.



All nucleated cells or platelets have MHC I

MHC-I → has 1 chain

MHC-II → has 2 chains.

If affinity of T cell is quite more towards self MHC

↓  
It may act as autoantigen

occurs in medulla

↓  
Hence undergoes apoptosis

# LYMPHOCYTIC HOMING

Thymus Dependent area

Thymus Independent area

Spleen Periarteriolar Lymphoid sheath

Cortical medullary follicular

Lymph node Paracortical area

germinal centre

T cell zone

B cell zone

In a person undergoing thymectomy <sup>(neonatal)</sup> at adolescence / LN Biopsy

↓  
Paracortical area undergoes atrophy  
↓  
as T cells are not formed.

## TOLL LIKE RECEPTORS

TLR1 → Mycobacteria + Gram -ve bacteria

TLR2 → Gram +ve bacteria  
Trypanosome  
Mycobacteria  
Yeast + other fungi  
Schistosoma

TLR3 → Virus

TLR4 → Gram -ve bacteria  
RSV  
Fungi

43

TLR5 → Bacteria

TLR6 → Mycobacteria  
Gram +ve bacteria  
Yeast & other fungi

~~TLR7~~ → Virus

TLR8 → Virus

TLR9 → Bacterial DNA  
Herpes virus

Malaria parasite heme byproduct

TLR10 → Unknown

TLR11 → Uropathogenic bact  
Toxoplasma

TLR12 → Unknown

TLR13 → Vesicular stomatitis virus

# ANTIGENS

Epitopes are found in antigen

↓  
They are separately immunogenic

## Antigenic Determinants

1) Size < 10k Dalton → Hapten

10-100k Dalton → Hapten or Immunogen

> 100k Dalton → Immunogen

All antigens are not immunogens but all immunogens are antigens.

## 2) Chemical Nature

Protein > polysaccharide > Lipid > Nucleic Acid

## 3) Susceptibility to tissue enzymes.

## 4) Foreignness.

directly proportional

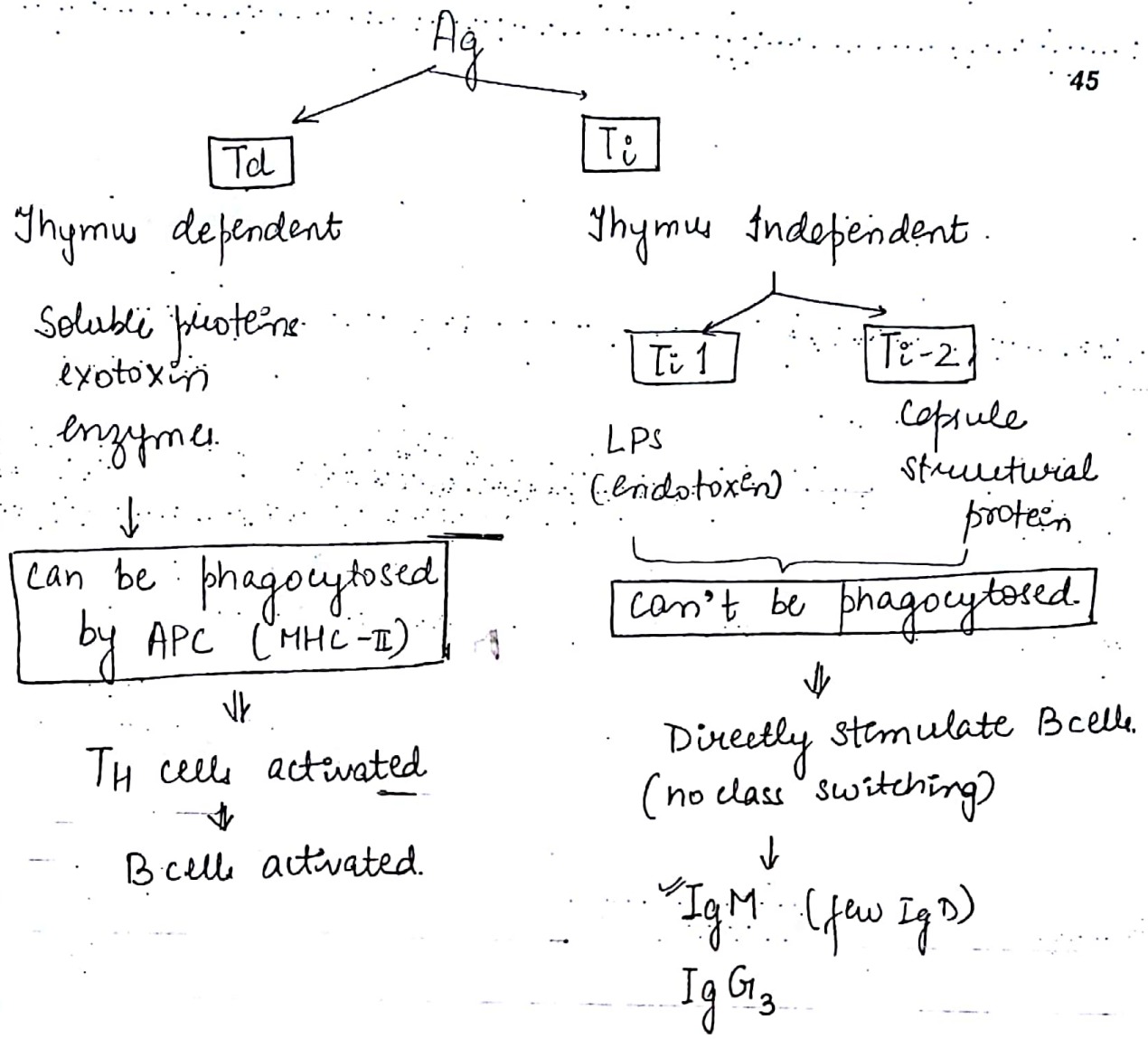
Sequestered Ag → lens protein  
sperms

↓  
can lead to  
autoimmunity

## 5) Immuno privileged sites

Cervix  
Uterus  
testis  
Brain } No immune response.



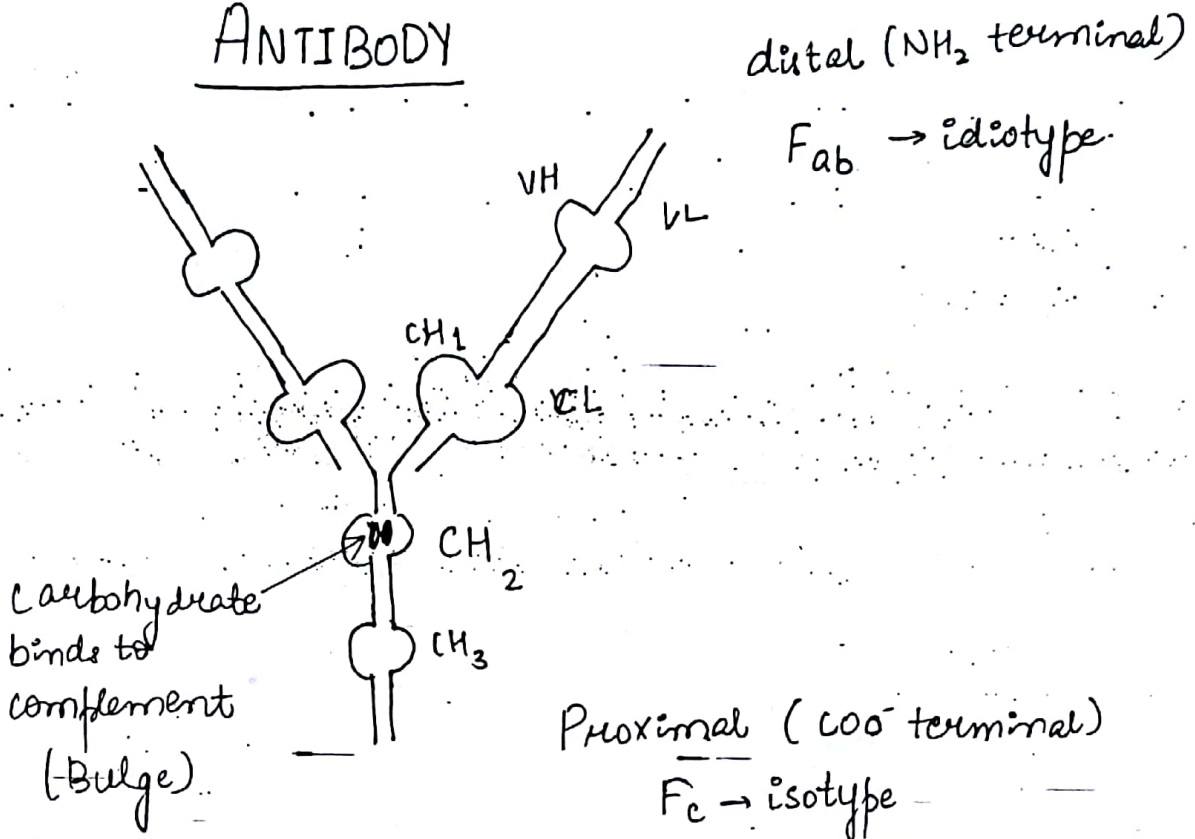


	Td	Ti
Class switching	(+)	(-)
Ab diversity	(+)	(-)
Affinity maturation	(+)	(-)
Memory response	(+)	(-)
Phagocytosis	(+)	(-)
Complement activation	(-)	(+)

B cell func<sup>n</sup> on cytokines released by T cells

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## ANTIBODY



IgM, IgE → additional CH<sub>4</sub> domain.  
Carbohydrate → CH<sub>3</sub>

Molecular wt:

IgM → 900 kDa (millionare)

IgA → 365 "

IgE → 190

IgD → 180

IgG → 150

Max carbohydrate content → IgE - 12%  
IgD - 13%

\* Idiotyp is determined by variable domain of both H & L chain. 47

↓  
Hypervariable region  
[Complementarity Determining Region]

↓  
Each Domain has 3 CDR:



3 Domain CDR

Paratope is the portion of Ab where Ag comes & bind.  
2 similar Ags can bind to both side.

Func<sup>n</sup> of Idiotyp - Antigen Binding.

\* Isotype is determined by constant region of mainly H chain & L chain

Func<sup>n</sup> ⇒ IgM → complement activation

IgG → opsonisation & ADCC (antibody mediated dependent cell mediated cytotoxicity)

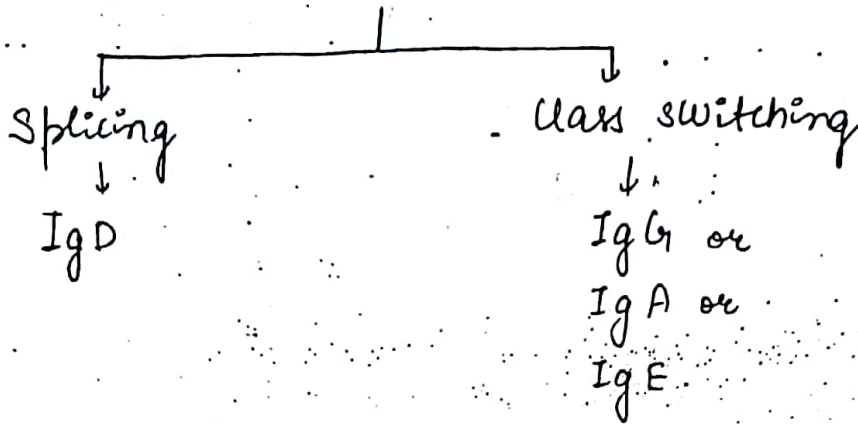
IgE → degranulation of mast cells  
basophils, eosinophils

IgA → destroy cell membrane of helminths

IgA → mucosal immunity

IgD → no biological func<sup>n</sup>

### MECHANISM OF ISOTYPE FORMATION :-



Genes encoding

H chain

L chain (kappa)

(lambda)

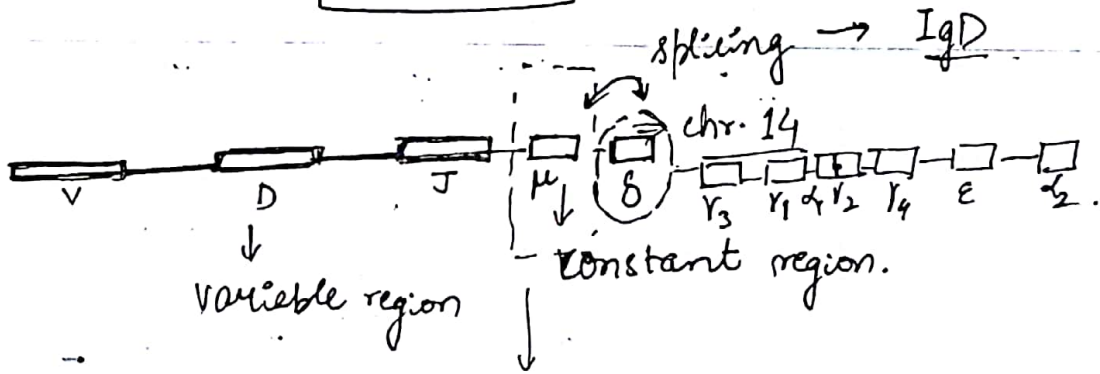
Located on:

- chr 14

- chr 2

- chr 22

$$K:\lambda = 2:1$$



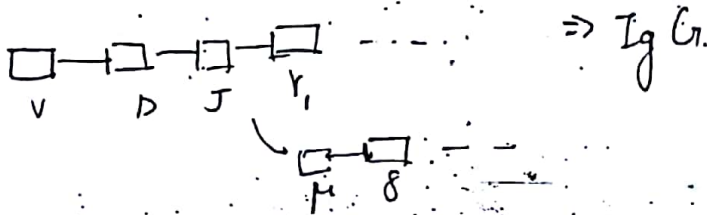
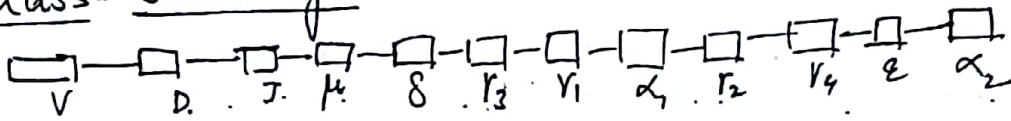
- Hence IgM is the 1st Ab formed
  - Marker of acute inf<sup>n</sup>
  - whenever B cell stimulated.
- IgM is always formed



Class-switching occurs in B cell only once.

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### Class-Switching:



1) enzymatic removal of isotype determining gene  
↓  
gene rearrangement

2) irreversible

3) occurs only once in one B cell

4)  $(T_H_2)$  (CD<sub>40L</sub>)  $\longleftrightarrow$  (CD<sub>40</sub>) B cell.  
↓  
B cell get activated.

5) Germinal centre of 2° lymphoid organs.

## Nude Mice [natural strain]

- No hair
- Chr 11 defect on Fox N1 gene
- vestigial thymus  
↓  
selective T cell deficient
- Gnotobiotic environment [Germ free environment]
- IgM (few IgD) → No class switching

## Di George Syndrome

Fish Mouth

Deformed ears

Wide set eyes

Cyanosis ← cong. heart Disease. [Fallot's M/c]

Seizure due to hypocalcaemia [no parathyroid]

No thymus

Defect - Chr 22 q11 defect

- failure of development of 3<sup>rd</sup>, 4<sup>th</sup> pharyngeal pouch

No class switching → only IgM Ab formed

# \* Hyper IgM Syndrome

defect in CD40L on T cell

↓  
selective B cell Deficiency

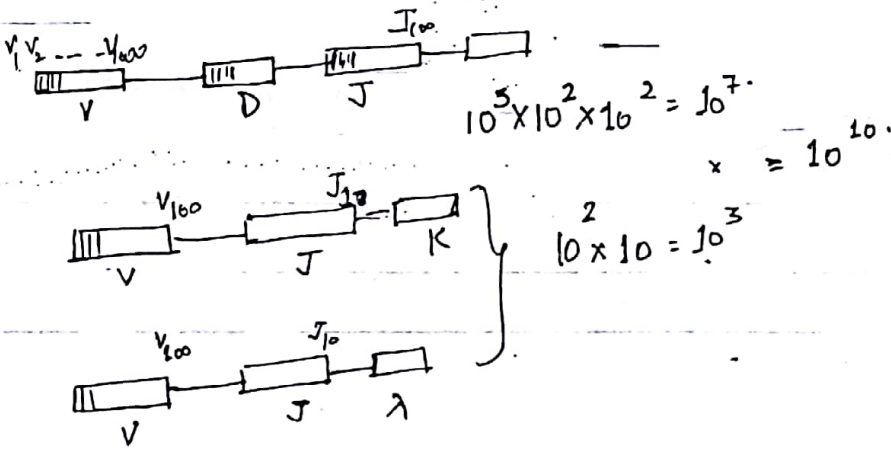
Classified on the basis of clinical outcome

- No class switching

-  $\uparrow\uparrow$  IgM  $\rightarrow 10 \mu\text{g/ml}$  [ $\text{N} \approx 1.5 \mu\text{g/ml}$ ]

## Ab DIVERSITY

Mechanism of idiotype formation



M/c. Helminthic infec<sup>n</sup> in HIV = Strongyloides.

- Recombination of multiple genes encoding for variable domain of both H & L chains

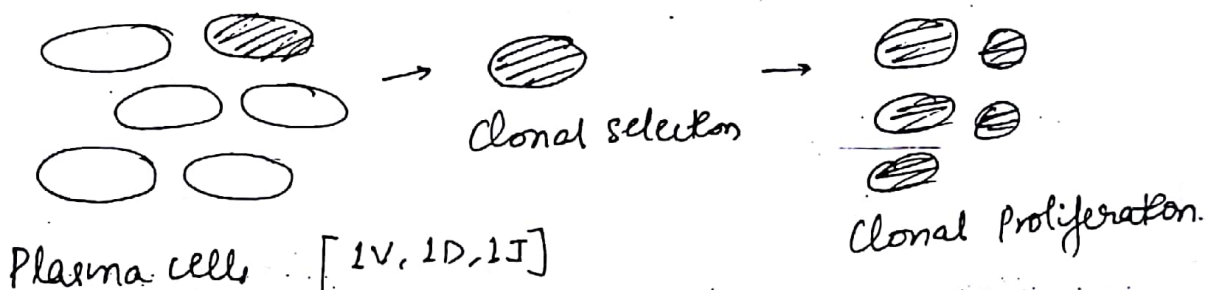
↓  
Recombination of every H chain  
= all different L chains

Nucleotides are getting added continuously, hence genes never get exhausted 52

Mutation also leads to addition of genes

- 1> Terminal nucleotide addition by tdt enzyme
- 2> removal of nucleotide by exonuclease

## AFFINITY MATURATION (Somatic Hypermutation)



Mutation in the gene in such a way that Ag binds with greater affinity in Domain.

By mutation in the variable gene,

↓  
↑ affinity

↓  
Clonal selection of that cell  
rest cell dies.

↓  
This cell undergoes clonal  
Proliferation.

Through affinity maturation we can know about present or recurrent infection.



past infect. → high affinity  
present " → low affinity

53

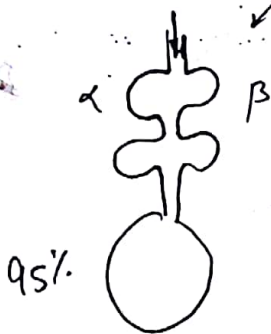
⊆ Ab has ↑ affinity?

IgG → since time taken is more

IgM → ↓ affinity → but highest ability (valency) 10

Valency of B cell Receptor = 2

T cell Receptor = 1.



18/2/18

M/c Mechanism responsible for idiotypes or Ab diversity  
= Recombination at the variable regions.

Affinity Maturation is due to mutation at the variable region genes.

⊆ ~~Isotype~~ Isotype has highest affinity IgG

⊆ " " lower " IgM.

A pt. suffering from COVID not susceptible to c Shigella species → Shigella dysenteriae<sup>54</sup>

The B cell stage predominant in Bruton hypogammaglobulinemia → Pre B cell

The cytokine responsible for pathogenesis of salmonella gastroenteritis → IL8

Negative selective helps in prevention of c type of disorder - Autoimmune

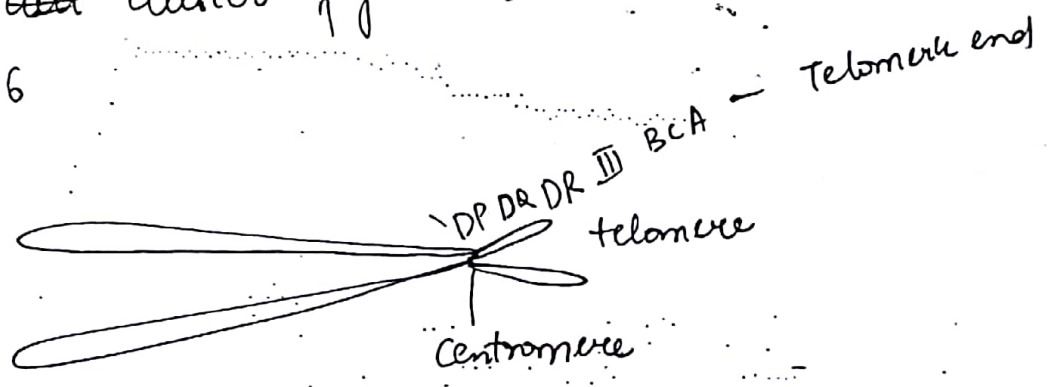
## ALLOTYPES

Subtle amino acid difference in the constant region of H chain & L chain.

↓  
Allelic Exclusion

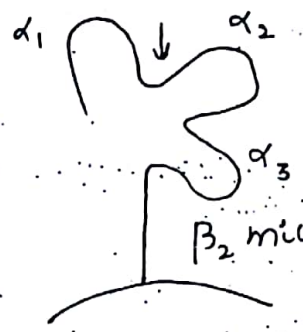
## MHC [MAJOR HISTOCOMPATIBILITY COMPLEX]

It is a ~~ext~~ cluster of genes located on short arm of Chr. 6



CD Player  
Centromere

MHC-I



$\beta_2$  microglobulin encoded by Chr 15

- Site of binding of Ag in MHC-I  $\Rightarrow$  Distal  $\alpha_1, \alpha_2$ .
- Peptide of length 8-10 AA can bind to this site
- Deficiency of  $\beta_2$  microglobulin leads to
  - Hereditary Haemochromatosis
  - Due to mut<sup>n</sup> in 283 position of Hfe gene



Due to this mutation

Cysteine is replaced by Tyrosine

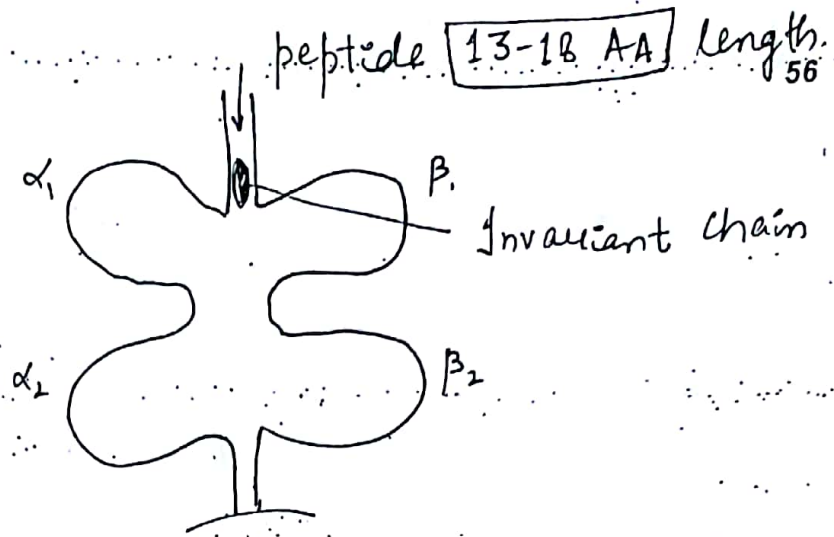


[ $\beta_2$  microglobulin regulates absorp<sup>n</sup> of Fe]



So Iron overload may occur

## MHC-II



- Invariant chain prevents blocks binding of normal peptide.

↓  
Hence MHC-II prevents autoimmunity

- Defect in Invariant chain leads to autoimmunity  
[is due to defect in DQ, DR region]

## MHC-III

It codes for complement protein  $C_4 + C_2$   
Heat shock proteins

TNF $\alpha$  +  $\beta$

21 Hydroxylase.



# ANTIGEN PROCESSING (Td)

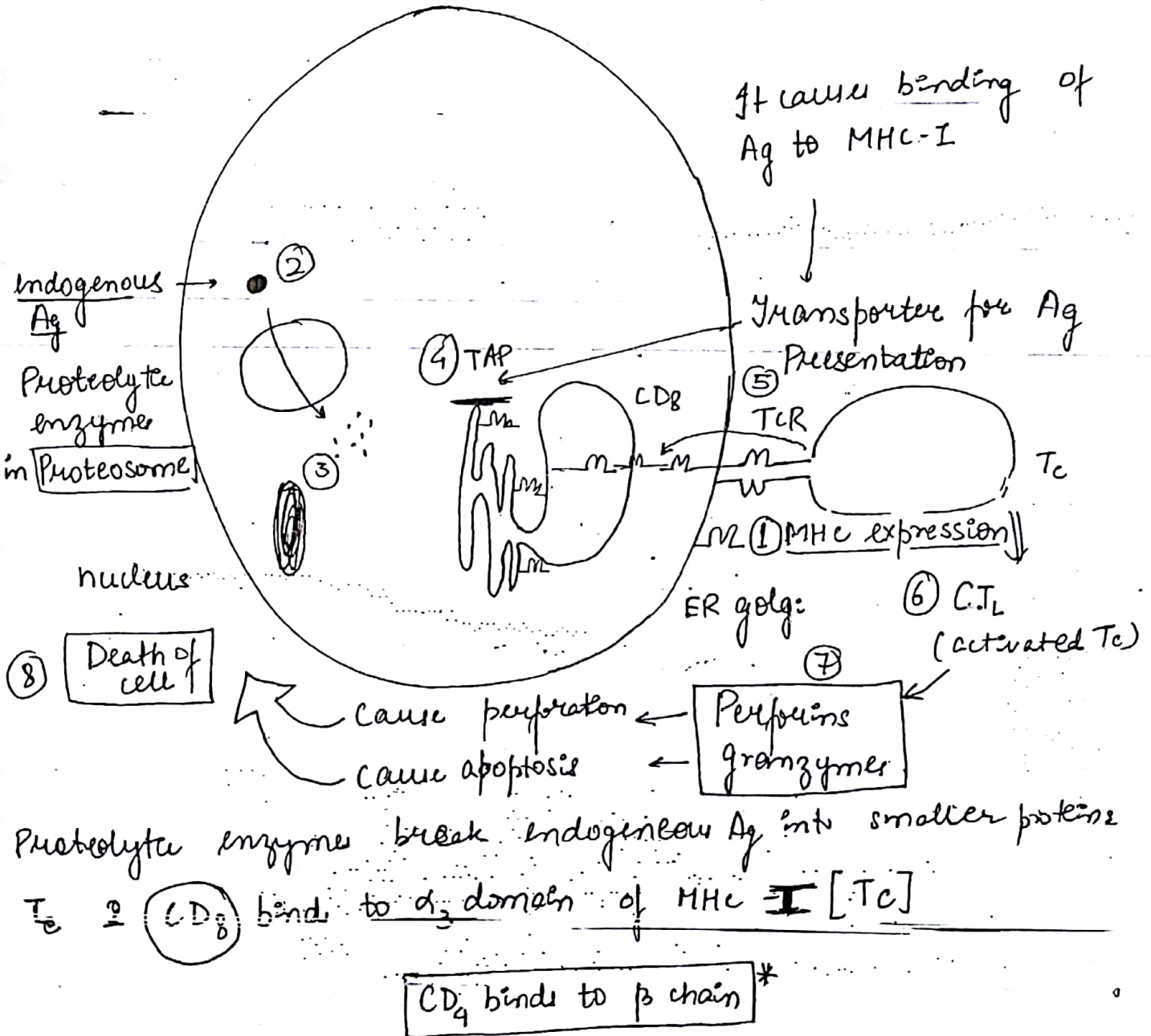
Td Antigens

Endogenous Ag

Exogenous Ag

↓  
 Tumour Ag cells  
 Virus infected cells

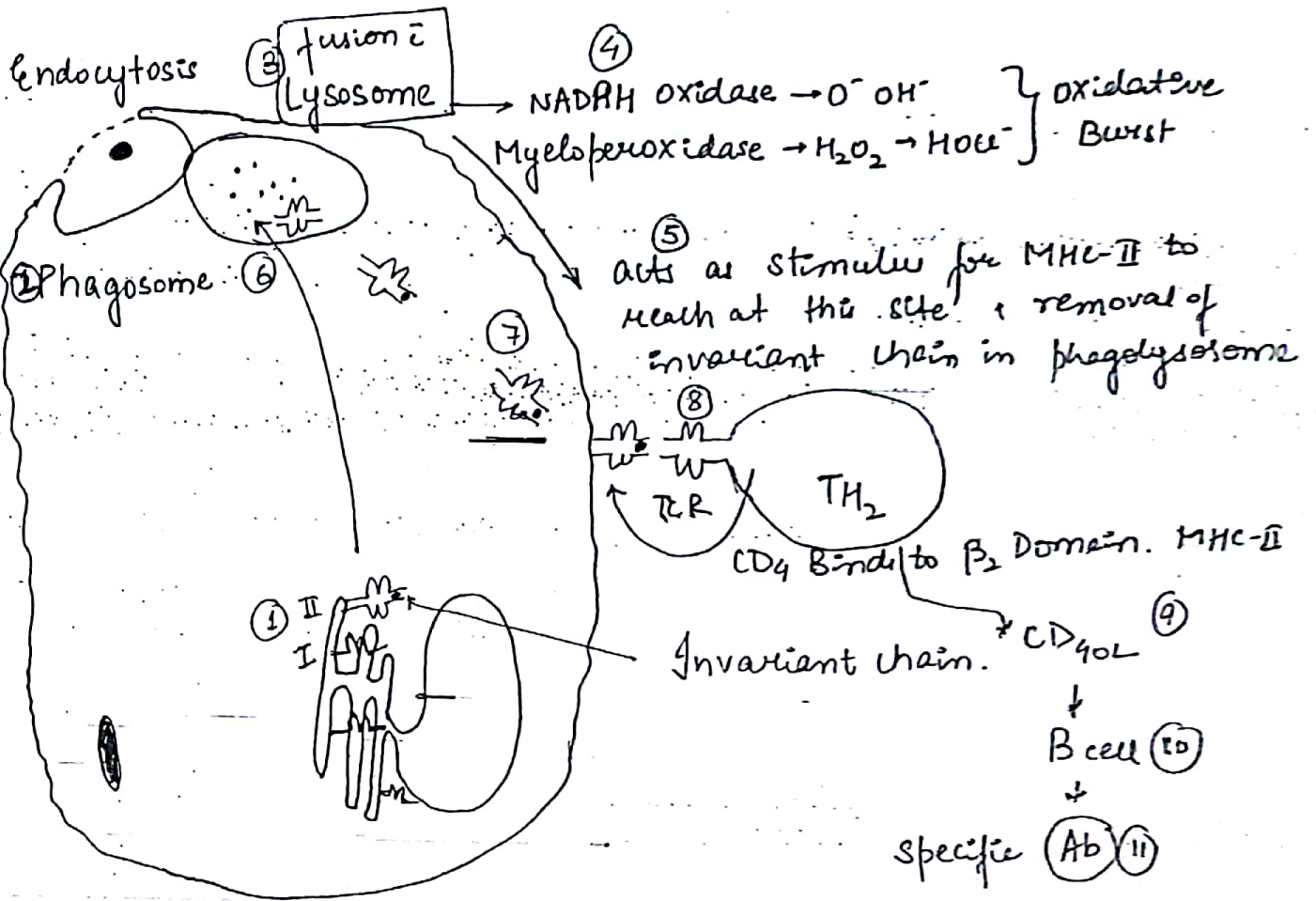
## I Endogenous Ag Processing





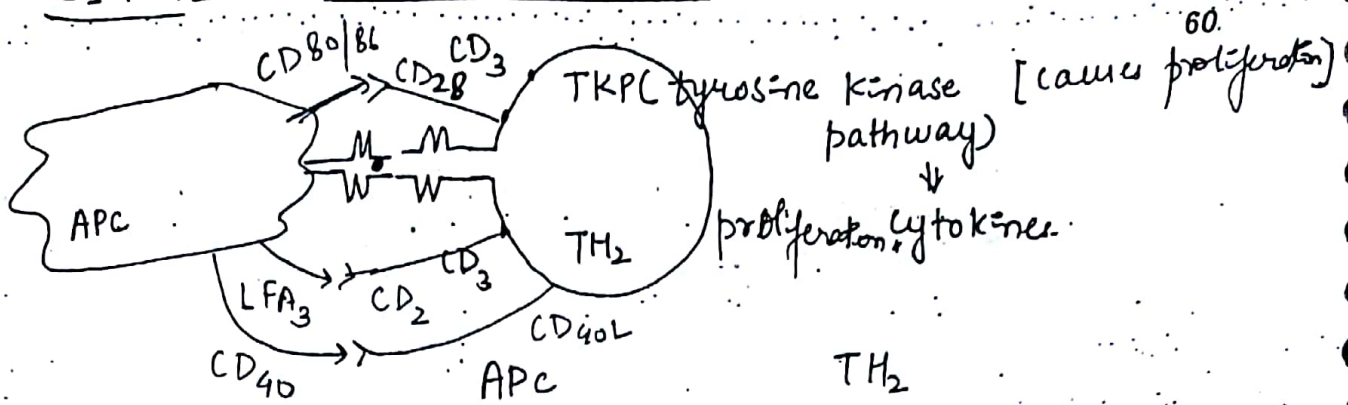
\* Exogenous Ag. Processing

B cell → pinocytosis 59



SIGNAL TRANSDUCTION OF T CELL

# SIGNAL TRANSDUCTION



1st Signal

Ag restricted  $\bar{c}$   $\leftrightarrow$  TCR  
MHC-II

2nd Signal

(costimulatory signal)

$B_7$  (CD80/86)  $\leftrightarrow$  CD28

LFA3  $\leftrightarrow$  CD2

CD40  $\leftrightarrow$  CD40L

3rd Signal

Signal Transduction

CD3 activated

$\downarrow$   
TKP activated

$\downarrow$   
Proliferation  
cytokines  $\uparrow\uparrow$



TH0 [Naive TH cells]

IFN $\gamma$

IL4

TH1

Produce

IL2

IL12

TNF $\beta$

IFN $\gamma$

form IgG

activate macrophages  $\bar{c}$   
(intracellular pathogens)

IFN $\gamma$   $\uparrow \uparrow \uparrow$

Neurosis

TH2

IL13

TGF $\beta$

IL4

IL5

IL6

IgA

Proliferation of many cells.

Class Switch B cells

IgE

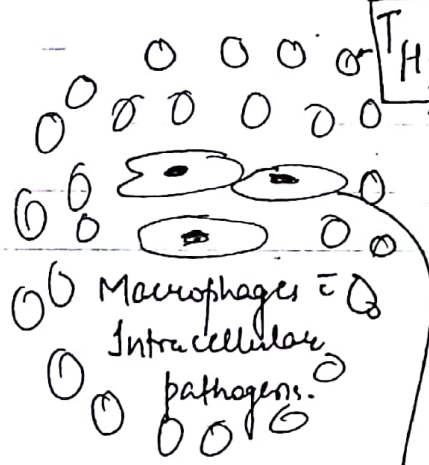
TH1  $\rightarrow$  IFN- $\gamma$   $\rightarrow$  IgG  
B cells  
TH2  $\uparrow$

For formation of IgG  
TH1 + TH2 both have to play the role as  
IFN- $\gamma$  is released by TH1

Plasma cells

Humeral Immune Response.

TH1 Lymphocyte



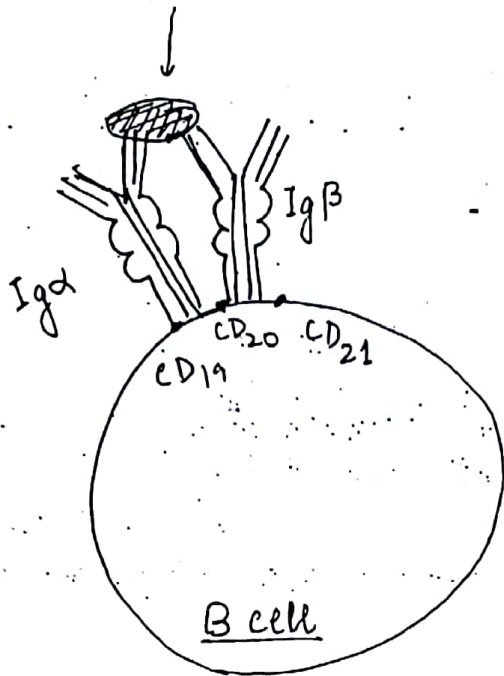
TH2 is not able to act + produce Ab through B lymphocytes.

epithelioid cells

Granuloma

Cell mediated Immune Response

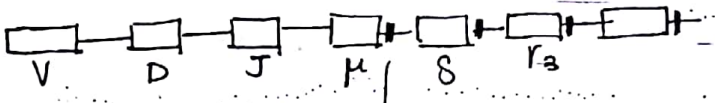
Ti Ag



Ig Superfamily

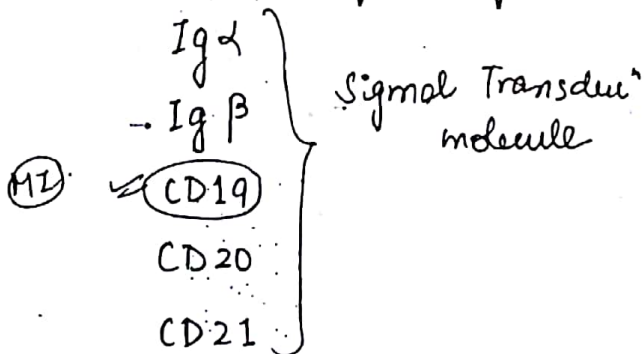
Ig $\alpha$  + Ig $\beta$

H + L chain



This gene encodes for Ig $\alpha$  - Ig $\beta$  chains } Differential RNA Splicing (Plasma  $\rightarrow$  Memory)

### Signal Transduction by Ti Ag



Rest is same [TKP activation + following]

IgM  $\rightarrow$  Complement System

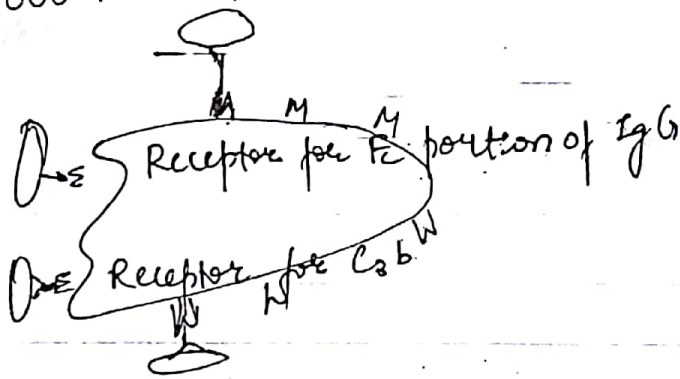
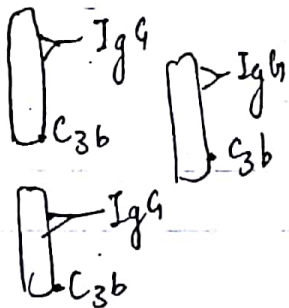
# COMPLEMENT SYSTEM

## Functions :-

- 1) Opsonisation (C<sub>3b</sub>)
- 2) MAc mediated lysis of bacteria (C<sub>5b-9</sub>)
- 3) Immune complex clearance (C<sub>3b</sub>)
- 4) Chemotaxis by smaller fragments (C<sub>5a</sub>)
- 5) Ag-Ab complex activation
- 6) Memory

## OPSONISATION

↑ phagocytosis ⇒ 4000x



## Opsonins

Innate response

- Collectins SPA,
- SP-D,
- MBL

L-ficolin

C1q

\* C<sub>3b</sub>

C<sub>4b</sub>

C<sub>3b</sub>

cleavage products (iC<sub>3b</sub>, C<sub>3c</sub>, C<sub>3dg</sub>)

Adaptive response

IgA

IgG\*

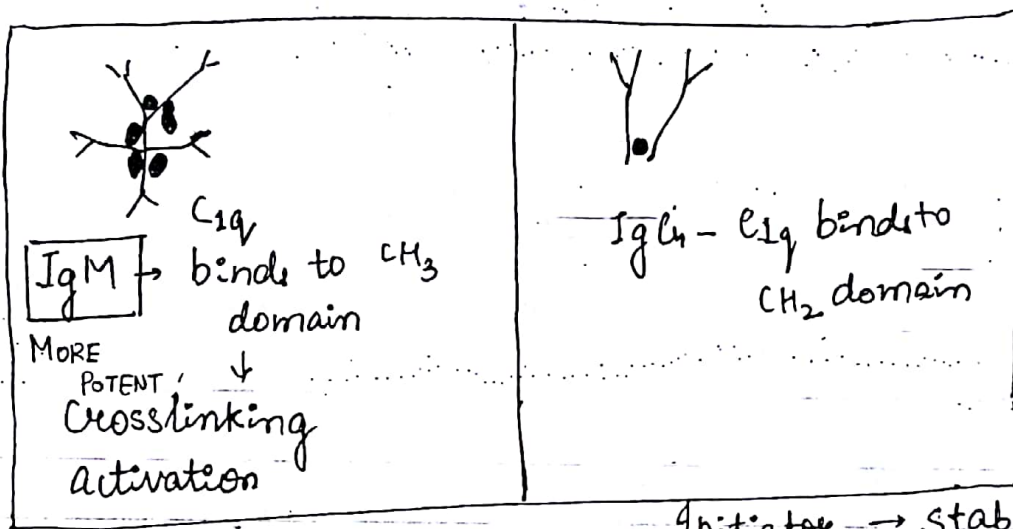
CRP

Max. complement protein  $\Rightarrow$  Bound  $C_3$ .

$C_3$  convertase bound  $C_3 \leftarrow \begin{matrix} C_{3a} \\ C_{3b} \end{matrix}$

(I) **Classical**  
IgM/IgG

(II) **Alternate**  
Free  $C_3$  in blood  $\leftarrow \begin{matrix} C_{3a} \\ C_{3b} \end{matrix} \rightarrow$  degrade



IgM/IgG binds to Ag +  $C_{1q}$

$C_4 \rightarrow C_{4a}$   
 $C_{4b}$

Factor D or Properdin  $\downarrow$

Factor B

Ba  $\leftarrow$  Bb

$C_{4b2a}$   $C_3$  convertase  $\rightarrow$  bound  $C_3$

$C_{3bBb}$   $C_3$  convertase

$C_{3a}$   $C_{3b}$

$C_5$  convertase  $\rightarrow C_{5b-9}$

$C_{3bBb3b}$   $C_5$  convertase



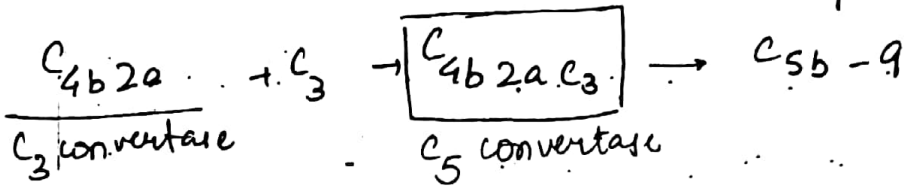
Neisseria Inf<sup>n</sup>  
It doesn't allow opsonisation.

← Lysis

MAC

65

So,



### Indicators of Alternate

- 1) Endotoxin
- 2) Snake venom
- 3) Yeelyporosoma protein
- 4) Zymosan on yeast
- 5) Nephritic factor
- 6) Dextran sulphate
- 7) Inulin
- 8) IgG, IgA, IgD.

$C_{4b}$  binds to  $C_2$ , expose it to the action of  $C_{1s}$

↓  
 $C_{1s}$  cleave  $C_2$  into  $C_{2a}, C_{2b}$

Yes Snake<sup>ne</sup> In & End Ymy, Kia → GRAD

IgE, IgG<sub>4</sub> doesn't activate complement

### (11) LECTIN PATHWAY

MBL (mannose binding lectin)

↓  
It activates  $C_{1q}$  directly

↓  
rest similar to classical pathway.

# REGULATION OF COMPLEMENT

66

- | <u>Protein</u>                                   | <u>Function</u>   |
|--|---|
| 1) CI Inhibitor                                  | → Dissociation of inhibition of $C_{1r, s_2}$ from $C_{1q}$                                       |
| 2) Decay accelerating factor (CD55)              | → Dissociation of $C_3$ convertase  |
| 3) <del>CR1</del><br>C4BP<br>Factor H            | } → Dissociation of $C_3$ convertase.<br>Cofactor for factor I                                    |
| 4) Factor I                                      | → Cleaves $C_4b$ & $C_3b$   |
| 5) Membrane cofactor of proteolysis<br>MCP       | → Cofactor for factor I   |
| 6) S protein or vitronectin                      | → Binds soluble $C_5b_6_7$ & prevents insertion into host.  |
| 7) Protectin (CD59)<br>Carboxypeptidase N, B & R | → Blocks binding of $C_9$ & formation of MAC<br>→ Inactivates the anaphylaxis $C_{3a}$ & $C_{5a}$ |

Deficiency of  $C_3$   $\Rightarrow$  No Opsonisation.  
(bound)

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$\downarrow$   
Pyogenic Infect.  
GN

Deficiency of Late complement proteins  $\Rightarrow$  Recurrent Neisserial  $\phi$ .  
Infection.

[MAC mediated Lysis  $\ominus$ ]

Deficiency of Decay accelerating factor ( $CD_{55}$ )  $\Rightarrow$  PNH.

Deficiency of  $C_1$  inhibitor = Hereditary Angioneurotic edema

$\downarrow$

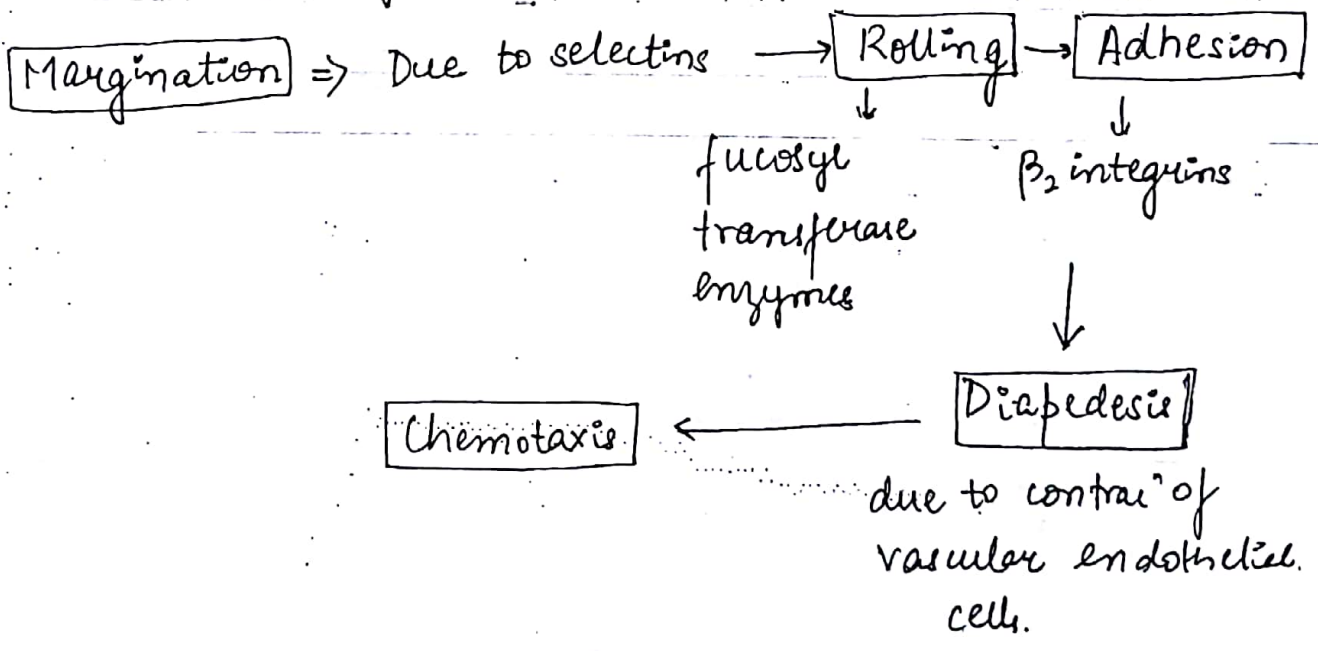
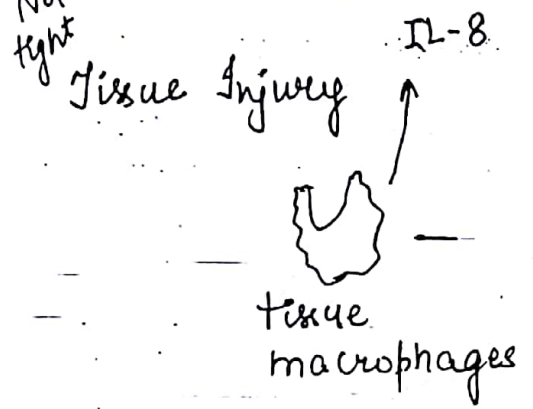
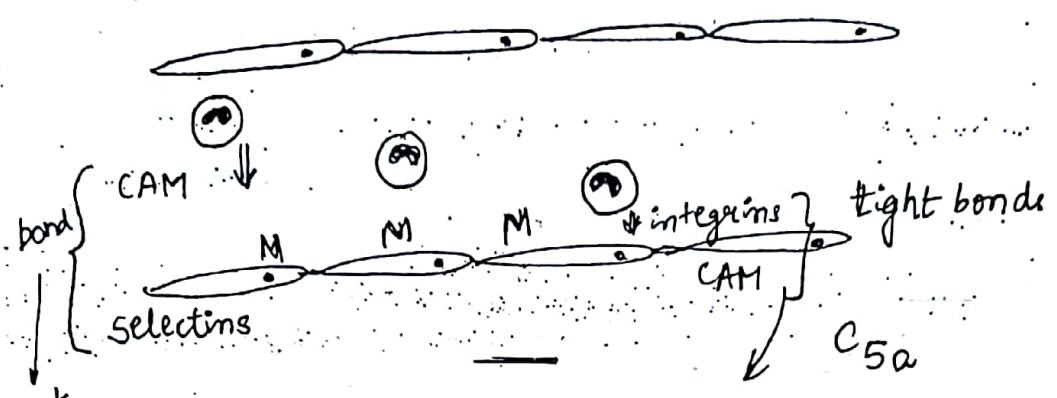
Swollen lips —  
Painful abdominal edema  
Laryngeal edema

$\Delta$  - Best marker

$\downarrow$   
 $\downarrow$   $C_4$  Level

# PHAGOCYTOSIS

## Extravasation of Neutrophils

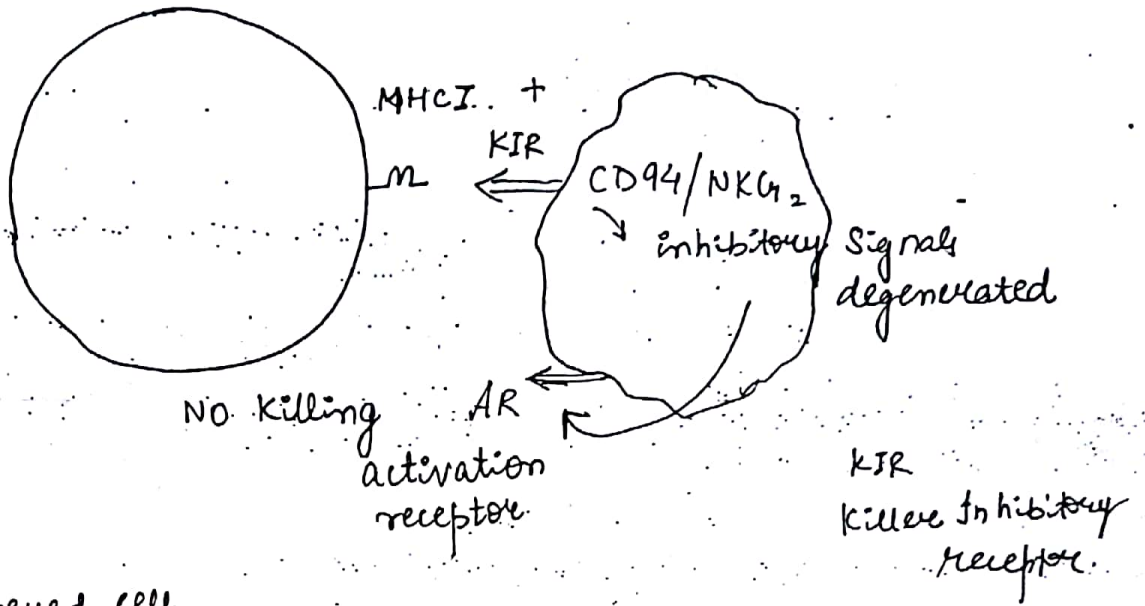




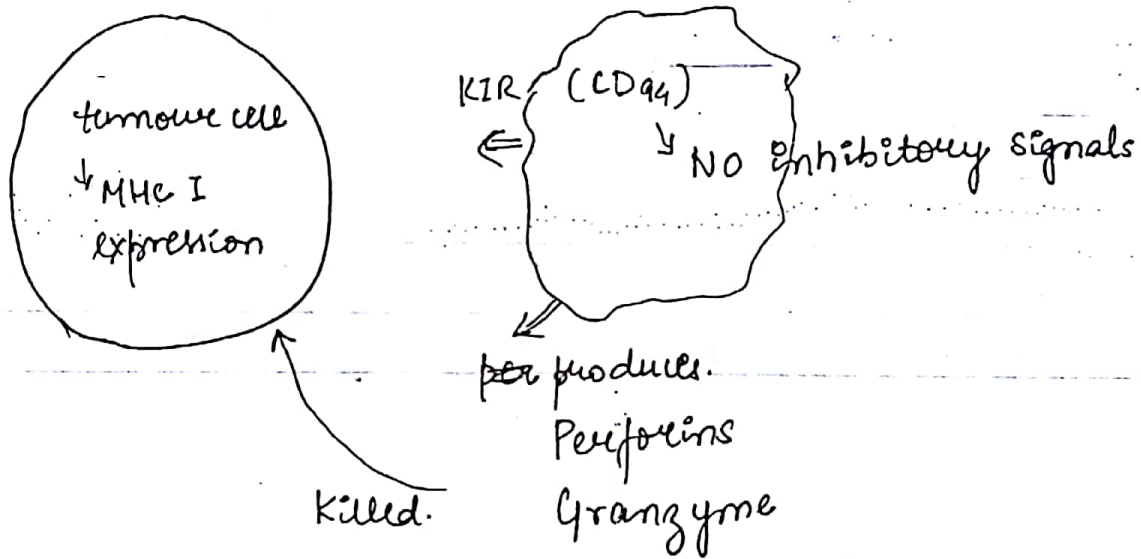
# NK Cell

MHC-I in exogenous Ag

Normal cell

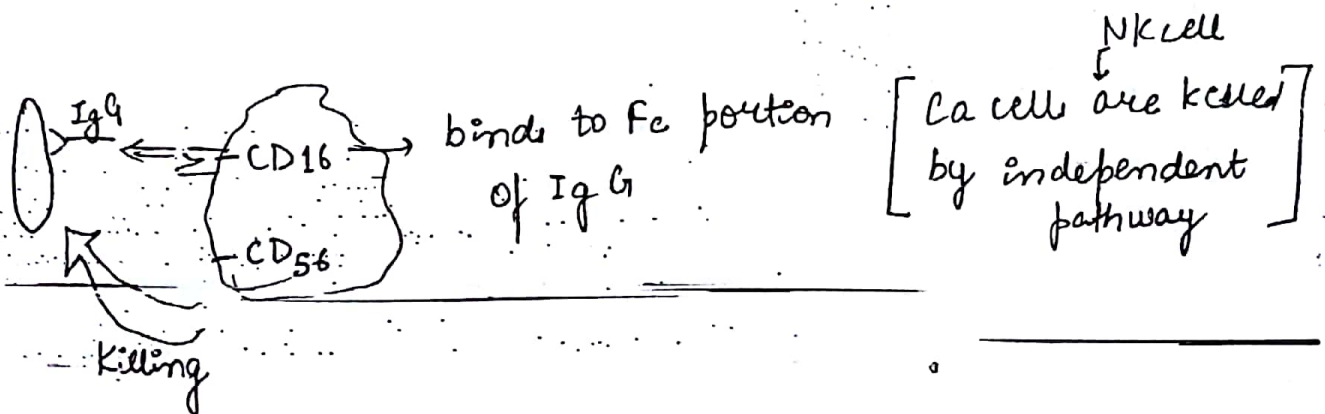


altered cell



Ab independent killing by NK cells.

NK cells → ADCC (Ab dependent cell mediated cytotoxicity)



# DEFICIENCY OF PHAGOCYTOSIS

70

## (I) Leucocyte Adhesion Deficiency (LAD)

Recurrent infections

No pus  $\rightarrow$  as neutrophils can't come out

Omphalitis

Extreme neutrophilia ( $>30,000/\mu\text{L}$ )

**LAD I**

$\rightarrow$

mutation in  **$\beta_2$  integrin (CD18)**, gene (adhesion)

AR

**LAD II**

$\rightarrow$

Deficiency of **fucosyl transferase** [selectin]

$\downarrow$   
rolling  $\ominus$

**LAD III**

Deficiency defect in **regulatory protein Kindlin**  
(fermt3)  $\subseteq$  activates ligand for  $\beta_2$  integrin.

**LAD I > LAD III > LAD II**

## (II) G6PD Deficiency

Deficiency of enzymes in **HMP shunt**

Same as CGD (chr 10p15)  $\bar{c}$  associated

Anaemia

III. MPO Deficiency  
Granule enzyme Deficiency

IV. Chediak-Higashi Syndrome

AR

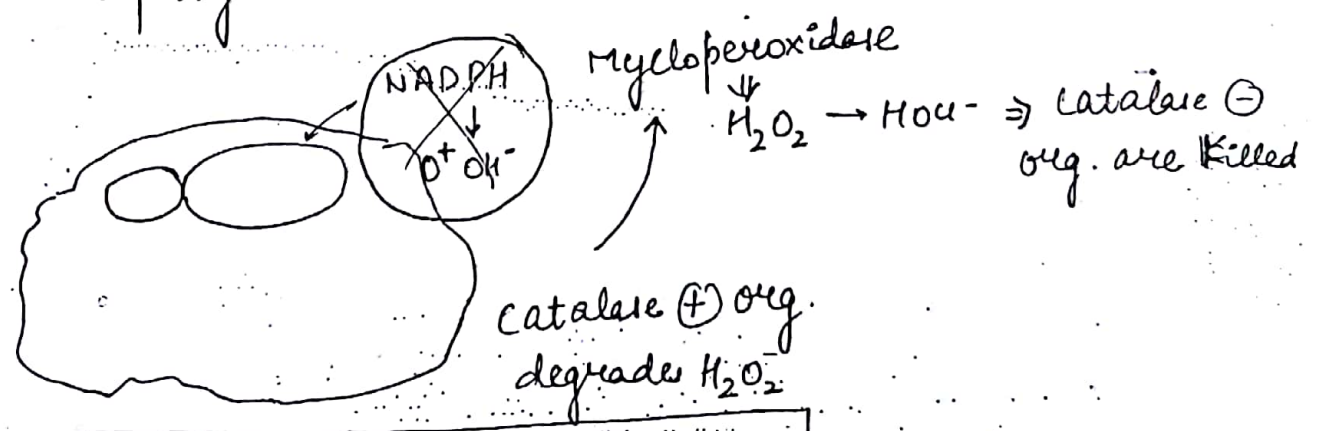
Defective Intracellular transport Protein (LYST).  
Recurrent infec<sup>n</sup>, Chemotactic factor, degranulation defect.

~~Absent~~ NK cells  
Partial albinism.

Δ - Giant Lysosome

V. Chr. Gr. Ds  
- XL or AR

- Deficiency of NADPH
- Recurrent Infection of  $\bar{c}$  Catalase +ve Bacteria & Fungi



Candida	Staph. aureus
Aspergillus	Haemophilus Inf.
	Mtb
	Enterobacteriaceae



① NBT (Nitroblue Tetrazolium) ⊖

2) Neutrophil Oxidative Index

if agammaglobinemia  
recurrent catalase +ve infec<sup>n</sup> ] ⇒ Bruton's  
NBT ⊕

B cell Deficiency

1) Bruton agammaglobinemia

Deficiency of tyrosine kinase B cell maturation  
XL

No Ig

Prec B cells in bone marrow  
narrow CMI

2) X Linked hyper IgM Syndrome

- Deficiency of CD40L on activated T cell

- Recurrent resp. + GI infec<sup>n</sup> [IgA is absent]  
↳ mucosal immunity

3) Selective IgA deficiency

IgA - M/c deficiency

Repeated sinopulmonary + GI infec<sup>n</sup>

No role of passive immunisation

↳

Passive Immunisation  
Delivered ⇒ IgG



#### 4) Common Variable Hypo $\gamma$ globinemia

Onset - Late teens

B cell  $\uparrow$  in blood

$\downarrow$  Ig over time

$\uparrow$  autoimmunity

73

#### 5) Transient $\gamma$ hypogammaglobinemia of infancy

Delayed onset of normal IgG synthesis

Detec<sup>n</sup> in 5<sup>th</sup> - 6<sup>th</sup> month.

Resolves by 2 1/2 years

#### 6) Job Syndrome [Hyper IgE syndrome]

Due to deficiency of T helper 17 [TH17] cells.

Retained 1<sup>o</sup> tooth

Cold abscess

Coarse facies

Eczema

TH17 cell  $\rightarrow$  encoded by chromosome 17.

Differentiated from TH0 cell by IL-6, TGF $\beta$ , IL-23

TH2 produces  $\rightarrow$  IL-17, IL-22

IL17A IL17F

$\downarrow$

Stimulate B cells to produce

$\uparrow$  Ab except IgE

$\downarrow$

- ① Prevent pyogenic fungal Inf
- ② Inflammation

③ autoimmunity

④ transplant rejection. (ADCC) 74

p40 knock out Mice → Deficient  $T_{H17}$

↓  
↑↑ IgE

## T cell Deficiency

1) Di George Syndrome

2) Bare Lymphocyte Syndrome (BLS) [Image]

Granulomatous necrotizing Lesion in mid face

Type I → TAP Deficiency → MHC I ↓

Type II → MHC II ↓

## Combined partial T + B cell Deficiency

Wiskott Aldrich Syndrome

XL

Defect in cytoskeletal glycoprotein (WASP)

Ataxia Telangiectasia

Defect in Kinase involved in cell cycle

Ataxia

Telangiectasia

Def of IgA + IgE.

# Complete Functional B & T cell Deficiency 75

## SCID

- **IL-2R $\gamma$**  or **JAK 3** deficiency [cytokine Receptor deficiency]  
Defective signals from IL4, 7, 9, 15, 21  
XL, AR

- Adenosine Deaminase **ADA** or **PNP** (Purine nucleoside phosphorylase) deficiency toxic metabolite in T, B cells.  
AR

- Zeta chain associated protein **ZAP** deficiency  
defective signal from **TCR**

- **Rag 1** or **Rag 2** nonsense mutation, AR  
No TCR or Ig gene rearrangement  
**Total absence of B & T cells**

# HYPERSENSITIVITY

76

	Immune mediator	Immune Response	Response Time
Type I	IgE	Humoral	2-30 min
Type II	IgG > IgM	"	5-8 hr.
Type III	Immune complexes (IgG)	"	2-8 hr.
Type IV	T cells	Cell Mediated	24-72 hrs.

## TYPE - I

→ 1° response to allergen.



Sensitisation of mast cell

$\bar{c}$  IgE



→ 2° response to same allergen



Cross-linking



degranulation





## Mediators of Type I HSN

1°

Histamine, Heparin

Serotonin

Eosinophil chemotactic factor

Neutrophil chemotactic factor

Proteases (trypsin, chymase)

2°

77

PAF

Leukotrienes (SRS-A)

PGI<sub>2</sub>

Bradykinin

Cytokines

IL 1, 3, 4, 5, 6, 10, 13

TNF $\alpha$

TGF $\beta$

GM-CSF

Egs.

1) Anaphylaxis

2) Atopy

3) Allergic rhinitis (Hay Fever)

4) Asthma

5) Food allergy

6) Allergic eczema

7) ABPA (Type I > Type III > Type IV)

[allergic bronchopulmonary aspergillosis.]

# Type - II

## Autoimmune

**Cytotoxic**

**Non-cytotoxic**



- ADCC  
↑  
Complement mediated  
autolysis

cellular func<sup>n</sup> altered by  
autoantibody.

eg. Grave's Ds

eg,  
1) Autoimmune haemolytic  
anaemia

2) Agranulocytosis, thrombocytopenia

3) ARF

4) Goodpasture Syndrome

5) Transfusion Reac<sup>n</sup>  
(ABO incompatibility)

6) Erythroblastosis fetalis  
(Rh incompatibility)

7) Drug induced haemolytic  
anaemia

[black water fever]  
[penicillin therapy]

8) ~~Myocarditis in Chagas Ds~~  
(American Trypanosomiasis)

9) Hyperacute Graft Rejection

1) Myasthenia Gravis

2) Grave's Ds

3) Type II non-insulin dependent  
DM

4) Pernicious Anaemia

## Type - III

Non-specific

79

Immune complexes



causes tissue damage



extravasation of neutrophils



reach site of injury



focal area of granuloma

Eg.s

1) SLE

2) Rheumatoid Arthritis [III > IV]

3) Polyarteritis Nodosa

4) Multiple Sclerosis

5) Serum Sickness

6) Arthus Reac<sup>n</sup>

7) PSGN

8) Leprosy Reac<sup>n</sup> Type II

9) Meningitis

10) SAGE

11) Hepatitis B + C (arthritis)

12) Mononucleosis

13) Dengue (arthritis)

14) 5<sup>th</sup> disease (step chick)

15) Nephrotic Syndrome in P. malariae

16) Katayama fever in schistosomiasis

17) African trypanosomiasis

18) Penicillin +

Sulphonamide allergies

## Type IV

80

### Intracellular Pathogens

- 1) M. Leprae, M.Tb
- 2) Listeria monocytogenes
- 3) Brucella abortus
- 4) Pneumocystis jirovecii
- 5) Candida albicans
- 6) Histoplasma capsulatum
- 7) Cryptococcus neoformans
- 8) Herpes simplex virus
- 9) Variola
- 10) Measles

### Skin Test

Tuberculin, Lepnomin

Montenegro, Fucc Test

Contact Dermatitis

Parvyl chloride

Hair Dye

Nickel Salts

Poison

Ivy

Poison Oak

Hashimoto's thyroiditis

Type I Insulin Dependent

DM

Guillain Barre

Cellar disease

Graft Rejection [IV > II]

Lepre Reac<sup>n</sup> Type I.

Hypersensitivity pneumonia

[IV > III]



# BACTERIOLOGY

81

## Drug Resistance

Chromosomal

Mech

→ Mutation in the chromosome

eg. Mtb

→ Transduction

eg. MRSA

Con

Plasmid

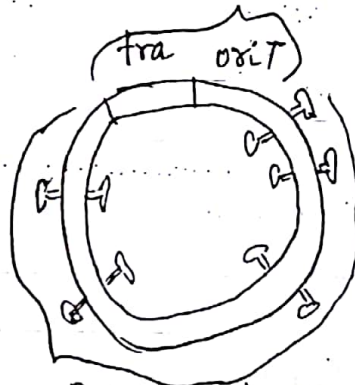
Mech

→ Insertion of Transposons carrying drug resistance genes in plasmid

↓

Antibiotic Selection Pressure

RTF = resistance transfer factor



Rd - resistance determinant

R plasmid = Rd + RTF

Combination = useful  
Rx

Metabolic Defects

↓

eg. INH (R) Mtb → catalase ⊖  
peroxidase ⊖

Not useful

> 8 drug (R) seen.

not seen.

(Plasmid doesn't code for metabolic)

# Anti-microbial Susceptibility Testing

82

M<sub>1</sub> Muller Hinton Agar

Middle Brook - M.tb.

Blood Agar - Haemophilus

Method

1) Dilution Method

Broth dilution

Agar dilution

2) Diffusion Method

More common

a) STOKES meth [European]

b) Kirbybauck  
(Disk Diffusion) [Imge]

as per CLSI

(Clinical Laboratory Std. Inst.)

USA

c) E-test

\* Broth Dilution method for MIC testing

Serial dilution of Ab

↑

Std. inoculum

Bacteria: 0.5 McFarland (MIC)

Fungi: 2 McFarland

↓

Density best measured by  
Spectrophotometer

Min. Bactericidal Conc<sup>n</sup> (MBC) ⇒ estimated by subculture

MBC ≥ MIC

MEC - Min. effective conc<sup>n</sup>

Done for Anti-fungal Susceptibility

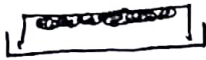
Min. conc<sup>n</sup> at which distortion of hyphae is seen  
morphological

# Disc Diffusion Test

- CLSI
- 9cm diameter Petri Plate
- 6 disc used each 6mm diameter.



Incubate



Hazy area  $\rightarrow$  resistance  
clear zone  $\rightarrow$  inhibition.

MIC can't be assessed.

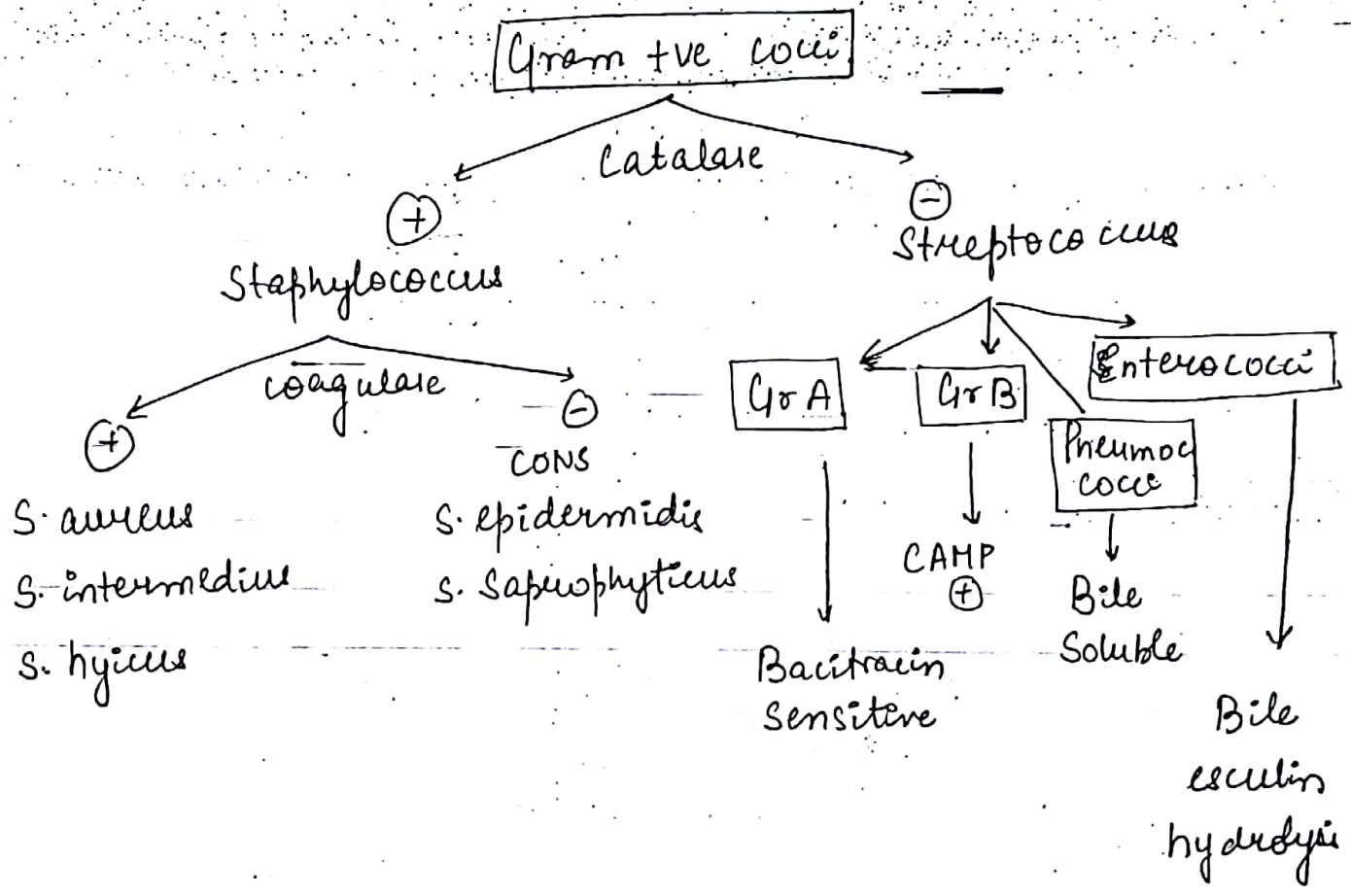
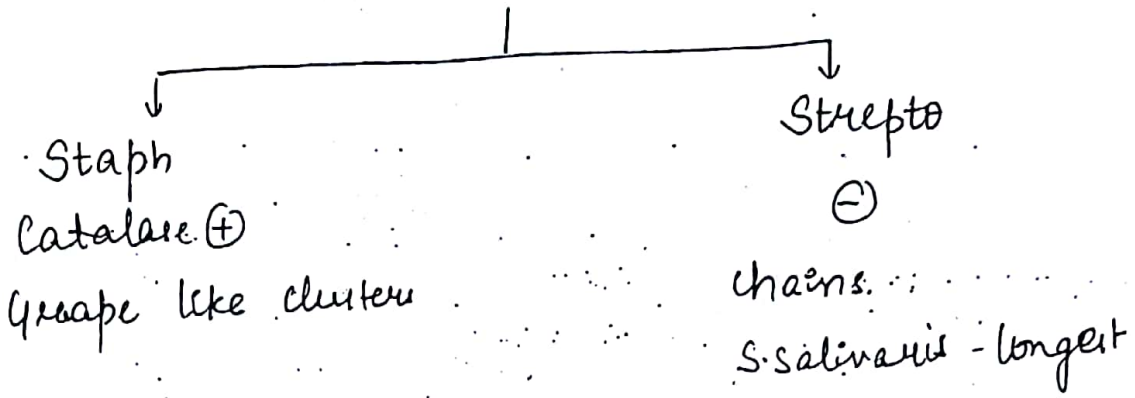
- E test (epsilon test) [Image]

Diffusing Ab Gradient on a strip  
Diffusion technique in  $\leq$  MIC can be tested.



$\leftarrow$  MIC

# GRAM +ve COCCI





# STAPH. AUREUS

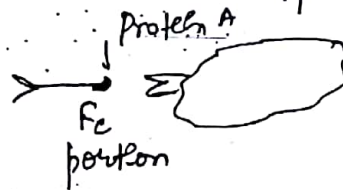
85

## I) Virulence Factors

↓ susceptibility to opsonisation

### 1) Protein A

- opsonin / Ab depletion by protein A
- binds to Fc portion of IgG. thus preventing binding of Ab to macrophages



- 90% of staph have protein A [COWAN I strain]

### 2) SCIN (Staphylococcal complement Inhibitor Protein)

binds + inactivates the  $C_3$  convertase of alternate pathway

### 3) Protease/

Degradation of IgG,  $C_3b$  by Staphylokinase

### 4) Clumping Factor activated Factor I.

### 5) Extracellular Fibrinogen Binding Lectin.

Inactivates  $C_3$  or prevents cleavage of  $C_3$ .

### 6) Capsule

prevents binding of phagocytes to opsonins

Other

1) Peptidoglycan

2) Teichoic Acid

3) Toxins

M/cc of  $\beta$  haemolysis,  $\uparrow$  virulent

a) Hemolysin -  $\alpha, \beta, \gamma, \delta$   
 $\beta$  hemolysis

$\beta$  toxin  $\rightarrow$  sphingomyelinase  
 $\downarrow$  secreted  
 $\downarrow$  virulent

$\gamma$  toxin  $\rightarrow$  Pantone Valentine leucocidin [PVL gene]  
2 component toxin S x F  
-  $\bar{c}$  binds  $\bar{c}$   $\gamma$  toxin  
Synergohymenotrophic toxin

Q. associated  $\bar{c}$  Community Acq MRSA

b) Epidermolytic Toxin / Exfoliative Toxin-

Destroys mucopolysaccharide of Stratum Granulosum

$\downarrow$   
BULLAE (Coalesce)

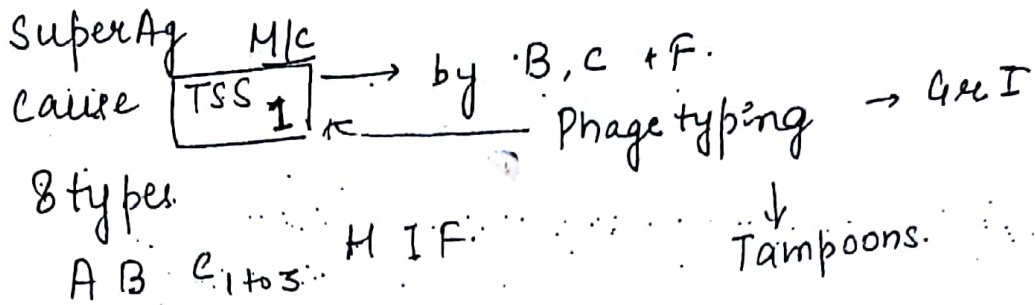
$\downarrow$   
SSSS [Staph scalded skin Syndrome] [Image]

children - Ritter's ds } fatal  
Adult - TEN

Pemphigus neonatorum }  
~~Bullous~~ Impetigo } milder form

$\Delta$  = toxin detection.

↳ Enterotoxin =



$\Delta$  - Nicolsky Sign +ve.  
desquamation on pressure

Food Poisoning

— Type A M/c

Due to preformed toxin

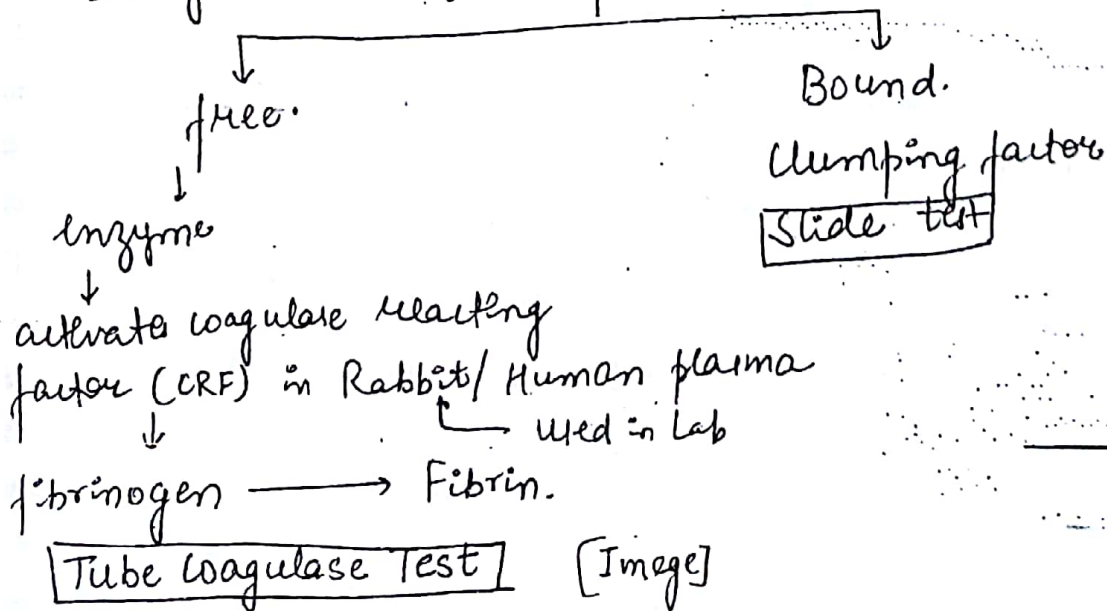
IP < 6hrs

vomiting (vagel nr stimulation)

'outbreak'

$\Delta$  - culture of vomitus / food.

4) Coagulase enzyme.



Tube coagulase

1:6 dilution plasma

Read at 4hr.

Released in log phase

5) Hyaluronidase ↑ virulent

It destroys tissues

II

M/c of boils / abscess

" osteomyelitis except

" Sickle cell anaemia → salmonella

" IV drug abuser → Pseudomonas

" epidural abscess

" native valve endocarditis

" Hospital acquired infection

III

Δ

Culture on 5-10% Blood Agar.

Selective media → Ludlam media

Salt milk Agar

Pigment → Nutrient Agar

(Golden Yellow)

glycerol monoacetate

Specific Test for S. Aureus = Mannitol Fermentation Test (+)

Phosphatase (+)

Heat stable nuclease (+)



S. intermedius

ZOO NOTIC → Dog

Q In 2 wks, 5 newborns in the NICU developed S aureus bacteremia. PFGE of the isolates were similar. Which of the following should be done next.

- a) Prophylaxis of all newborns in the NICU = I/V vancomycin
- b) Protective isolation of all newborn
- c) Ensure strict hand hygiene
- d) Collu<sup>n</sup> of nasal swab of health care workers.

Best Typing Method → PFGE (pulsed field gel electrophoresis) or sequence based typing.

Phage Typing [Image]

↳ susceptibility to different phages among S. aureus strains

for virus = agarose gel electrophoresis preferred

Lysis out ⇒ If 1 bacteria infected = many phages. (undergoing any replication) of phage



Uses :- 1) S. aureus

M/c phage Type. In India = Group II  
Most useful in epidemiology

2) Shigella

3) Vibrio

M/c = Eitor

4) Salmonella

M/c - E1

Least useful in epidemiologically

Vi phages used in salmonella

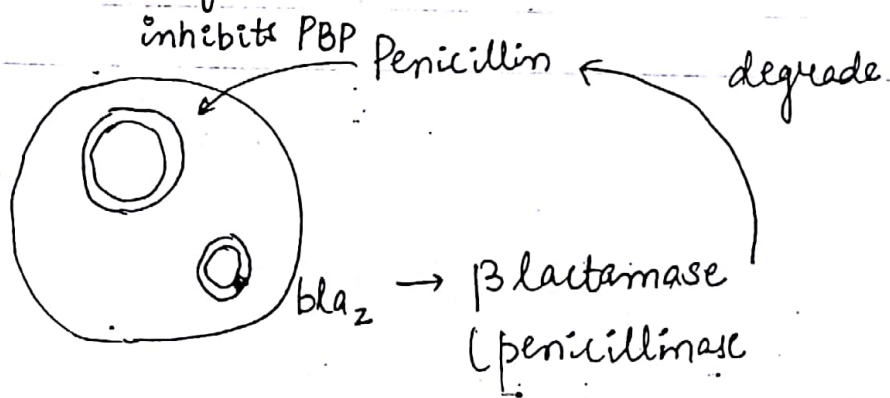
- Vi Ag = S. Typhi
- S. Parahaemolyticus Typhi
- S. Dublin
- Citrobacter

Father of Hand Hygiene = Edward Semmelweis  
 obstetrician  
 5<sup>th</sup> of May - Hand Hygiene Day

5 moments

- Before & after seeing a pt
- Before & after a procedure
- Contact & pts. surrounding

### IV Drug Resistance

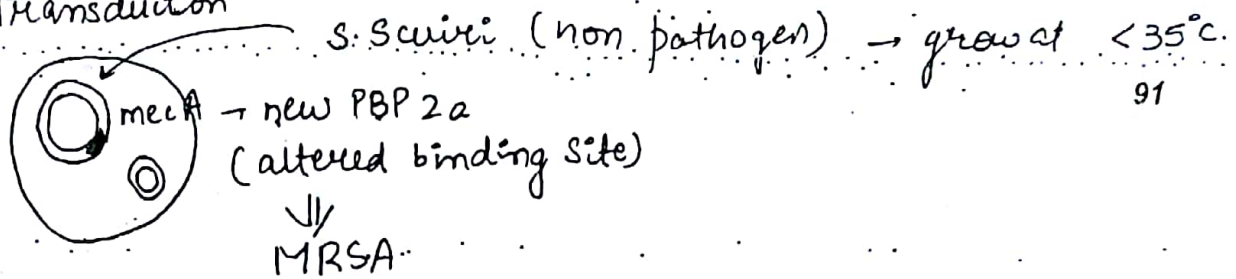


- Plasmid encoded Drug (R) → ~~By Transformation~~
- By Transduction - 90%
- Conjugation - 10%

Penicillinase Resistant Penicillin ⇒ ⊖ PBP → ⊖ degrade Penicillinase

Methicillin	Dicloxacillin
Oxacillin	Carbenicillin
Cloxacillin	Nafcillin

## Transduction



## MRSA

Hospital Acquired Infection

Chromosomal

DOC → for t/ting MRSA → Vancomycin

Screening → Nasal swab (50% colonisation)  
Hand 40% colonisation

↓  
Mannitol Salt Agar ± Cefoxitin

↓  
yellow colonies  
30°C → incubation [mecA gene expression  
best at 30°C]

TcBC media for Vibrio.  
Green coloured due to citrate

Q. 25 yr old girl present to OPD ± carbuncle at the back of neck. Pus aspirated reveal MRSA ± is true about the strain.

a) ↑ Resistance

b) ↓ virulence

c) Associated ± SCC mec

I II III

[Staphylococcal cassette chromosome → pathogenicity island]

id) — " — " ~~the~~ pvl



# Criteria of HAI :-

after 48 hrs of admission  
or

in 2 weeks of Discharge from hospital  
or

in 12 ~~weeks~~ months of Discharge in case of prosthetic implant

HA-MRSA

↓ virulent

SCC<sub>Hec</sub> I II III

↑ Resistance

↓

Clindamycin (R)

D test (+)

CA-MRSA

new strain

↑ virulent

SCC<sub>mec</sub> (IV) V VI

prb associated

↓ Resistance

↓

Clindamycin susceptible

(-)

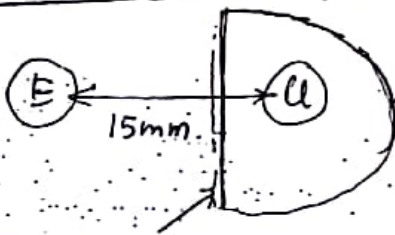
## Q D Test

To detect Inducible Clindamycin Resistant Strains  
expressing Ecm gene

↓

Erythromycin → induces Clindamycin (R)

This can't be done by Diffusion Test.



← susceptible strain  
grows away as resistant  
is not developed this side

Resistant strain

due to erythromycin grows  
towards clindamycin



VRSA

Plasmid encoded

VanA gene derived from Enterococci

$\geq 16 \mu\text{g/mL}$

↓ common.

93

VISA (Vancomycin Intermediate (R) *Staph. aureus*)

↑ cell wall synthesis

4-8  $\mu\text{g/mL}$

VSSA (Vancomycin Susceptible S.A.)

$\leq 2 \mu\text{g/mL}$

MIC → Done for VRSA  
Neisseria  
Done by Broth Dilution

Q. Accessory Gene Regulator (agr)

MSCRAMM (Microbial surface component recognising  
Q adhesion matrix molecules)

↓

Protein A, Clumping Factor, Lectin.

Modified Hodge Test  
for Carbapenemase (R)

# CONS

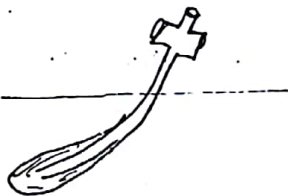
① *S. Epidermidis*  
75% infe<sup>n</sup>

Skin commensal → Lysostaphin

↓  
⊖ *S. aureus*

R/F - IVC (central line)

Intercellular adhesion → adhere to cellular tip



↓  
thin polysaccharide  
BIOFILM (300 nm)

↳ antiphagocytosis  
Antibiotic (R)

C/F

△ M/c cause of prosthetic valve endocarditis  
(early onset) - ~~H~~ < 12 months HAI

Late onset - *Strept. viridians*

M/cc of CR BSI (Catheter Related Blood Stream Infection)

△ of CRBSI → Culture of catheter tip (5cm)

Congo Red stain → biofilm.

Confocal microscope

↓  
multiple focal point image

Superimposed

In situ △ of CRBSI → BACTEC blood culture of 2

samples

- catheter

- peripheral vein

1/  $> 2hr$  difference in positivity  $\rightarrow$  CRBSI

2/ ~~2hr~~ Differential to time test positivity

95

$\downarrow$   
Bet<sup>n</sup> the +ve of 2 samples

Catheter      Peripheral ven.

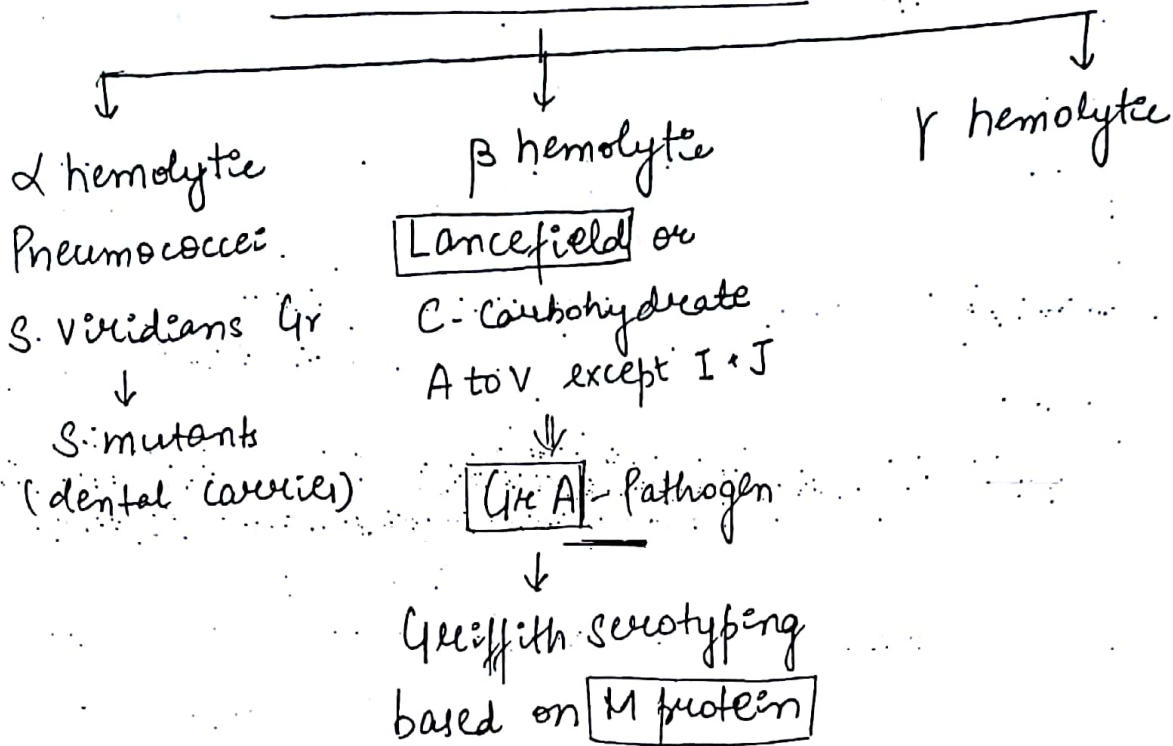
(II) *S. Saprophyticus*

UTI

Novobiocin (R)

# STREPTOCOCCUS

96



Gr. D (M/c - γ hemolysis)

Intestine

Enterococci

{ S. faecalis M/c  
S. faecium Most (R) }

They Don't grow in 6.5% NaCl. PYR (+)

S. Bovis (S. Gallolyticus) → Grow in 6.5% NaCl  
↳ a/c ca colon. PYR (-)

## GRP-A STREPTO

### Virulence Factors

1) Pyrogenic Exotoxin (exfoliative)

↓  
Scarlet Fever

→ Rash (pastia's line)  
Sandpaper appearance  
Strawberry tongue  
M<sub>28</sub> - M/c serotype



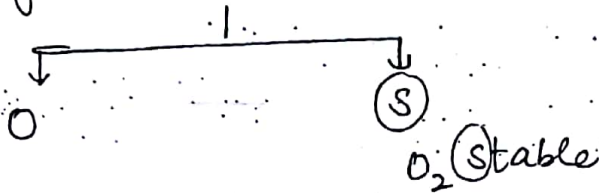
2> **M Protein**

antiphagocytosis  
Serotyping



T & R protein  $\Rightarrow$  No virulence.

3> **Hemolysin (Streptolysin)**



O<sub>2</sub> labile  
active in reduced or  
anaerobic state

**ASO titre**

ARF > 200 IU  
low in PSGN & Pyoderma  
Tonsillitis strains M<sub>1, 5, 12, 24</sub>.

Molecular mimicry  $\rightarrow$  Ab against cell wall proteins of  
Group A strept. cross reacts w  
myocardium.

4> **Streptodornase**

destroys DNA

Anti streptodornase B  $\Rightarrow$  Marker for PSGN &  
Pyoderma

↓  
Skin pathogens

M<sub>25 43 53-55 59-61</sub>

5)

**Streptokinase**

dissolve clot

used as thrombolytic agent → source is Gr C  
*S. equisimilis*

6) Hyaluronic acid in capsule

7) **Hyaluronidase** → destroys tissue

Flesh eating bacteria

Gr A strepto M<sub>1</sub> to 3

↓  
Necrotising Fasciitis

Diagnosis

PYR ⊕

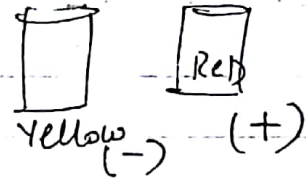
Bacitracin sensitive.

**PYR Test**

Aminopeptidase enzyme

Releases free β naphthylamide

pyrrolidonyl β naphthylamide



**PYR**

⊕ Gr A strept., enterococci

⊖ Gr B strept., bovis

# GR. B. STREPTO [S. GALACTRIAE]

99

↳ Bovine mastitis

20-40% female → genital tract



Neonatal Meningitis (direct spread)

M/c

Δ - Hippurate hydrolysis ⊕

CAMP ⊕ IOC

## Hippurate Hydrolysis Test

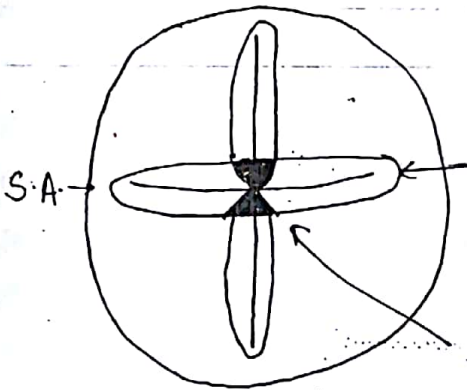
[Image].

Glycine = ninhydrine purple colour.

Hippurase enzyme acts on Hippurate

### CAMP

Grp B strepto → stimulate Staph to release β toxin



β hemolysis & toxin

Butterfly hemolysis

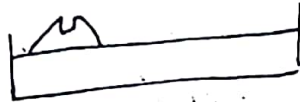
β hemolytic strepto

# PNEUMOCOCCUS

Lanceolate shaped diplococci  $\bar{c}$  capsule



Draughtsman colony (cannon coin)



## Virulence Factor

- 1) capsule
- 2) Ig A<sub>1</sub> protease
- 3) Pneumolysin } Not secreted
- 4)  $\alpha$  toxin

Autolysin  $\rightarrow$  destroys bacteria to release

## C/F

- M/cc of pyogenic meningitis
- " " community acquired pneumonia
- " " Hosp. acq. pneumonia (VAP) except
- after 5-6 days } Pseudomonas MDR strain
- " " Otitis media in  $<5yr$

## $\Delta$

① Inulin Fermentation (+)

② optochin (3)  $\rightarrow$  screening test

③ Bile Solubility  $\rightarrow$  confirmatory



Rx

Meningitis → start ceftriaxone + vanco

101

↓  
Penicillin

Other infec<sup>n</sup> → start c penicillin

↓  
ceftriaxone + vanco

### Vaccine

Adult → PPSV (polyvalent polysaccharide vaccine  
23 seroprevalent strains)

↓  
High Risk

- splenectomy
- cochlear implant
- > 65yr
- chr. lung, kidney, liver, heart Ds
- immunocompromised.
- Diabetics
- Hospitalised pts w/ H/O smoking alcohol.

child - PCV13 [Pneumo conjugate vaccine]

2 - 24 months

OSLER TRIAD / AUSTRIAN SYNDROME :-

Pneumococcal endocarditis (Aortic valve) + Meningitis + Pneumonia

# ENTEROCOCCUS

102

↑ Resistant → UTI, HAI

↳ (R) to penicillin - hyperprod<sup>n</sup> of PBP<sub>5</sub>

6.5% NaCl

9.6 pH

46°C

40% Bile

Δ → PYR (+)

Bile esculin hydrolysis (+)

~~S. Bovis~~ → PYR (-)

[Gallolyticus] ⇒ Bile esculin Hydrolysis (+)

~~DOE~~

VRE

plasmid

VAN A (nc) → (R) to both vancomycin + Teicoplanin

Van B, C, E → (R) to vanco only

Substitution of terminal

D alanine = D serine/  
Lactate

↓  
Elimination of Target

HLAR → high level aminoglycoside (R)

## SUPER ANTIGEN

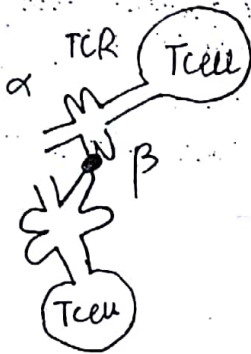
MHC unrestricted

Bind to V $\beta$  region of TCR

↓  
polyclonal activation

↓  
20% of Total cells. [N] → 0.1% of total cells  
in monoclonal activation]

↓  
↑↑ Cytokines.



## Pathogen showing superantigen

- 1) Staph aureus
- 2) G $\mu$ A strepto
- 3) M:tb. including BCG
- 4) Rabies
- 5) HIV
- 6) EBV

IgA → Mucosal Affinity

⊆ Ig doesn't cross placenta

a) Ig G<sub>1</sub>

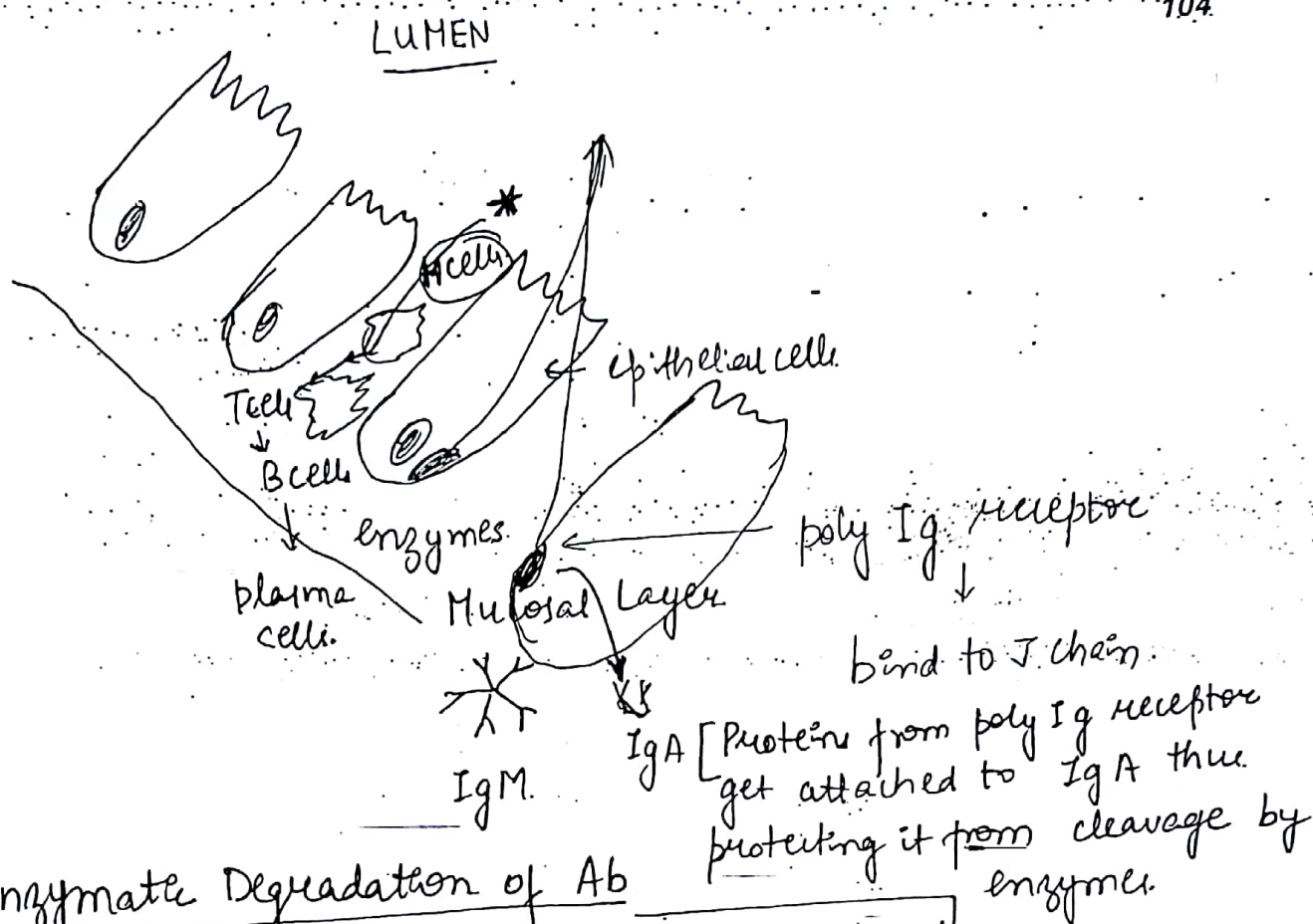
b) Ig G<sub>2</sub>

c) Ig G<sub>3</sub>

d) Ig G<sub>4</sub>

→ placenta doesn't have receptor for  
Fc portion of Ig G<sub>2</sub>

[Receptor mediated Trans cytosis of Ab]

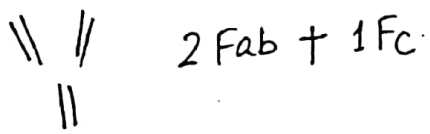


Enzymatic Degradation of Ab

Pepsin → cleaves below di-sulphide Bond.

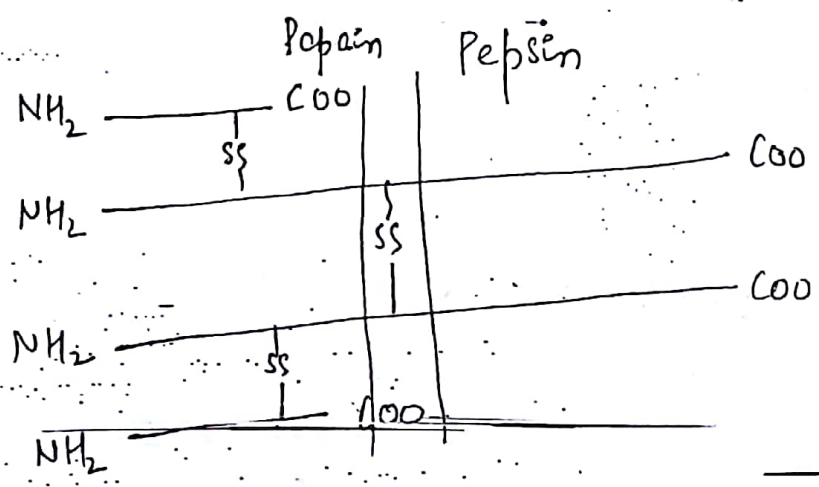


Papain → cleaves above disulphide Bond



secretory portion

↓  
It derived from part of poly Ig receptor.]





H chain is cleaved → Isotype func<sup>n</sup> is lost

L chain is intact → Idiotype intact  
Ag. Binding occurs.

Valency (monomer)  
Pepsin → unchanged.  
Papain → 2 → 1.

2-ME - 2 Mercaptoethanol → cleaves disulphide bond

2H + 2L chains

Idiotype, Isotype all lost

DIFFERENTIATE S. AUREUS FROM MICROCOCOCCUS

Micrococcus

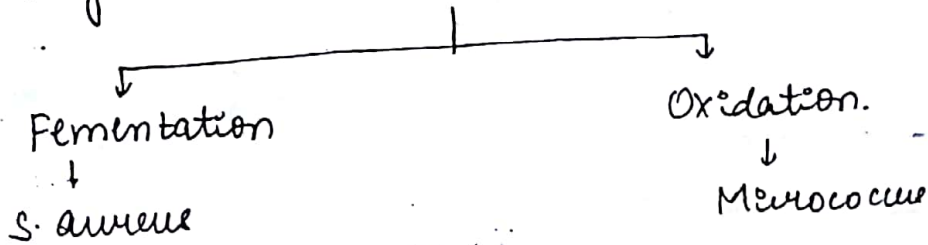
Obligate aerobe

Non-pathogen

Tetrad.

Skin commensal

Hugh & Leitsons Oxidation Fermentation Test



DIF in Blood Agar

Group A strepto → Bacitracin (S)

Pneumococci → Optochin (S)

24/2/18

\* Non-Streptococcal Catalase -ve Gram +ve Cocci 106

→ Pediococcus + Leuconostoc → Vancomycin Resistant

→ Abiotrophia + Granulicatella species

nutritionally variant streptococci → require Vit B6 ⊕

Doc → Gentamicin + Penicillin (to avoid resistance)

\* S. Angiosus Gr

Agglutinate A, C, G, (F) antisera

VP test ⊕

Butterscotch, or caramel odour.

## GRAM +ve BACILLI

### ① LISTERIA MONOCYTOGENES

1) Only Gram +ve Bacillus e has Endotoxin.

2) Intracellular pathogen.

Int. A & B toxin → helps in internalisation.

3) <sup>(LLO)</sup> Listeriolysin O secreted by Listeria destroys phagosome & thus escapes phagocytosis.

Listeriolysin + Cytotoxin ⇒ helps in escaping phagocytosis

→ Haemolysin  
show phemolysis

### 3) Actin Filaments

helps in intracellular & intercellular motility

↓  
BLEB Formation

[Image]

47 Cold Growth. (2-8°C)

57

CF → Neonatal Meningitis

↓  
Early Onset

Granulomatosis infantiseptica

Intracellular Transmission.

Mother is asymptomatic

Disseminated local

↓  
Late Onset

↓  
10-30 days after birth

Neonatal Meningitis

Mother is asymptomatic

Spread through faecal  
contamination by health  
care workers.

Adult ⇒ Food Poisoning

By consumption of Refrigerated food.

67 A → ① Blood Agar → β hemolysis

② CAMP test → (+)

③ Anton test → (+)

④ Tumbling Motility at 21°C.

⑤ Ab detection

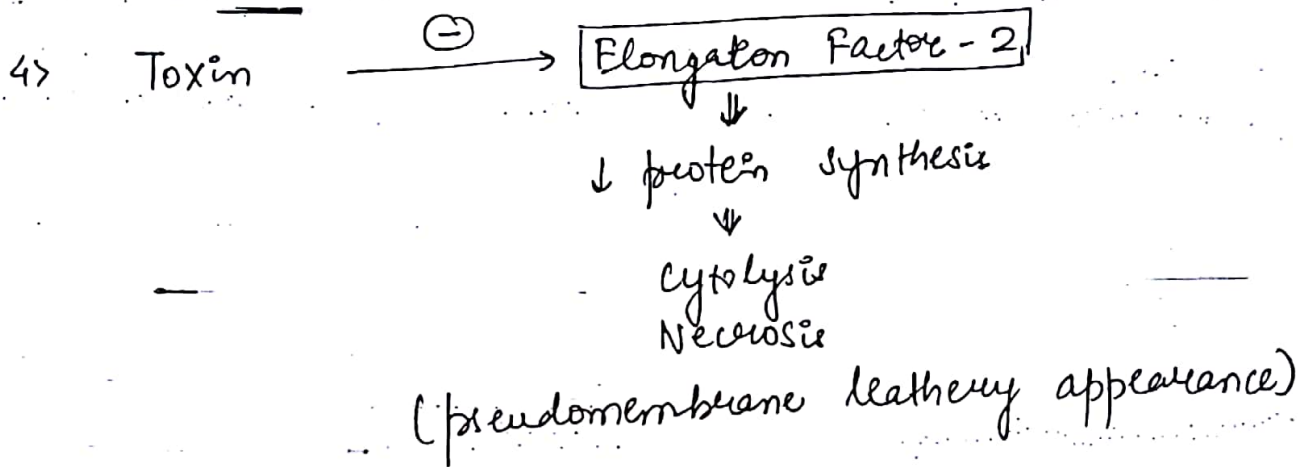
⑥ PCR (↑ sensitivity) — Good method nowadays.

# II CORYNEBACTERIUM DIPHTHERIAE

108

- 1) Gram +ve Bacilli
- 2) Metachromatic Granules are not seen in Gram +ve
- 3) For Pathogenesis, toxin is important

↓  
It depends on Iron Conc<sup>n</sup>  
[0.1 mg/lc]



- 4)  $\Delta$
- a) Culture → Loeffler's Serum slope (c<sub>tn</sub> 4-6 hrs)  
↳ patient.  
→ Tellurite [carrier → selective media]  
preferred for carrier state

If not specified patient or carrier → go for LSS

→ Hoyle, Tinsdale  
Miss Serum water

- b) Specimen → Throat Swab



# GRAVIS

Daisy Head Colony

More virulent

# INTERMEDIUS

Frog Egg Colony

~~POACHED EGG COLONY~~  
MITIS

Poached Egg Colony

c) Microscopic exam of Throat Swab  
↳ for toxigenicity.

- i) Albert stain
- ii) Neisser stain
- iii) Pender

Metachromatic  
or  
Volutin  
or

Baker Ernest granules.  
↓  
Pathogenic.

## d) Toxigenicity

In vivo → Guinea Pig  
(250-350gm)

Rabbit - 500gm  
Guinea Pig - 250gm  
Mice - 20gm

Subcutaneous → Intracutaneous

s/c inj<sup>n</sup> of test strain  
↓  
death in 46 hrs

I/c inj<sup>n</sup> of test strain  
↓ after 4hrs  
50 IU of diphtheria antitoxin intraperitoneally

Use control → s/c inj<sup>n</sup> of test strain + 500 IU of diphtheria antitoxin intraperitoneally  
Protective Dose

Cutaneous Lesions in 48-72 hrs.

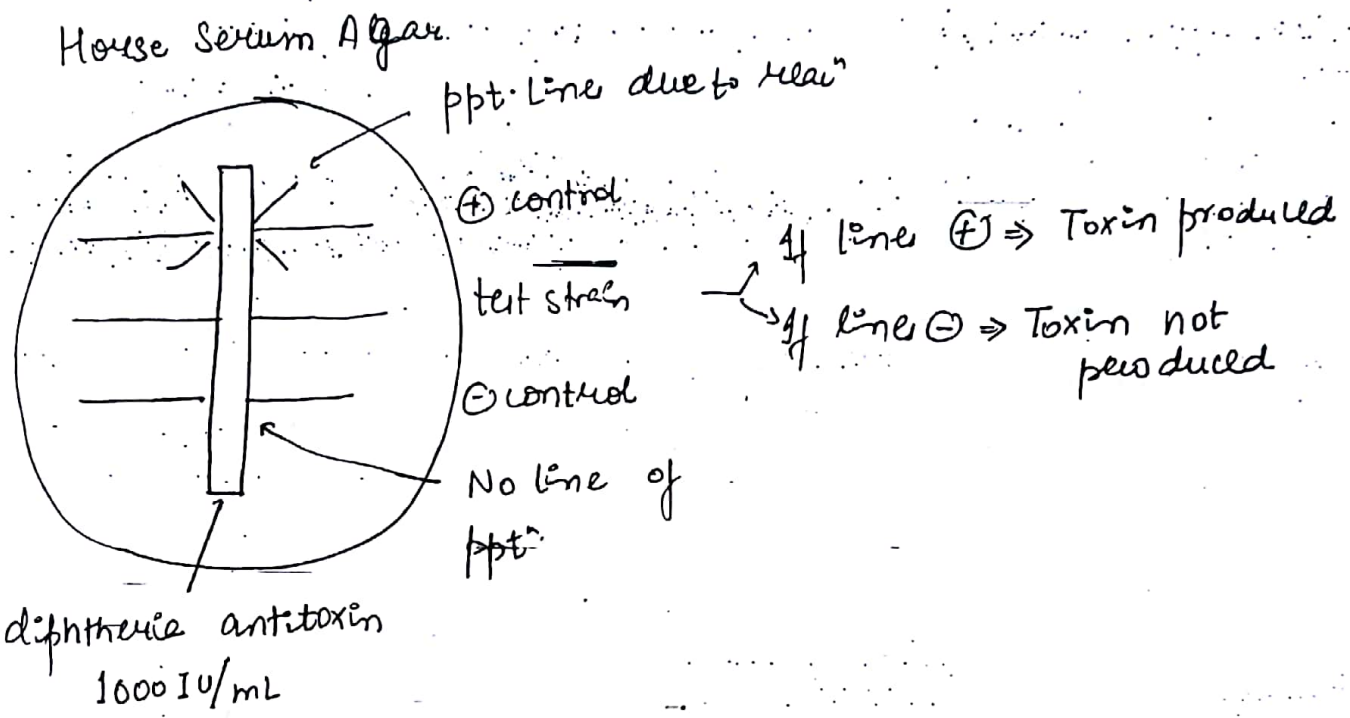
4 Guinea Pig ⇒ 3 for test + 1 control used

Advantage - 10 test performed on 1 guinea pig

1 IU of Antitoxin = Min. amount Required to neutralise 100 fig of toxin.

In Vitro → Elek's Gel precipitation

↓  
Double Diffusion in 2 directions  
[Ouchterlony Procedure]



- *Corynebacterium Jeikeium* ⇒ MDR
- " *Urealyticum* ⇒ urease +ve (UTI)
- *Aerobacterium haemolyticum* ⇒ Reverse CAMP test (+)

SCHICK TEST (Neutralization Test)  
for susceptibility

Toxin  
(one arm)

Positive (susceptible) (+)  
Negative (Immune) (-)  
Pseudoreac<sup>n</sup> [immune but Hypersensitive] (-)

Heat inactivated toxin  
(other arm)

(-)  
(-)  
(+)

Combined Reac<sup>n</sup>

(susceptible + Hypersensitive)

(+)

(+)

111

Toxoid is safe than heat inactivated Toxin.

So, no fear of anaphylaxis

DIPHTHERIA TOXOID

5-25 Lf units/dose [0.1ml]

[Lf = Limes flocculation]

TT → 10 Lf

dT → low dose → 1-2 Lf

↓  
used in adults.

## BACILLUS ANTHRACIS

Zoonotic Disease

Non-Motile.

Seasonal outbreak - summer season.

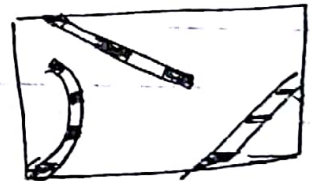
Dry Grass ← feeding Cattle

↓  
gets injured

(spores + nt in soil) Spores enter

↓  
Haemorrhage

↓  
Death.





Urbanesiu Anthrax

INCIDENCE

MORTALITY

Hide porter Disease

45%

20%

112

Malignant pustule

Black Eschar

Pulmonary Anthrax

5%

95%

Wool sorter

Intestinal Anthrax

Rare

95%

Virulence Factor

1) Capsule

} Plasmid

2) Toxin

3)  $\rightarrow$

Oedema factor (  $\uparrow$  cAMP )

$\rightarrow$  Protective factor } cytotoxicity

$\rightarrow$  Lethal factor }


Diagnosis

Transportation of sample  $\Rightarrow$  Triple Layer packaging (used in infectious samples)

Culture on PLET Media

[Polymyxin, Lysozyme, EDTA, Thallus acetate]

$\downarrow$

Medusa Head Colony  $\rightarrow$  

String of Pearl  $\rightarrow$  oooooo



# Bamboo stick or Box Car Appearance

B. anthracis  
(obligate aerobe)

↓  
also seen in Clostridium Perfringens.  
(obligate anaerobe)

Inverted Fir Tree → on gelatin stab culture



McFadyen Reac<sup>n</sup> → for capsule

## Confirmation

1) γ phase lysis

or

2) MLST (multilocus sequence typing)

by PCR

[to distinguish other spore bearing, aerobe organism]

Bioterrorism → Category 'A' agent

- B. anthracis
- Yersinia Pestis
- C. Botulinum
- Francisella tularensis
- Small Pox
- Agent of Viral Haemorrhagic fever

→ Most potent

→ epidemiologically important.

## B. CEREUS

Motile

- cause food poisoning - By RICE

Food poisoning

Emetic

vomiting

preformed toxin

< 6 hrs IP

↑↑ bacilli in stool

Enteric

diarrhoea

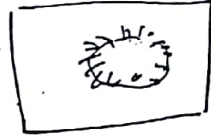
> 12 hrs IP

↓↓ bacilli in stool

Δ - Culture - MYPA media

(mannitol Yolk Phenol Red novobiocin agar)

ACTINOMYCES



Gram +ve Bacilli [Image]

Filamentous

Obligate Anaerobe

A → Actinomyces

B → Bacteroids [Gram-ve]

C → Clostridium

No ~~of~~ superoxide Dismutase

Δ No Peroxidase

Gram +ve Branching filamentous rods

actinomycetes

Nocardia

# Actinomyces

Anaerobe  
 Non Acid Fast  
 Endogenous Infect<sup>n</sup>  
 ↳ oral cavity  
 Lumpy Jaw - M/C

GIT

Genitourinary Tract

Thoracic Actinomyces

↳ aspiration of oral secretions

Δ of Actinomyces

Actinomyces involvement of pelvis occurs most commonly in association with an IUD.

↳ Sulphur Granules

filamentous bacteria with peripheral clubs.

Ag-Ab complexes.

↳ Sunray appearance.

**MYCETOMA**

swelling

sinus

granules

Actinomycetoma → actinomyces, Nocardia + streptococcus

Eumycetoma → Fungi pigmented

Botryomycosis → S. aureus (M/C)

## Actinomycetoma

acute inflammatory cond<sup>n</sup>

Purulent D/C

white or yellow sulphur granules  
 except actinomadura pelletiere  
 ↳ Red granules

# Nocardia

115

Aerobe

Acid Fast (1% H<sub>2</sub>SO<sub>4</sub>)

Exogenous Infect<sup>n</sup>

Nocardia	Mycobacteria
entangled red bacilli	Long, slender Beaded

## Eumycetoma

Chronic

~~Purulent D/C~~

Serous D/C

Brown & Blue stain.

Gram stain.

116

27 CULTURE → Molar Tooth Colony  
Spiderly colonies

### Δ of NOCARDIA

1° infection in Lungs → Brain + Kidney Abscess.  
subcutaneous Infection → Mycetoma

- Gram stain

- Acid Fastness

- Culture using Paraffin Bait Technique

BACTEROIDES FRAGILES [ Gram - ve  
Anaerobe ]

17 Virulence Factors

Capsular polysaccharide

Protease, neuraminidase

Cytolysin

Enterotoxin

LPS - 1000 times less Biologically potent

Drug resistance to  $\beta$  lactamase



## 2) LEMIERRE'S SYNDROME

117

Jugular thrombophlebitis

Fusobacterium necrophorum → Bacteroid species

Lung emboli sepsis

3) Δ → culture Media

- a) Trypticase Soy Agar
  - b) Schaedler's blood agar
  - c) BHI (Brain Heart Infusion)
- Kanamycin

## CLOSTRIDIUM

Obligate anaerobe

No O<sub>2</sub> / ↓ Eh (Redox Potential)

### Cl. TETANI

Drumstick appearance

Virulence Factor -

Tetanolysin

No virulence

Tetanospasmmin

- plays role in virulence
- ⊖ GABA + glycine
- ↓
- ⊖ of presynaptic receptors
- ↓
- Spasmodic  
Spastic paralysis

Δ - Robertson Cooked Meat Broth

Thioglycollate Broth

118

↓  
Blood Agar

↓  
Macintosh Field

(Molybdenum catalyst)

→ [Image]

Candle Jar  
↓  
for capnophiles

BI for checking  $O_2$  free environment

in Macintosh Field ⇒ 'Pseudomonas'

↓  
It does not grow if completely  $O_2$  free

GAS PAK JAR

→ Better, safe

Prevention :- Active Immunisation + Passive immunisation.

PEP for tetanus

Immunity Category	Simple wound	Contaminated wound
Cat A	Nothing Req'd	Nothing Req'd
Cat B	Toxoid 1 dose	Toxoid 1 dose
Cat C	Toxoid 1 dose	Toxoid 1 dose + HTIG
Cat D	Toxoid complete dose	Toxoid complete dose + HTIG.

A - taken complete course of TT/Booster in part 5 yrs  
119

B - " " " " " " in part 5-10 yrs

C - " " " " " " in part >10 yrs

D - Not taken complete course of TT/booster in part 5 yrs.

Simple wound - <6 hrs, clean, non-penetrating, negligible tissue damage.

Booster recommended every 10 years.

1° Immunisation - 1st 3 doses on 6, 10, 14 weeks.  
Safe vaccine.

Pertussis vaccine - cause fever as cellular component is used.

## C. PERFRINGES / WELCHII

12 toxins

$\alpha$  toxin  $\rightarrow$  Most virulent

$\downarrow$   
lecithinase c / phospholipase

C/E

① Gas Gangrene

② food poisoning

$\rightarrow$  C. perfringens 60%

C. novyi / septicum 40%

Type A

Type C

Drambrand.

Germany

Pigbel

Papu New Guinea

Enteritis Neurotoxic

endemic

World wide

Necrotising enterocolitis

$\alpha$  toxin  $\rightarrow$  ileum  $\rightarrow$  Necrosis + Gas

$\alpha$  toxin  $\rightarrow$  jejunum  $\rightarrow$  Neurosis + Gas

Neurotoxic enterococci

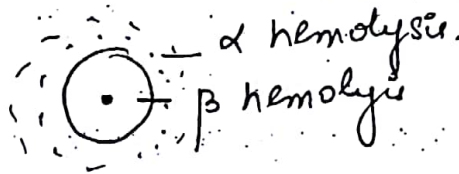
120

↓  
Fever In The Belly.

1 - Target Sign.

around clostridium  $\Rightarrow$  zone of  $\beta$  hemolysis due to  $\theta$  toxin.

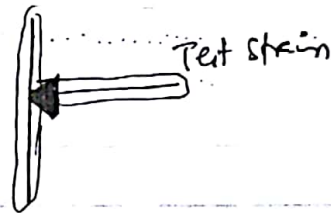
zone of  $\alpha$  hemolysis due to  $\alpha$  toxin



2) Reverse CAMP Test (+ve)

- Group B streptococcus used for
- \* Test strain is inoculated.

Arrow type hemolysis.



Bow Type hemolysis

Toxin of clostridium goes to strepto + causes more hemolysis.

Group B Streptococcus

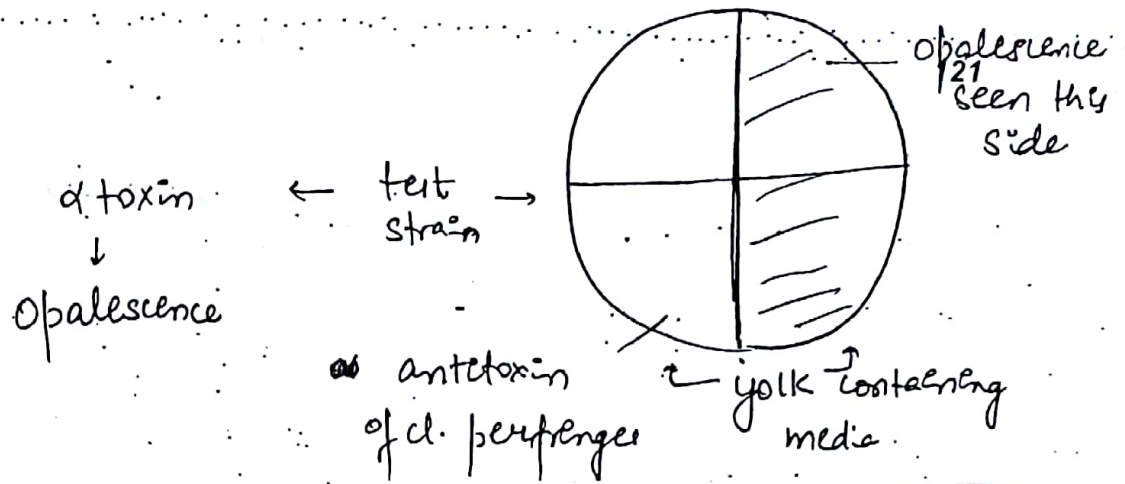
Toxin of strept. goes towards clostridium & causes more hemolysis

3) Stormy fermentation on litmus milk

4) Nagler Test

Volk containing media





## C. BOTULINUM

### \* Virulence Factor

Toxins → all are neurotoxins. except  $C_2$  (enterotoxin)

Botox Injec<sup>n</sup> → anti-aging

Biorevoviem

### \* Pathogenicity -

1) ⊖ Ach → Flacid Paralysis.

2) food poisoning

preformed toxin. in home made canned food.

IP - 8 to 36 hrs Q.

3) Floppy Child Syndrome  
spores (HONEY)

Infant gut microbiota starts developing in 4 hrs.

4) ~~Wound Botulism~~

due to spores.

# Δ - Toxin Detection

## Cl. DIFFICILE

Cause pseudomembranous colitis due to Clindamycin, cephalosporin [2<sup>nd</sup> & 3<sup>rd</sup> generation] (MC)

### \* Virulence Factor

Toxin - A - enterotoxin } Both toxin glycosylate GTP  
          - B - cytotoxin } binding proteins of Rho subfamily  
                                  ↓  
                                  protein synthesis

### \* Pathogenicity -

Sammit Lesions



Pseudomembrane



Necrosis



No Gross Blood in stool.

\* Δ

Test	Sensitivity	Specificity
Stool culture Best test for confirmation. (Gold Std)	++++	+++ <sup>123</sup>
Cell culture cytotoxin test on stool	+++	(+++)
ELISA for toxin A + B in Stool	++	+++
ELISA for C difficile common Ag in stool	++++	+++
Best Test NAAT for toxin A + B gene in stool	(+++)	(+++)
Colonoscopy or sigmoidoscopy	+	++++

1st Rx (1) DOC - Metronidazole  
or

Vancomycin → ↑ effective → used in severe cases.  
To prevent incidence of VRE.

(2) Fecal Transplantation

# GRAM -ve Cocci

124

## NEISSERIA

Intracellular, diplococci

Oxidase (+)

Media req. for growth - chocolate agar  
Thayer Martin  
New York City  
Martin Lewis

Candle Jar useful → as they are capnophilic

Oxidase Test

Kovac Reagent [p-phenylenediamine hydrochloride]

↓ cytochrome oxidase

Blue (indophenol)

(+) → Pseudomonas

Aeromonas

Neisseria

Campylobacter

Haemophilus

\* (-) → Enterobacteriaceae



# N. MENINGITIDIS

125

Virulence factors

- 1) Capsule
- 2) Endotoxin
- 3) ~~Pili~~ Pili

C/F  $\Rightarrow$  Pyogenic Meningitis + Rash.  
lead to outbreak.

Waterhouse Friedrichson Syndrome [due to endotoxin]

Serotypes A, B, C  $\rightarrow$  outbreak.

Y, W<sub>135</sub>  $\rightarrow$  sporadic cases.

X, Y, Z, 9E  $\rightarrow$  in HIV pts.

others - carriers

$\downarrow$   
Nasopharyngeal 5-10%.

$\downarrow$   
during outbreak 80-90%.

chemoprophylaxis - Inj<sup>n</sup> ceftriaxone > cefuroxime > Rifampicin.

Affects European countries. In India - Meghalaya.  
 $\downarrow$   
we use cipro.

Vaccine - polyvalent polysaccharide vaccine

$\downarrow$   
A, C, Y, W<sub>135</sub>.

(Gr B)  $\rightarrow$  Inj<sup>n</sup> + out deficiency of Late complement proteins properdin.

$\rightarrow$  M/c Inj<sup>n</sup> in infants

$\rightarrow$  capsule is non-immunogenic

Group B vaccine: based on subcapsular antigens.

not in cell wall.

- outer membrane vesicle.
- neuraminidase
- adhesion factor A.
- Factor H binding protein.
- neuraminidase heparin binding antigen

\*Rapid Carbohydrate Utilisation Test :-

ONPG test [ o-Nitrophenyl  $\beta$ -D-galactopyranoside ]

$\beta$ -galactosidase

galactose + o-Nitrophenyl.  
[ yellow ]

N. lactamica  $\rightarrow$  ferments lactose unlike other N. species

N. Meningitis  $\Rightarrow$  Maltose fermentation.

N. Flavescence  $\Rightarrow$  F/Sucrose "

N. Gonorrhoea  $\Rightarrow$  glucose "

### MORAXELLA CATARRHALIS

Cause - Otitis Media  
Sinusitis.  
COPD - AE

$\Delta$  - - Hockey Puck Sign  
colonies can be slid across the agar surface.  
no disruption

- Lacks Carbohydrate Fermentation  $\rightarrow$  used to differentiate from saprophyte
- Produce DNase  $\rightarrow$  Butyrate Esterase Neurine

# Drug Resistance to $\beta$ Lactamase.

127

## N. GONORRHOEA

### Virulence Factors

- 1) Pili Adhesion.
- 2) Opa (Protein II) - adhesion  
• transient ↓ in CD4
- 3) Porin (protein I)  
• endocytosis, invasion  
• ⊖ Complement
- 4) Rmp (protein III) - Blocks Ab block effect of bactericidal  
— Ab to porin + LOS leading to reinfection  
(lipooligosaccharide)
- 5) Ig A1 protease  
Fbp (ferric Binding protein)  
Lip (HB)

6) LOS - endotoxicity → Chronic Infection.  
Pili, opa, LOS express Phenotypic variation  
Por of different strains express different Ag typing

Proteins inside human body doesn't change → they remain same.  
↳ It shows variation in different strains.

### Resistance

PPNG -  $\beta$  lactamase producing strain of N. gonorrhoeae  
(Mlc) (plasmid) encoded  
Common



CMRNG - chromosomal encoded high level resistance to penicillin. 128

note

TRNG - High level Tetracycline resistance (MIC  $\geq 16$  mg/L)  
- tet M gene on conjugative plasmid.

Ceftioaxone resistant - chromosomally encoded pen A gene  
expressing PBP 2a.

Gonococcus doesn't affect vagina.

I.P. - 3-4 days. [Chlamydia - 7 days]  
MIC

Chlamydia diagnosed  
by NAAT

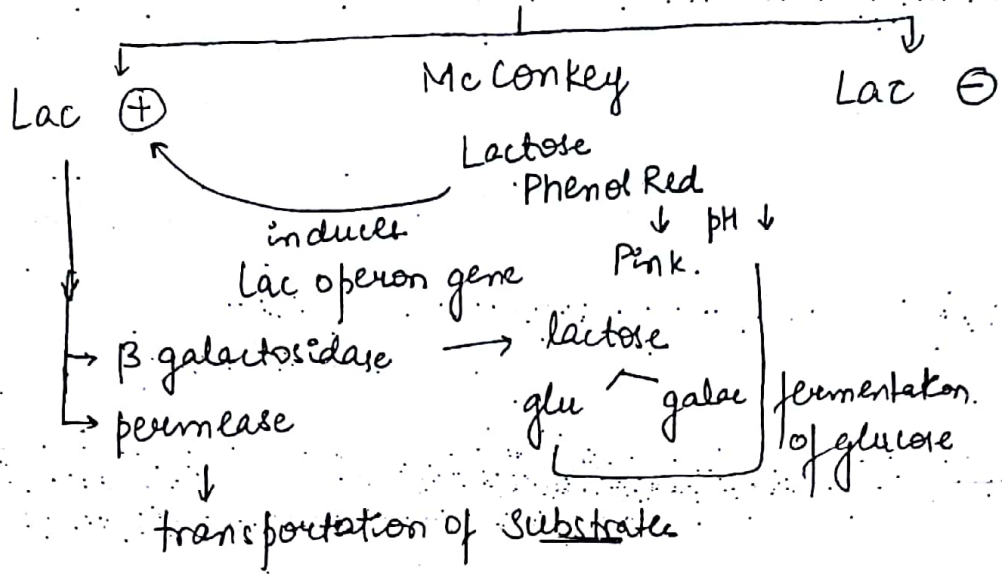
GRAM -ve BACILLI

ENTEROBACTERIACEAE

- ① Ferment Glucose
- ② Motile / Non-Motile  
↓  
peritrichous flagella
- ③ Catalase (+) except *S. dysenteriae* Type I
- ④ Oxidase (-)
- ⑤ Reduce nitrate to Nitrite



# Enterobacteriaceae

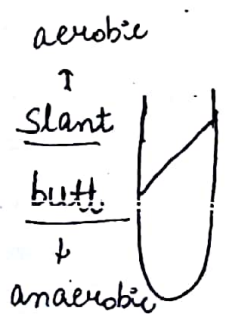


- E. coli - [except. Enteroinvasive E. coli]
- Klebsiella
- Enterobacter
- Serratia
- Edwardsiella
- Citrobacter
- Aerizona
- Providencia
- Leuwinia
- Shigella sonnei

- Salmonella
- Shigella
- Proteus
- Yersinia
- Enteroinvasive E. coli

## Triple Sugar Iron Agar Media (TSI)

[glucose, lactose, sucrose]  
 disaccharide  
 1 : 10 : 10



[Slant] → oxidative deamination  
 ↓  
 peptides (AA) broken down to  $\alpha$ -NH<sub>2</sub>  
 ↓  
 ↑ pH. [Red]

Butt → fermentation → ↓ pH [yellow].

130

Lactose ⊕



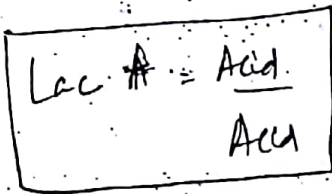
Initially, glucose fermentation occurs  
↓  
yellow colour.

↓  
Later glucose is finished.

↓  
Then Lac ⊕ organism causes  
Lactose metabolism

↓  
So pH ↓

↓  
Again Yellow



Lac ⊖



Initially, glucose fermentation occurs  
↓  
yellow colour

↓  
Later glucose finishes

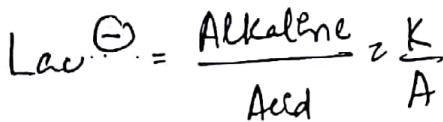
↓  
Then Lac ⊖ can't metabolise Lactose

↓  
No new acid production

So, oxidative deamination continues. ⚡ out any  
neutralisation.

↓  
↑ pH

↓  
Red colour



Non-Fermenter.



Non-fermenter =  $\frac{\text{Alkaline}}{\text{Alkaline}}$

Oxidative Deamination. occur.

Vibrio - Lac<sup>-</sup>  $\left. \begin{matrix} \text{glucose} \\ \text{Sucrose} \end{matrix} \right\}$  fermentation (+)



for Vibrio - TSI + McConkey Req.

as in TSI resemble Lac<sup>+</sup> Organisms

H<sub>2</sub>S producing Enterobacteriaceae

TSI media since contain Fe

Fe + H<sub>2</sub>S  $\Rightarrow$  Black colour.  $\Rightarrow$  BLACK COLONY

Salmonella

Proteus

Citrobacter

Edwardsella

Media used

- 1) Bismuth sulfite citrate sulfide
- 2) Deoxycholate citrate agar (DCA)
- 3) Xylose Lysine ~~deoxy~~ deoxycholate (XLD)
- 4) Salmonella shigella Agar (SS)
- 5) Hekton enteric agar (

Fe

- 6) Lysine agar
- 7) TSI, <sup>Fe</sup> Kligler Fe Iron (KIA)
- 8) SIM (Sulfide Indole motility medium)
- 9) Lead Acetate Agar

tube

KIA. Contains - glucose : Lactose  
1:10

It is used in vibrio non-endemic area as it doesn't contain sucrose as sucrose is for vibrio

## E. COLI

**UTI** - ~~fib~~ fimbriae P, Fe, S, Type 1.  
Lower serotypes O<sub>1</sub> O<sub>2</sub> O<sub>4</sub> O<sub>6</sub> O<sub>7</sub>.

→ Screening -  
M/E

Guiness Nitrate Test

Comp<sup>n</sup> { culture of urine.  
Significant ~~bacteremia with~~ bacteremia }  $> 10^5$  c/fu/ml.  
Midstream urine  
Suprapubic aspiration.  
KASS CONCEPT

except → Urem +ve org. }  $10^2$  to  $10^5$   
Catheterised patient

Urine culture

std. loop technique

Miles & Mitty

**Quantitative**

**Semi-Quantitative**

1 ml of urine in media

4mm internal diameter

Coulter Counter

0.001 ml approx.

Colony counter





Media -

17 McConkey + Blood Agar

133

↳ used so that *Staph aureus* if present

2) CLED (cysteine Lactose electrolyte Deficient media)

- differential media
- prevents swarming of proteus
- ~~prom~~ promotes growth of *Staph.* + *Candida*  
Q [enriched media not required]

## Diarrhoea

Serotype

Virulence factors

1) STEC  
EHEC

— Lambda like Stx1 or Stx2 encoding  
bacteriophage

2) ETEC

CFA, LT + ST (plasmid)

3) EPEC

EPEC adherence factor (plasmid)  
Locus for enterocyte effacement (LEE) -  
chromosomal pathogenicity island

4) EIEC

Invasion, Intracellular spread.  
cell to cell transmission (plasmid)

5) EAEC

Adherence + toxin genes (chromosomal  
plasmid)

**EPEC**

- Paediatric  
M/C infant children.

Non-inflammatory Diarrhoea  
& Adhesion on HELA/HEP cell lines

↓  
Enteroadhesive E. coli

~~EHEC~~

Δ - Serotyping

**ETEC**

- T- Traveller's Diarrhoea M/C

CFA

Toxins

← LT (labile)

↓  
↑ cAMP

Δ - Rabbit ileal loop  
ligation test



Ballooning

**BIKEN test**

(ppt)

→ ST (stable)

↓  
↑ cAMP

Infant or mice ileal  
loop ligation

Ag detected by ELISA  
using mouse Ig

\* Serotyping for ETEC.

ETEC causes non-inflammatory diarrhoea

EIEC → atypical E. coli  
Non-motile / Lac<sup>-</sup>

135

Inflammatory Diarrhoea

Δ - Sereny Test [conjunctivitis in Rabbit eye]  
serotyping

EHEC → Inflammatory Diarrhoea & Blood.  
due to stx (shiga like toxin) or verocytotoxin

↓  
haemorrhagic colitis

HUS → EHEC (H1C)  
90% association.

Stx<sub>2</sub> > Stx<sub>1</sub>.

M/c cause of outbreak.

Δ - For screening ⇒ Sorbitol MacConkey media.

↓  
EHEC doesn't ferment sorbitol

serotyping

EAEC → BRICK STACK PATTERN  
Non-inflammatory Persistent Diarrhoea

# KLEBSIELLA

136

Necrotising Pneumonia  $\bar{c}$  Empyema

Lower Lobe  $\rightarrow$  Lobar pneumonia

HAI. (hosp. acquired)

Hypervirulent *Klebsiella pneumoniae* (hvKP)

1) Hypermucoviscous phenotype

2)  $\uparrow$  dissemination

3) String Test (>5mm long)  $\rightarrow$  also seen in *Vibrio*

4) *Klebsiella* - lysine decarboxylase

	E. coli	<i>Klebsiella</i>
Motility	(+)	(-)
Urease	(-)	(+)
Mucoid colony	(-)	(+)
IMVic	++--	--++

Indole

## Indole Test

~~Indole~~ Tryptophan

$\downarrow$  Tryptophase

Indole; Pyruvic acid -  $\text{NH}_3$

$\downarrow$

red complex aldehyde group.

in KOVAc or Ehrlich reagents



- (+) → E. Coli
- (-) → Klebsiella  
Enterobacter.  
Hafnia  
Serratia.

**Methyl Red**

pH indicator - Red at 4.4.  
 (+) for E. Coli

**Voges Proskauer**

Acetoin detected.  
 (+) for Klebsiella  
Enterobacter  
Hafnia  
Serratia.

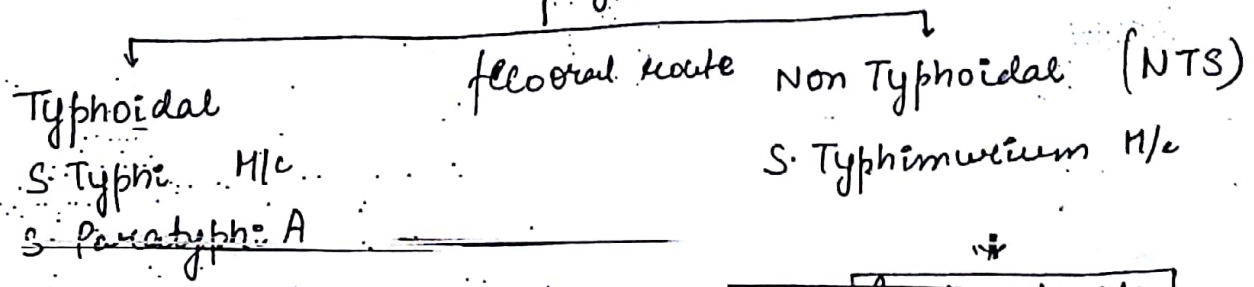
**Citrate**

utilisation of citrate  
 when citrate utilised, Green → Blue colour change

SALMONELLA

Salmonella enterica subspecies enterica

Serotypes



S. Paratyphi B  
 Enteric fever, Neutropenia

Gastroenteritis.  
Neutrophilia.

Serotype - motile except *S. Gallinarum* +  
*S. Pullorum*.

138

## NTS

Salmonella → Toxin → causes endothelial intestinal  
epithelial damage

↓

IL8

↓

Neutrophilia

— No blood or mucus

Pus (+) in stool.

Rx - not required

no antibiotics

Since antibiotics will lead to ↑ fecal shedding

↓

'outbreak'

## ENTERIC FEVER

Salmonella multiplies in Peyer's patches [MALT]

↓

Spleen, Lymph node.

Bone marrow.

↳ it gets blocked  
leading to neutropenia

Neutropenia

• Hepatosplenomegaly

Lymphadenopathy

⊕

Other features  
~~constipation~~

Rose spots

Bradycardia

Step ladder fever.

Pea soup stool [constipation/diarrhoea]

For Confirmation

Blood culture - 1st + 2nd week 1:10 dilution. 139

Widal test - 3rd wk onwards.

### WIDAL TEST

Tube agglutination.

To detect O & H Ab in pt serum using Widal Ag.

T<sub>O</sub> - 'O' Ag of S. Typhi

T<sub>H</sub> - 'H' Ag of S. Typhi

A<sub>H</sub> - 'H' Ag of S. paratyphi A

B<sub>H</sub> - 'H' Ag of S. paratyphi B.

'O' Ab appears early 'H' Ab persists longer.

'O' agglutinin is granular → round bottle FELIX TUBE

'H' " " fluffily → conical DREYER TUBE

Mirror is used for looking at the agglutination at the bottom.

16 tubes are used (4x4)

Widal Ag extraction - (O-901 strain of salmonella used)

'O' Ag → Heat stable

Smooth strain (LPS = OAg)

grow in phenol Agar. H-O variation. [loss of flagella]

ethanol / chloroform

Not total loss

'H' Ag. → Heat labile

Rough strain (No LPS - No Ag)

140

S → R variation (Loss of virulence)

"old culture"

autoagglutinable.

grow in Craigie tube.

H antigen and migrate.

salmonella



semisolid  
agar media

→ formalin is used for killing

Factors affecting Widal

1) Endemicity

(single serum sample)

cut off titre

O Ab  $\geq$  1:320

H Ab  $\geq$  1:640

(94)

4 fold rise in titre

2) Anamnestic Reac<sup>n</sup>

Transient rise in widal titre due to some other infection in apparently a previously salmonella infected pt.

IgG is responsible for it

3) False +ve Reac<sup>n</sup>

due to some other infect<sup>n</sup>.

4) Rx

5) Vaccination.

— TAB (killed) , Ty 21a (live) polyvalent



Vi - polysaccharide - monovalent

↓  
↳ Do not affect widal titres.  
from 2yrs to any age group.

141

Rx Ciprofloxacin - DOC  
2nd line - ceftazoxime

### [CARRIER

By Bile culture

Stool culture

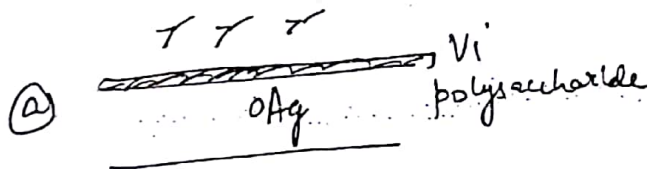
Vi Ab detection using ViAg (source is Citrobacter)

Serotyping in salmonella  
Kauffman White scheme.

False negativity while  
serotyping is removed by  
V → W variation achieved  
by heating

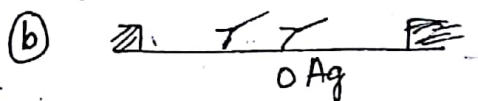
Best specimen - Bone Marrow culture

(painful but ↑  
sensitivity)



V forms fresh isolates.

Non-⊙ agglutinable



W forms

⊙ agglutination.

# SHIGELLA

142

- Non-Motile
- ~~Produce Gas (due to pyruvic acid)~~
- No Gas:- can't cleave pyruvic acid.  
(anaerogenic)
- Infective Dose - 10-100 bacilli
- Cause outbreak

## S. DYSENTERIAE

chromosomally encoded toxins  
↓  
verocytotoxin (stx)  
enterotoxin  
neurotoxin (acts on blood vessels)

12 serotypes

3.

## S. FLEXNERI

6 serotypes - X + Y Variant  
More common in India

## S. BOYDI

18 serotypes

## S. SONNEI

no serotypes

→ antigenically homogeneous.

Most common worldwide

✓ Colicin Typing (Bacteriocin Typing)

Invasion → By Virulence marker: Ag (plasmid)

143

Δ - Stool culture. (Salmonella + shigella)

\* Enrichment media [liquid = inhibition].

↳ Tetrathionate Broth

Selenite F Broth.

SS Broth

\* Selective media → media for H<sub>2</sub>S detection

### PROTEUS

Swarming  
Pleomorphic

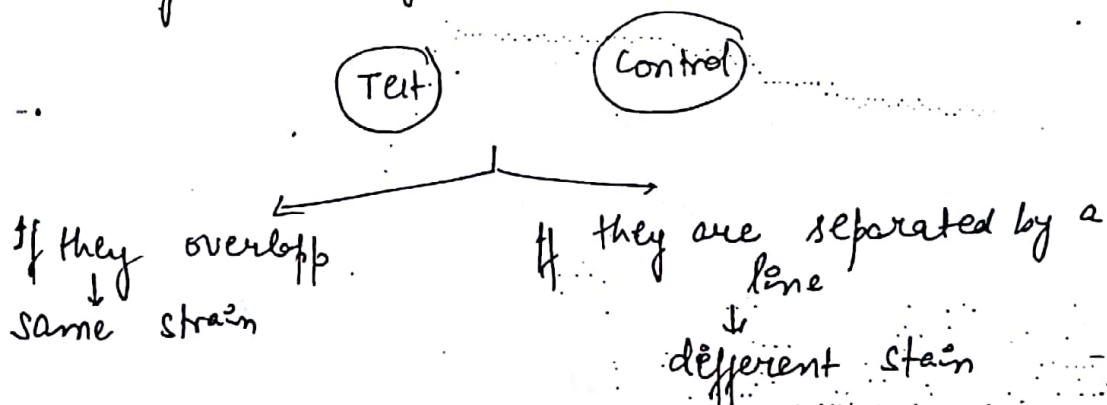
produce urease → cause UTI = calculi  
(Struvite)

Δ - PPA test (Confirmation for Proteus, Providencia + Morganella)

↓  
Phenyl pyruvic acid

Diene phenomenon for typing

↓  
using swarming



# YERSINIA PESTIS

144

VF:- Toxin → No virulence

- VW antigen
- F<sub>1</sub> protein (plasmid)
- Coagulase
- Phospholipase

Virulence +  
helps bacilli to survive at  
27°C in flea midgut

## Plague (Black Death)

↓

- Bubo
- pneumonic bubo → if enters lung
- Septicaemic
- outbreak

## Endemic Area

- ① Beed - Latue
- ② Kohlu
- ③ Rohru (shimla)
- ④ Uttarakhand.

Δ - Wayson Stain  
safety pin Bacilli  
due to bipolar staining

Ghee Buoth - Stallactite Growth.  
at 27°C



# YERSINIOSIS

145

Gastroenteritis = mesenteric involvement

*Y. enterocolitica* M/c

*Y. pseudotuberculosis* M/c mimics appendicitis.

M/c Bacteria  $\hat{=}$  mimics appendicitis  $\Rightarrow$  *Pasteurella multocida*.

$\Delta$  of yersinosis -

Stool culture on buffer saline. Q.  
'cold enrichment'

Selective Media  $\rightarrow$  CIN (Cefsulodin Irgason  
Novobiocin media)

## CALYMMATOBACTER (KLEBSIELLA) GRANULOMATOSIS

Intracellular

- Donovanosis / Pseudobubo / Pseudoelephantiasis.

[no lymphadenopathy]

- Safety pin appearing Bacilli in mononuclear cells (PUNO cells)  $\rightarrow$  Diagnostic

## H. DUCREYI

$\downarrow$   
Pain.

Chancroid  $\rightarrow$  Painful

Requires only Factor X for growth.

A school of fish or Tramtrack or Rail Road

Extracellular

Gram -ve Cocci bacilli

BURKHOLDARIA PSEUDO MALLEI

shows safety Pin Appearance

Gram -ve.

Safety Pin Appearance

Vibrio parahaemolyticus

Pasteurella

Burkholderia pseudomallei

Calymmatobacter

Yersinia

FERMENTORS

VIBRIO

VIBRIO CHOLERAE

Comma shaped - single Polar Flagella.

Virulence

→ Endotoxin - no virulence

→ Exotoxin

CT (Cholera toxin) Most Imp

ACE (Accessory Cholera exotoxin)

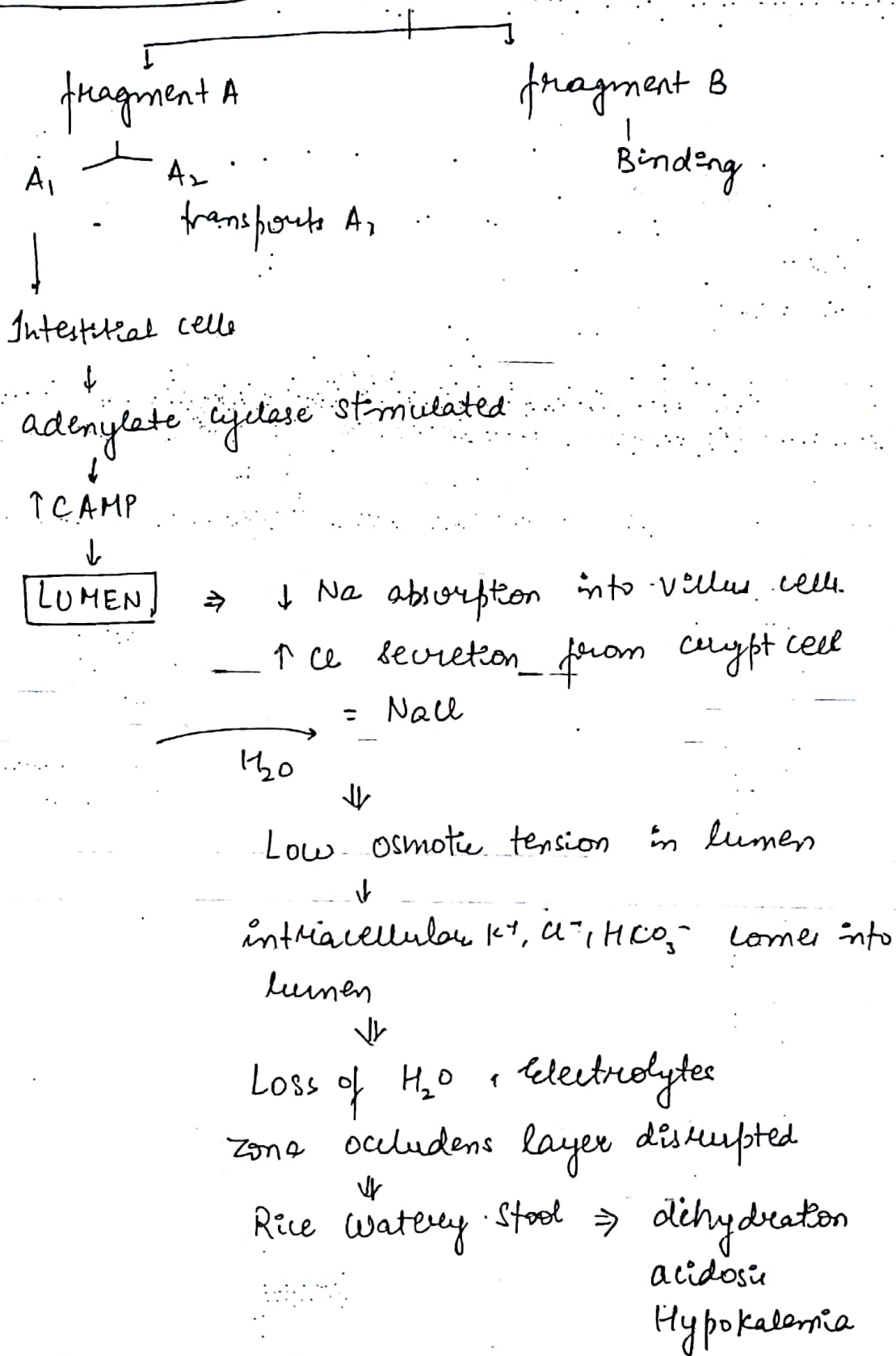
ZOT (Zona occludens toxin)

TCP (Toxin coregulated pilus)

found in O<sub>1</sub>, O<sub>139</sub>

# Cholera Toxin

147



## Diagnosis

① Stool Culture

② Enrichment Media → Alkaline Peptone Water  
Monsieur Tauxocholate broth

① Transport Media - Carvey Blair

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VR media

autoclaved sea water

② Selective Media - Bile Salt Agar

GTTA (Gelatin Tawochdate Tellurite)

TCBS (Thiosulphate Citrate Bile Salt  
↓  
sucrose)

*V. cholera* → yellow colonies

Halogenic vibrio → Green colonies. ~~Halophilic~~ [Halophilic].

17 *V. parahaemolyticus* → causes necrotizing enterocolitis

Kanagawa  
phenomenon

← [β hemolysis on High salt Agar  
containing Blood Agar Media

[Wagatsuma Media]

2) *V. vulnificans*.

Darting Motility  
other tests -

• String Test

• Oxidase (+)

• Cholera Red Reac" (+)

↳ Nitroso indole formed.

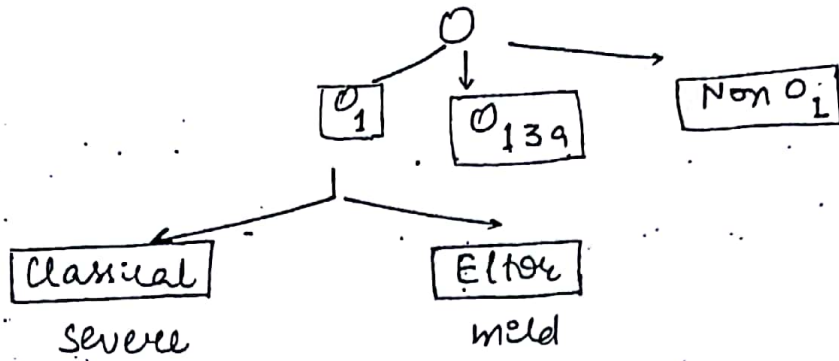
\* Epidemic Period → Source is Human

\* Interepidemic Period → Source is crustaceans in sea water



# Typing of Vibrio

Serogroups



1st 6 pandemic originated from Bengal

7th pandemic from Indonesia

Best for typing  
Biotyping  
(VP Test)

Agglutination of RBC

Hemolysis of RBC

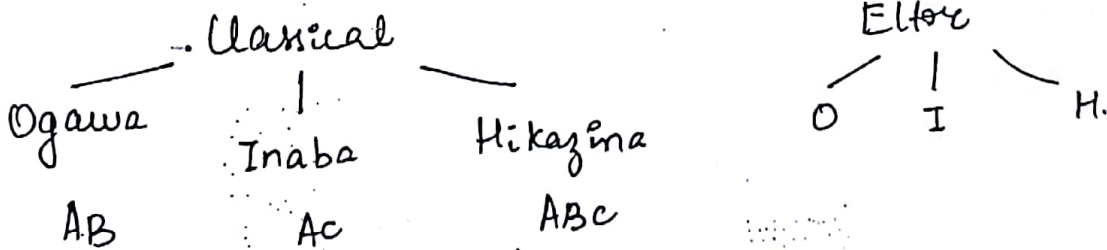
Polymyxin B

Phage susceptibility

VP Test	(-)	(+)
Agglutination of RBC	(-)	(+)
Hemolysis of RBC	(-)	(+)
Polymyxin B	(S)	(R)
Phage susceptibility	IV	V

'Mukharjee'

~~Biswas~~ Biswas Biogroups - classical & Eltor



O139 - Capulated. ↳ capsule contains O139 Ag

↓  
1st isolated in Chennai.

Bengal Strain → 1992-94 outbreak in Bengal

It caused outbreak.

So, Non-pandemic strain.

150

O<sub>139</sub> is antigenically, epidemiologically distinct from O<sub>2</sub>

Clinically similar

NO cross immunity seen.

### Vaccine

Killed vaccine oral vaccine - classical, Eltor -  
ogawa, Inaba

↓  
↑ Mucosal Immunity

'Copies ~~Ab~~ Q.  
Ab

in stool.

### NON-FERMENTORS

PSEUDOMONAS

[Obligate Aerobe]

→ environment

Colonies have shiny "Gun Metal" appearance.

fruity odour / Grape odour

NLF, Oxidase +ve

Grows at 42°C

Pigments :-

Pyocyanin - blue.

Pyoverdins - green

Pyorubins - red

Pyomelanin - black

## Virulence Factors

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- ① Pili - Adhesion to cells
- ② Flagella - Adhesion, motility, inflammation
- ③ Capsule - Biofilm
- ④ LPS - Bind to CFTR for internalisation  
Anti-phagocytosis  
Inflammation
- ⑤ Type III Secretion System - Exo S  
Exo U  
Exo T  
Exo Y  
Anti-phagocytosis
- ⑥ Type II Secretion system - Exo A - inhibition of EF2  
Cytotoxicity of phagocyte
- ⑦ Phospholipase - Cytotoxicity of phagocyte
- ⑧ Proteases - Proteolytic activity

## Complement System Evasion by Pseudomonas

Elastase & alkaline phosphatase



degrade C1q

C3b / C3b

Δ - Cetrimide Agar - Selective Media

# Typing - Bacteriocin Typing

[Image]

Difference antimicrobial Agents secreted.<sup>152</sup>  
by different strains

Shigella Sonnei - colicin  
Klebsella - Klebocin



Grow strains in between  
1 Remove it next day

↓  
Chloroform application.

↓ standard  
Now put again strain.

↓  
Growth differs due to  
antimicrobial substance released.

Rx

↳ Neutropenic host or HDR

↓  
Cefipime

② Non-neutropenic host

↓  
Monotherapy

↓  
Ceftazidime

↓  
Combination

↓  
Pip/taz or

Carbapenem or  
Aminoglycosides



# BURKHOLDARIA PSEUDOMALLAI

153

① Capsulated

② Most virulent amongst Non-fermenters.  
It has all ~~the~~ skeleton system + capsule.

③ ~~Med~~ Melioidosis → Pulmonary Infection  
Abscess

pharyngitis

necrotizing lung Disease

skin ulceration

Lymphadenopathy

④ Bioterrorism - Cat II agent

⑤ Long Latent Period ⇒ 'Vietnam Time Bomb Disease'

⑥ Δ → Gram -ve  
Safety Pin

Culture - Ashdown media -

cornflakes head wrinkled

Purple colonies

Doc - Meropenam / TMP-SMX.

# STREPTOTROPHOMAS MALTOPHILIA

(Non-fermenter)

154

Gram-ve.

Motile (poor)

Environmental

HAI

Δ - culture ✓ Lavender Green

✓ Gray colour colonies on blood agar

oxidase ⊖

Oxidation of glucose + maltose

Doc- TMP-SMX \* Ticarcillin - clavulanic acid

## ACINETOBACTER

Environmental, HAI

Non-motile

oxidase ⊖

R<sub>x</sub> - same as pseudomonas.

Non-Fermenter (ZOO NOTIC)

## BURKHOLDARIA MALLEI

Equinus (Horse)

→

Glander's

## BURKHOLDARIA CEPACIA

Causes Cephalic Syndrome

↓  
associated w/ sepsis

Δ - Colistin containing ~~media~~ agar.

Pneumonia in Cystic Fibrosis = mucoid colonies

↳ B. cepacia

155

## BACTERIAL VAGINOSIS

caused by Gardnerella

Bacteroides

Mobiluncus sp.

↓  
Lactobacillus

Nugent score  $\geq 7 \Rightarrow$  Diagnostic

Based on less no. of Lactobacilli & more pathogens.

Clue cell - epithelial cells studded = Gram -ve bacteria

Whiff test - Amine (fishy odour)

pH  $> 4.4$ .

## CHROMOBACTERIUM

## VIOLACEUM

Purple coloured.

Lead to lung & wound infection

Gram -ve

## SERRATIA Marcescans

Pseudomonas

# CAPNOCYTOPHAGA

Slow growing

Capnophile

Gram.-ve

- Fusiform. filamentous

Gliding Motility

Modifies chemotactic ~~factor~~ activity of neutrophils

Fulminant Injct in aplenia or allohem.



Fusiform  
Gram-ve

## HACEK

Haemophilus parainfluenzae (HC) sp isolated from

- HACEK endocarditis

A. actinomycetemcomitans - Prosthetic valve endocarditis.

Cardiobacterium Homini → aortic valve

Eikenella corrodens → Least common cause of HACEK.  
endocarditis

Human Bite → clenched fist injuries

Kingella Kingae - septic arthritis in children. (M/d)  
Purpura Rash similar to meningococcus



LEGIONELLA PNEUMOPHILIA

BCYE

157

\* Charcoal in the ~~BYCE~~ media used to isolate Legionella pneumophila

↳ (Buffered charcoal Yeast Extract)

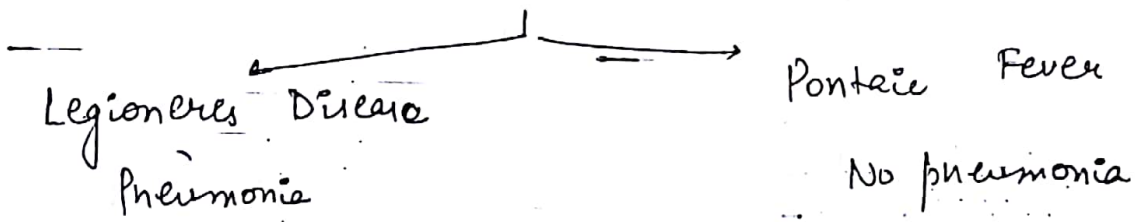
↓  
detoxifying agent

[Black colonies]

\* Contaminated water (amoeba/algae)

↳ aerosol through A/c  
aspiration.

→ No person to person transmission



Δ ① Neutropenia

② Culture on BYCE media [charcoal for detoxification]

↓

Monoxenic media

(Requires 1 biological agent in media)

③ used in practice cultures

③ Immunofluorescence - sensitive method

Pfeiffer's Bacillus

aerobe  
oxidase ⊕  
pleomorphic

Heb. sample - coccobacilli  
CSF - filamentous.

1st organism - entire genome sequenced

2 months to 3 years - No Ab to PRP

Biotype 1 is M/C cause of meningitis

Virulence

capsule → 6 serotypes (a to f)

Type b → capsule has PRP  
[95% infection] (polysaccharide ribitol PO<sub>4</sub>)  
↓  
causes invasion.

5% of infection - Non-capsulated or  
Non-Typable strains

Vaccine - Heb  
(monovalent polysaccharide conjugate  
vaccine)

△ Require X, V factor for growth.

159

① Culture

a) chocolate agar - factor V released

b) Blood agar  $\bar{c}$  S. aureus

c) Nutrient agar  $\bar{c}$  disc of X, V factor

d) Levinthal agar (Capulated strains produce incidence)

e) Fildes Agar - peptic digest of blood in nutrient agar.  
(Best)

— Satellitism in H. influenzae is due to factor V.

~~Colon~~ Bigger colony around S. Aureus.

↓  
It stimulates produc<sup>n</sup> of V factor

### H. AEGYPTICUS

Koch's weeks Bacilli or Brazilian purple fever  
Egyptian conjunctivitis Red eyes.

# BORDETELLA PERTUSSIS (Gram -ve coccobacilli)

Whooping cough → inspiratory stridor

160

100 day fever

No animal reservoir unlike B. Bronchoseptica

Virulence -

① Capsule → No Role in Virulence

② Pertussis Toxin (Type I, IV) - ~~secretory~~ secretory system  
activate adenylate cyclase → ↑ cAMP

③ Tracheal cytotoxin

④ Adenylate cyclase Toxin

⑤ Dermonecrotic toxin

⑥ Endotoxin

⑧ Adhesin (Type V) → FHA, pertactin, fimbriae  
secretory system BAKA protein

⇓

↑ Histamine

↑ insulin

Lympholysis → huge

△ ① Thumb Print appearance

② Regan Lowe → Mercury Drops or Busted Pearls

(Charcoal + 10% horse blood +  
cephalexin) Regan Lowe ⇒ Transport media



③ Bordet. Gengou → Potato Blood Agar

Vaccine

Whole celled

④ Ab detect by ELISA

161

⑤ PCR IS481 - PT promoter gene

acellular region

contains ↓ more safe

PT - pertussis toxin

FHA1 - fimbriae

FHA2 } filamentous

FHA3 } haemagglutinin Ag

Pertactin

Thiomersal - preservative

FRANSICELLA

TULARENSIS

Zoonotic

- Transmission - ticks / deer fly or direct contact
- rabbits / mink rats
- penetration of skin M/c
- inhalation, ingestion

Culture media

✓ chocolate agar

✓ MTM (mod. Hayler Marten)

✓ BCYE

Gram -ve Cocci bacilli

Δ - Serology

(M/c)

R<sub>x</sub> - resistance to  $\beta$  lactams

162

streptomycin / gentamicin.

tetracycline

chloramphenicol

FQs

CAT A agent of Bioterrorism.

(BSL III) req for culture.

M. TB

① Obligate aerobe

② Micro-aerophilic → M. Bovis.

③ Virulence factor

- CORD factor.

$\Delta$  -  $\nabla$  M/E → ↓ sensitivity

$10^4$  bacilli/ml of sputum is req.

a) Zn stain - slender, long, curved.

[for confirmation] beaded

b) Auramine Rhodamine - Direct fluorescence assay

↓  
↓  
Binds to mycolic acid

Highly sensitive → used for screening

## 27 Culture

LJ media.

163

Egg containing media = asparagine  
Malachite Green (Selective)

M. Tb → Eugonic growth

M. Bovu → Dysgonic growth (sparse)

Petroff's Method → sputum processing.  
(15-20 min)

↓  
4% NaOH  
N-Acetyl Cysteine (mucolytic agent)

↓  
Centrifugation at ↑ RPM

⇓  
BSL III (Biosafety Level)

Best  
CBNAAT (Cartridge Based Nucleic Acid Amplification Test)

- PCR

- BSL - II

INNO-LiPA (Line probe assay)

Reverse Hybridisation technique.

20 DNA probes of different mycobacterial species  
are immobilised on nitrocellulose strip. The

amplicons (amplified DNA) are hybridised

to the probes

Chromogenic Reagents are used instead of radioactivity

GTMD (Genotype Mycobacteria Direct)

NASBA (NA. where RNA is amplified & reverse <sup>164</sup> hybridized = RNA probe)  
↓  
Nucleic acid sequence based assay  
on strip  
Only 5 species detected

IS6110 - Best for Genotyping

Best Culture Technique - BACTEC NGIT

↓  
✓ fluorescent  
✓ 2 days - 7 days

Latent TB

Mantoux Test → 5 TU of PPD-S  
or 1 TU of RT<sub>23</sub> strain ← RNTCP  
after 72 hrs ↓  
in ⊙ forearm.

>10mm induration along short axis  
on ⊙ forearm

⇓  
Positive → exposed

Quantiferon Gold Test / IFN-γ Release Assay IGRA

Whole blood  
↓  
Stimulate = M.Tb Ag. eg. -  
E.CAT.6  
CFP 10  
M.Tb 7.7 ⇒ less false +ve  
Lymphocyte → IFN-γ  
↓  
⊕  
exposed



Q No. cross Rx = BCG or atypical Mycobacteria  
or NTM or MOTZ  
except M. Kansasi

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## ATYPICAL MYCOBACTERIUM

↳ Runyan classification

Photochromogenic → Pigments in Light

M. Kansasi

M. Maximum [swimming pool / Fish tank granuloma]

Scotochromogenic → Pigments in dark

M. Scrofulaceum

M. Szulgai

[crow gae in dark]

Non-Photochromogenic - No pigments.

M. ulcerans → Buruli Ulcer

M. Avium → < 50 CD4 cell. in HIV

M. Avium

M. intercellularis (Batey Bacilli)

Rapid Growers -  
(< 7 days)

M. Chelonae

M. fortuitum

} → cause post trauma abscess

[loan + fortune = rapid growers]

MTB

Atypical

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① Niacin (+) (-)

② ~~Amu~~ Amyl Sulphatase (-) (+)

M. LEPRAE [acid fast staining w/ 5% H<sub>2</sub>SO<sub>4</sub>]

SSS (short, stout, straight bacilli) → [Image]

- acid fast B. in palisade

750 bacilli - globi

in histocytes - FOAM cells

specimen

SSS - split skin smear (6 specimen → 4 skin, 1 ear lobe, 1 nasal mucosa)  
+ nt in edge of lesion

Ab to PGL2

Δ - M/E - ↓ sensitivity 10<sup>4</sup> bacilli / gm of tissue.  
IOC

culture - Best Armadillo (natural infection)

M/c - Mice (suckling)

↓

Thymectomy

(to induce lepromatous leprosy)

~~LEPTOSPIRA~~

Leptospira → ?

Dark Ground Microscopy ← Treponema

Light microscopy → Borrelia

# SPIROCHAETES

Spiral organisms & endoflagella → cork screw <sup>167</sup> motility

T. Pallidum → venereal Syphilis

1<sup>o</sup> stage → Hunterian chancre

↓  
painless, indurated.

self-limiting in 4-6 weeks.

2<sup>o</sup> → Diminution.

after 2 to 6 months.

↓

Condyloma

Latent → No sign & symptom

3<sup>o</sup> → complications.

Aortic aneurysm  
Tabes Dorsalis

Δ -

1<sup>o</sup> stage → ① Dark Ground Microscopy

↓  
only reflected light through from object  
through special condenser

Resolution power not increased.  
only optical illumination used.

② EIA (Enzyme Immuno Assay)

Iol

2° stage → TPPA (Treponema Pallidum Particulate Agglutination)

168

(Easy)

↓  
gelatin  
✓ Most sensitive, specific. Overall  
Used for Confirmation.

Screening → VDRL  
slide flocculation Test

↓  
Cardiolipin Ag [Calcutta Ag]

(derived from ox heart)

Reactive → clumps. [Image]

Non-reactive → Scattered

Using microscope → floccule seen.

RPR

Cardiolipin + carbon particles  
Particulate agglutination based test

Nichol Strain → Pathogenic Treponemes

TPI

not safe but ↑ specificity

TPHA - haemagglutination assay

↓ sensitivity

FTA-ABS

Reiter's Strain → non-pathogenic treponemes

(Difficult)

+  
Absorption of Non-specific Ab in serum

Nichol's Strain used

[Indirect Immunofluorescence]



# LEPTOSPIRA

- ① Curved at 1 end. ? interrogans.
- ② M/c Zoonotic disease.
- ③ 3R → Rats, Rainfall, ricefield  
rat urine → penetration of skin.

affect liver & kidneys

Jeterior haemorrhage fever. & myalgia

## WEIL'S Ds. / Andaman Fever

1-① Culture on Korthoff, Fletcher, EMJH media

② Ag detect<sup>n</sup> in urine

③ Typing → Serum is used.  
(Leptospira difficult to grow)

Reference Lab

Macroscopic agglutination Test (MAC)

↳ Killed Leptospira Ag used to detect Serogroups  
↓  
Serotypes having similar Ag

Passive Agglutination

Serogroups detected.

Microscopic agglutination test → Serotypes detected

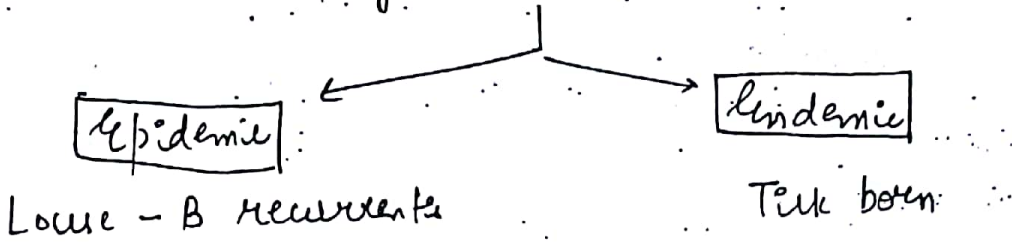
Live Leptospira  
immobilisation test → Dark Ground Microscope used

# BORRELIA

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Thick → can be seen in Light microscope

Causes Relapsing Fever



B. Burgdorferi



LYME'S Disease

Erythema chronicum Migrans

[Image]



Concentric Centrifugal Rash

Δ

PG6 Ab by western Blot Assay  
(Non-specific But Best)

25/2/18

## RICKETTSIACEAE FAMILY

- Rickettsia
- Orienta
- Coxiella
- Ehrlich

weil ~~fe~~ Felix Test



Heterophile Agglutination Test



Non-motile Proteins strains

P. vulgaris

Ox 19

Ox 2

P. mirabilis

OxK.

Russia in endemic for Typhus Fever

# TYPHUS FEVER.

Epidemic

Endemic

Brill Zinsser<sup>171</sup>

(Recurrent)

Louse

Flea

causative

R. Prowazeki

causative

R. Typhi

P. Prowazeki

Some of the elderly not suffering from Disease

§ Spotted Fever

Tick Borne.

- Ox2. - Rocky Mountain - R. Rickettsiae  
Fever

Indian tick typhus

Japanese " "

Siberian " "

R. conorii

mite → Rickettsial pox → R. Akari Q

Scrub Typhus

Oxk

Thrombiculid

mite (Larva)

→ orientia

tsutsugammshi

Epidemic Typhus

Endemic Typhus

Neil Moore or

Tunica vaginalis test

⊖ in

R. Prowazeki

⊕ in

R. Typhi

LV  
AV  
TV



# ✓ Q FEVER / ABBATOIR / QUEENSLAND FEVER

→ Tick act as reservoir → transmit to sheep, goat, cattle <sup>172</sup>  
but not to humans

→ Transmission to humans by respiratory route -  
aerosol from soil, tissue or during parturition.  
only rickettsial infect<sup>n</sup> can be transmitted out arthropod i.e.  
man to man → resp. route

→ Acute Infection → Influenza like

→ Chronic " → Infective Endocarditis M/c

→ Risk Factors - Pre existing valve anomaly  
- Immunocompromised

Δ - Culture only in cell lines [BSL 3 lab]

- Δ Immunofluorescence 10c  
Giemsa stain.

PCR

≡ Coxiella Survival Holder's Method by pasteurisation.

↓  
(63°C) for (30 min).

[Flash method - (72°C) for 13-15 sec].

Ehrlichia → Morula (vacuoles in phagocytes)

sp  
Sennetsu

→ raw fish.

Chaffensis

Phagocytophilia

} - tick.

→ affects monocyte

→ affects granulocytes.



# CHLAMYDIA

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Gram -ve cell wall But no peptidoglycan. (no neuraminic acid)

Lack ATP synthesis  $\rightarrow$  Energy Parasites

Infectious form k/n/a Elementary Body  
(50-330nm)

① Inclusion Body  
(Reticulate Body)

② MacLoy Cell Line

$\Delta$  form  $\rightarrow$  Reticulate body

Pathogenicity :-

C. Trachomatis  $\rightarrow$  H.P. Body (Heidstaden Proxazeki)

serotype A-C  $\rightarrow$  Trachoma

D-K  $\rightarrow$  Inclusion Conjunctivitis  
NGU

$\rightarrow$  Fitz Hugh Curtis  
 $\rightarrow$  Reiter's syndrome  
e  
conjun urethritis Poly arthri- tis

LGV<sub>1 to 3</sub>  $\rightarrow$  Lymphogranuloma Venereum  
 $\rightarrow$  painless ulcer, painful bubo

$\Delta$  - TRIC Serovars.

M/E  $\rightarrow$  Giemsa, Gimenez, Casteneda;  
Machiarella.

LGV  $\rightarrow$  Miyagawa corpuscle

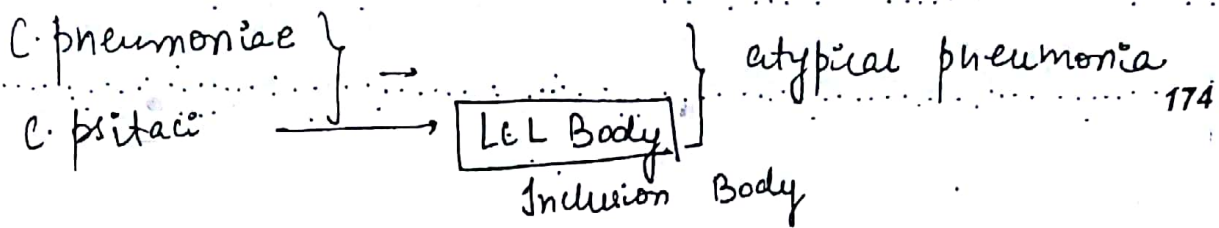
$\rightarrow$  culture on MacLoy cell lines

$\rightarrow$  Ab detection by ELISA

$\rightarrow$  NAAT by PCR.

LGV Serovars  $\rightarrow$  Free Test

Ab detect' by Micro Immunofluorescence Assay



### Typing:-

- 1) LPS → Genus Specific Ag (CF Test)
- 2) Envelope Ag → Species specific Ag
- 3) Outer Membrane protein → serovar or serotype specific Ag [Micro IF test]

### MYCOPLASMA

PPLO or Eaton Agent  
[pleuro-peritoneal like org]

My fried egg glided in dining while eating

M/c cause of atypical pneumoniae or walking pneumonia

✓ No cell wall

✓ Sterols +nt in cell membrane

No turbidity in liquid media → also seen in B. anthracis

Δ

① Culture in media  $\bar{c}$  cholesterol

⇓  
⇓ Diene stain of isolates [Methylene Blue + Azure]  
fried egg colony

② Serology → Heterophile agglutination Test

a) Cold agglutination → 'O'-ve RBC at 4°C

b) Streptococcal MG Test

# HELICOBACTER

- 1) Deep in mucous layer on epithelial side (pH - 7.4) 175
- 2) ~~Protects~~ → modifies gastric mucosa - reduce diffusion of acid.  
Proteases
- 3) Urease activity → Ammonia - buffering of acid
- 4) Complement system evasion by H. Pylori  
Protectin inhibits MAC → C<sub>5</sub>-C<sub>2</sub>

Δ

1) Warthin Starry silver stain ⇒ Sea gull wings.

also used for

Campylobacter

microaerophilic

media: Skirrow's

for Both. Butzler's

CAMPY Blood agar. grows at 42°C

HLA: B27 - Reactive arthritis.

Triggers Guillain Barre syndrome (serotype 019)

gullwing shaped, darting motility

✓✓ - comma shaped  
darting motility

Microaerophilic (5% O<sub>2</sub>)

Pathogenicity:-

Helicobacter → peptic ulcers

Adenoma / Maltoma

Campylobacter → Gastroenteritis & blood.

Reservoir (poultry)

Gold std. for Δ of Helicobacter → Urea Breath Test

Biopsy x urease ⇒ for dysplasia



# BARTONELLA BACILLIFORMIS

176.

## CARRION'S DISEASE

Oraya Fever - Acute phase

← [Female Lutzomyia]  
(sandfly)

- fever, other constitutional symptoms
- severe anaemia, jaundice, Hepatomegaly
- Lymphadenopathy, myalgia

— Verruga Peruana - after resolution of oraya fever  
[Image] Non tender,  
red to purple  
nodular lesion.

Δ:-

M/E → Warthin Starry or Giemsa stain  
⇒ Bacillin in RBC

Culture on blood agar at 35°C

Ab detect

PCR.

B. QUINTANA →

French fever (5 day fever)

By louse

caused outbreak

B. HENSELAE → Cat Scratch Disease → Cervical LN  
Basillary Angiomatosis

Cat Bite → Pasteurella



# RAT. BITE FEVER

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Spirillum Minor

3-5µm

negod spiral org.

Rat bite fever (Sodoku)

Local lesion, regional gland.

swelling, skin rashes.

Culture - inoculation in guinea  
pig / mic

Flagella at Both ends

Streptobacillus moniliformis

Gram (-ve)

Aerobic

Pleomorphic bacteria that  
forms irregular chains of bacilli  
interspersed with fusiform  
enlargements

L-forms seen.

Rat bite fever - Septic fever  
Blotchy

Petechial rashes

painful Polyarthritide

Ingestion of milk -

epidemics of Haverhill  
fever

Culture - trypticase soy  
enriched in 20% blood.

# BRUCELLA

Zoonotic

(from animal milk)

affects the reticulo-endothelial system

Intracellular

✓ Hepatosplenomegaly

✓ Lymphadenopathy

Malta or undulating fever (typhoid like illness)

✓ Neutropenia

Triad

- 1) undulating fever  
fever in night sweats
- 2) arthralgia
- 3) hepatosplenomegaly

DOC - Doxycycline + streptomycin.

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Δ - <sup>BE</sup> Casteneda Blood culture.

'biphasic media'

Serology

→ coombs test

→ std. agglutination test

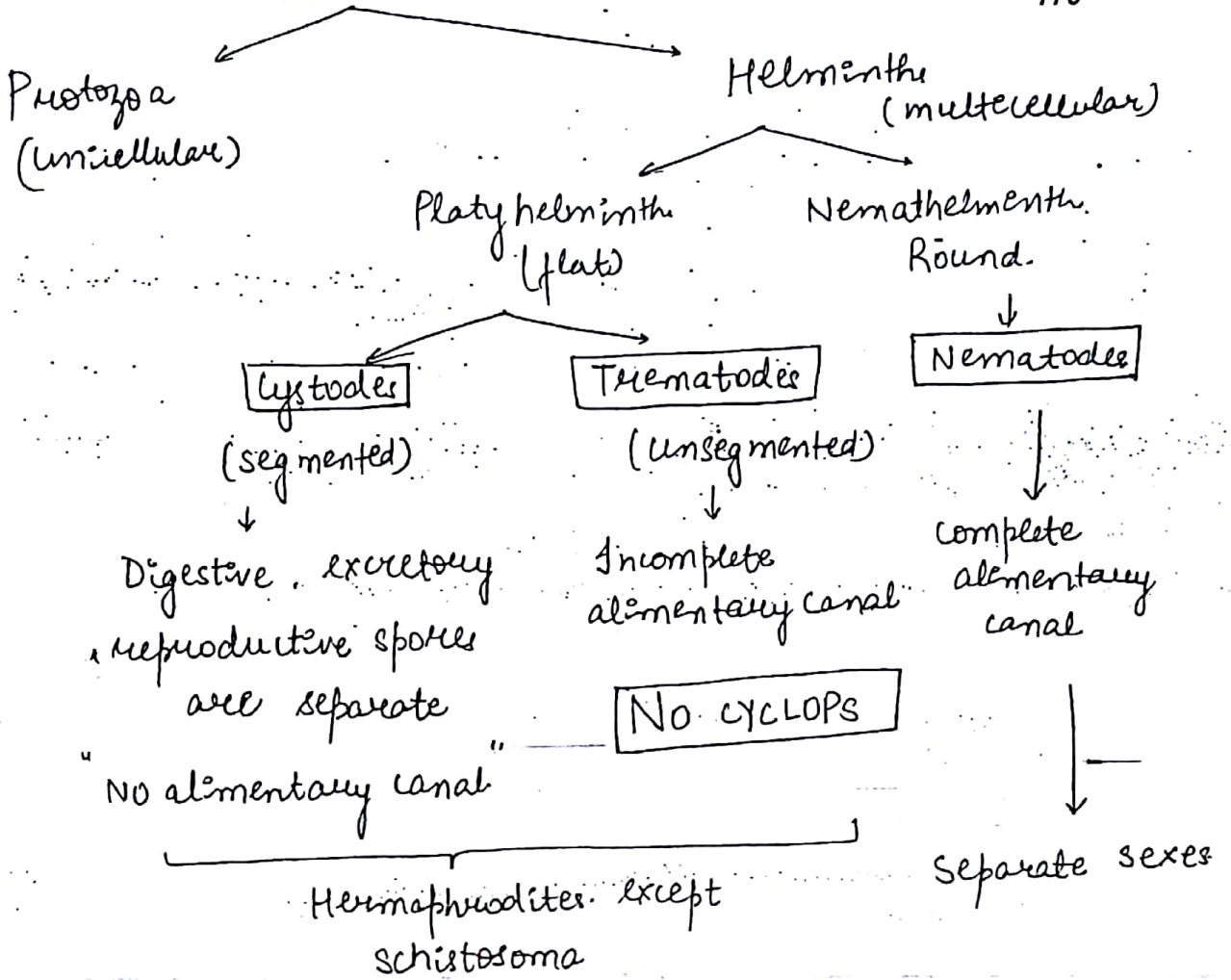
11 tube. dilution.

"PROZONE" Phenomenon.

→ PCR. → Gold std

Silver Impregnation → Leivaditi / Fontana  
for spirochaetes.

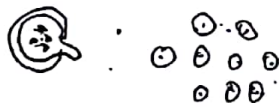
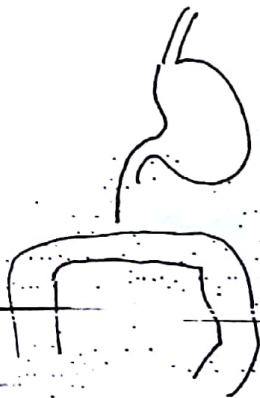
# PARASITOLOGY



## INTESTINAL PROTOZOA

ENTAMOEBAS HISTOLYTICA (pathogen) [Image]

Infective form  $\Rightarrow$  Quadrinucleate Cyst  
 Excystation occurs in ileum ( $\uparrow$  pH)  
 cyst  $\rightarrow$  Trophozoites



M/c site - Sigmoid colon  
 caecum (M/c)



## Virulence factors :-

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- ① Galactosamine Lactin → binding
- ② Protease → degrade collagen
- ③ Calmodulin } inhibit phagocytosis  
phosphatase A }
- ④ Thioredoxin Reductase → degrades toxic  
O<sup>-</sup> & N<sup>•</sup> radicals.

Af. Distal colon → encystation occur.  
Trophozoites → cyst

## Diagnosis :-

① M/E of stool. (wet or iodine or trichrome stain)

	<u>Pathogens</u>	<u>Non-Pathogen</u>
<span style="border: 1px solid black; padding: 2px;">Trophozoite</span>	15-20 μm active	20-30 μm sluggish
	RBC or cellular debris in trophozoite [leukophagous]	Bacteria & trophozoites
<span style="border: 1px solid black; padding: 2px;">Nucleus</span>	central	acentric
	Ectoplasm & Endoplasm - [thin] [granular]	Endoplasm
<span style="border: 1px solid black; padding: 2px;">Cyst</span>	6-15 μm 1-4 nuclei	15-20 μm 1-8 nuclei
<span style="border: 1px solid black; padding: 2px;">Glycogen Mass</span>	uninucleate stage	Binucleate stage
<span style="border: 1px solid black; padding: 2px;">Chromatoid Body</span>		

NO difference Q.



Δ Rectal Biopsy ⇒ Flask shaped ulcer.



Other ways of A

① Nested PCR

② zymodene assay → isoenzyme pattern on electrophoresis

③ galactosamine Lectin Ag detection by ELISA ] IOC for  
Gal/Gal NAC ELISA ] Invasive amoebiasis

## EXTRAIESTINAL AMOEBIASIS

~~Hem~~ Hematogenous Route

Liver (MIC)

Lung

Brain

↳ Posterosuperior quadrant of R lobe

Cutaneous (directly)

↳ cutaneous amoebiasis.

Δ

① ME of Liver aspirate ⇒ Anchovy sauce pus trophozoites ↓

② PCR → most sensitive

③ Radiology

④ Clinical diagnosis



Trophozoite is at periphery

## FREE LIVING AMOEBIA

Found in natural water source

Acanthamoeba → 1° lung infection

↳ haematogenous

Granulomatous Amoebic encephalitis.

# Keratitis (contact lens)

Δ - CSF ⇒ Both Cyst + Trophozoite

Naegleria → flagellated amoeba

↓  
travels via olfactory n/v

↓  
pierces cribriform plate

↓  
1° amoebic meningoencephalitis

↓  
fatal

Δ → CSF only trophozoites

- A of free living Amoeba

→ culture on non nutrient agar c̄ E. coli → Tracts.

→ M/E of CSF

• H. E. stain - [ Cyst + trophozoite → acanthamoeba  
Trophozoite → Naegleria.

• Immunofluorescence [Image]

## GIARDIA INTESTINALIS

Infective form - Quadrinucleate Cyst

Excystation takes place at the Jejunum - M/E site

Trophozoites bind c̄ ventral sucking disc

↓  
disrupt brush border enzymes

↓  
Malabsorption  
Steatorrhea

foul smelling stool  
Anaemia (↓ IF) - Megaloblastic  
Pernicious

## Diarrhoea

Stool → "Loose Greasy" yellow colour

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Δ -

- ① Stool M/E for trophozoites + cyst (M/c)  
wet mount, trichome, Kohn, Giemsa
- ② Direct Immunofluorescence assay → auramine
- ③ Ag Detection by ELISA, rapid immunochromatographic assay
- ④ Other test - Antero-test → string ingestion + then exam.  
Duodenal Biopsy  
NAAT

Each Trophozoite has 4 pairs of flagella [Image]

→ [ 1 ant  
1 middle  
2 post. ]

## BALANTIDIUM COLI

Ciliated.

↳ LARGEST PROTOZOA

Infects Pig, humans (occasionally)

Asymptomatic (M/c)

Persistent Intermittent Diarrhoea.

Doc - Tetracycline

Δ - stool M/E → trophozoites (cysts rarely seen)

Tissue staining  $\bar{c}$  H&E after endoscopy

[Differential Interference Contrast → for cilia]

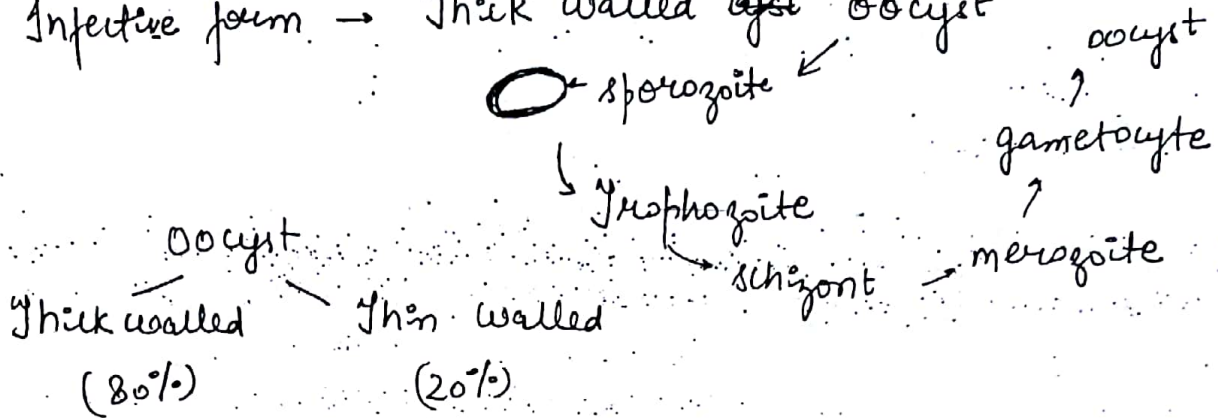
# COCCIDIAN INTESTINAL PROTOZOA

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Diarrhoea in HIV pt + extremes of age

## CRYPTOSPORIDIUM PARVUM (6µm)

Infective form → Thick walled oocyst



No t/t, only improve immune system Persistent Diarrhoea

## CYCLOSPORA (10µm)

autofluorescence. seen.

## ISOSPORA

① Kinyoun stain [Modified ZN stain] ⇒ Acid fast  
Δ :- ~~Kinyoun~~ stain cold technique oocyst

[Cryptospora - 6µm  
Cyclospora - 10µm  
Isospora - 25-40µm]

A Decolorising Agent → 0.5% H<sub>2</sub>SO<sub>4</sub>.

\* ~~ghost cells~~ → unstained cells



② Immunofluorescence Assay by auramine

MICROSPORIDIUM

↳ earlier considered coccidia

- Fungi
- cause diarrhoea + ocular infec<sup>n</sup>
- species seen
- Δ :- "Weber stain"

PLASMODIUM

Pigments

- ?

Peripheral Blood Smear - Gold Std

Δ :-

P. Vivax

P. Falciparum

RBC

Enlarged + Pale

Normal

Schuffner's - yellow

Maurer's dot - Black

Single infection

Multiple infec<sup>n</sup>

~~1 RBC~~

1 RBC = one parasite

1 RBC - multiple parasite

All stages

Early + Late stages

↓  
SEQUESTRATION

Mech: of

↓  
Vasculare endothelial cells

a) Cytoadherence.  
- adhesion molecule

b) Rosette formation  
- 2-3 parasite stick together

c) Agglutination - surface adhesion on RBC



P. vivax



Ring form

Romanowskii stain

[Early Trophozoite]

Amoeboid form

[Late Trophozoite]



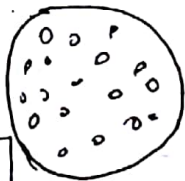
Schizont



completely fills the RBC

Merozoites

> 12 in no.



Gametocyte

P. Falciparum

causes

Renal failure



acrole forms

'signet Ring'

Divided Nucleus is also seen here

Crescent or Sausage



Gametocyte

P. MALARIAE

causes Nephrotic Syndrome

RBC - normal

Band forms



Basket forms



Merozoites



≤ 8 No.

Zeimann's Dots  
(light Brown)



\* Transfusion Malaria / Mother to child.

M/c: Trophozoites  
Schizonts  
Merozoites

short I.P.

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as No pre-erythrocytic cycle

\* Screening of blood Serology

→ Rapid. Immunochromatographic Assay

Advantage of Rapid.

No expertise required.

Falciparum

quinine therapy. - Black water Fever

↓  
acts as hapten

quininised RBC



↓  
autoimmune HSN II  
hemolysis of RBC.

complement mediated

quinine → cause hypoglycemia  
hence should be accompanied  $\approx 25\% D$ .

BABESIA MICROTI - Protozoa invading RBC

Tick Borne

RIF → splenectomy

Maltese cross tetrads. → seen in RBC.

$\Delta$  form



# HAEMOFLAGELLATES

Leishmania  
Trypanosoma 489

## LEISHMANIA

L. Donovanii } → Visceral Leishmaniasis  
L. infantum }  
L. chagasi } - new world.

Infective form → Promastigote (flagellated form.)

↓  
deposited on the skin by

Sand fly

Phlebotomus - old world

Lutzomyia - new world

↓  
Penetrate the skin  
through tracks

AMASTIGOTE

Spleen  
95%

BM  
65-80%

LN  
50%

↑↑ IL-10

↓  
inhibits TH<sub>1</sub>

↑↑ TH<sub>2</sub> response

↓  
IL-6 released

↑  
Proliferation

↓  
Massive splenomegaly  
Kalaazar.

# Δ M/E of Bone Marrow.

Bert  
Test

Macrophage → LD Body

↳ kinetoplast on amastigotes

↓  
'Dot & Dash' [Image]

② Napier Aldehyde / antimony test  
Serology

③ Montenegro Test  
Negative except in Sudan

④ Culture on NNN medium.

↓  
Rosette formation of promastigotes.



## Cutaneous LEISHMANIASIS

L. Tropica M/c

Delhi Boil or Oriental Sore.

Mucocutaneous - L. Braziliensis M/c

L. Mexicana complex

PKDL - Nodular Lesion on face after Visceral Leishmaniasis

↓  
East Africa [Sudan] - after 2-6 months

Indian Subcontinent - (Bangladesh)

> 3 years.

India - W.B., Bihar, Assam.

# TRYPANOSOMA

T. CRUZI

T. BRUZI

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## T. CRUZI

causes Chagas Disease



Chagoma

Infective form →

Trypanomastigote

↓  
deposited by

Triatoma / Reduvid / kissing or  
Assassin



chagoma - skin lesion.

cardiomyopathy

megacolon

Romana Sign ⇒ u/L Periorbital edema

Δ - Amastigote in (heart) tissue.

C-shaped Trypomastigote in blood

## T. BRUZI

causes Sleeping Sickness

Infective form → Trypomastigote



vector - Tsetse Fly



inhibition of Insomnia Receptors.

Winter Bottom Sign ⇒ cervical L.V. enlarged

Δ - NO amastigotes.

ELONGATED PROMASTIGOTES

glycoprotein switching

↓  
Immune evasion.

TRICHOMONAS VAGINALIS

[Image]

- 1 morphological form →
- 4-5 ant. flagelle + 1 along-undulating body



- Greenish frothy D/c
- Whiff Test +ve

LMP TOXOPLASMA

A ♀ = H/O recurrent abortions present to ANC in her

1st trimester	TORCH screen	IgG.
	↳ IgM.	
	Toxo +	+
	Rubella -	-
	CMV -	+
	HSV -	+

Next Management ?

a) Start spiramycin

b) advise MTP

c) IgG avidity test

d) IgA detection



# Toxoplasma Gondii

Transmission through cats & canines.

Infective form  $\Rightarrow$  oocyst in cat faeces

$\downarrow$   
Ingested

Main - accidental host:

$\downarrow$   
Bradyzoites

$\downarrow$   
Tachyzoites (motile)

$\downarrow$   
tissue cyst (Bradyzoites) (dormant)

Sign, symptoms develop in immunocompromised,

♀

Reactivation

HIV pt.

$CD_4 < 50$

$\swarrow$   
Fetal encephalitis

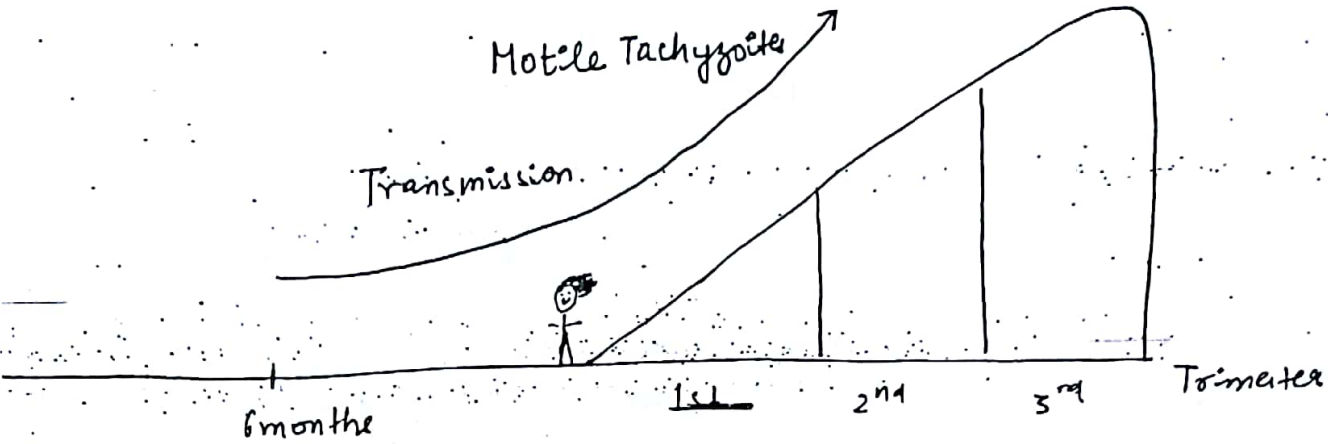
Δ - ① No IgM, low IgG titre

② MRI - Crescent shaped multiple ring enhancing lesion.

Eccentric target sign

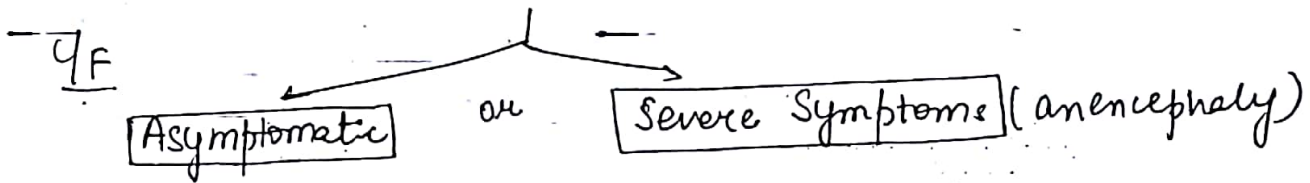
③ PCR of CSF.

$\downarrow$  spiramycin - DOE



Beyond 6-months  
no tachyzoites  
↓  
no transmission

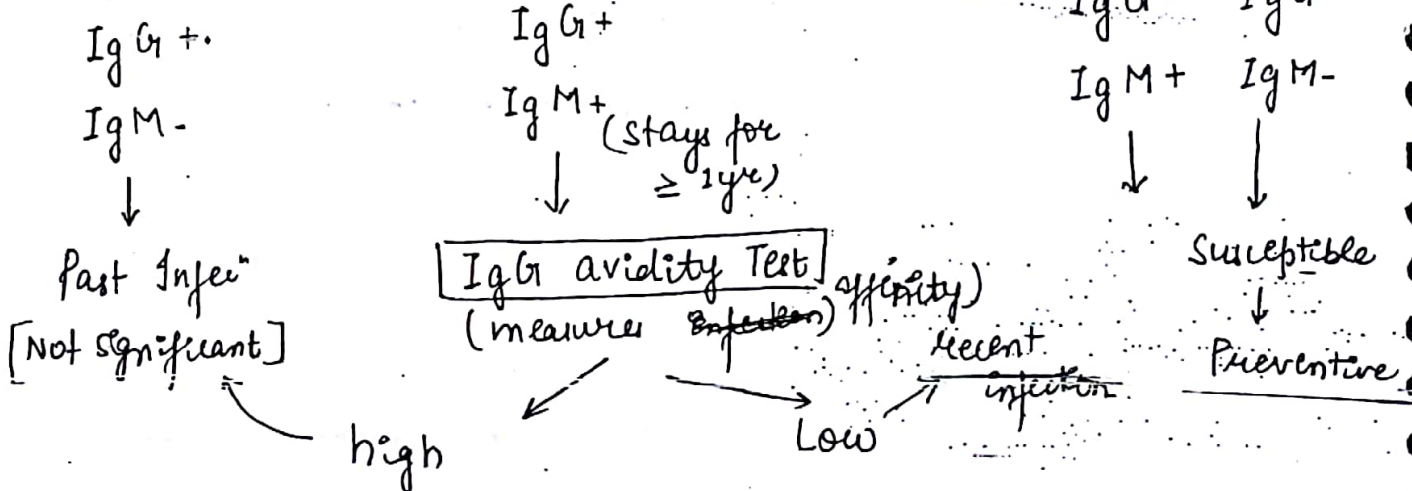
← foetal affection more in earlier parts



Late sequelae of Choro-retinitis  
↓  
Lead to Blindness

Risk Assessment In Cong. Toxoplasmosis :-

By Serology



IgA detection Low IgG avidity

↓  
more sensitive test for recent inf.

Sabin Fieldman dye Test.  
to detect Ab

↓  
Not recent Inf.

CYSTODES

Developmental stages

Eggs - Coracidium - Procercoid Larva - Pleurocercoid Larva

Trematodes Developmental Stage  
Eggs - Miracidium - sporocyst - Radial - Cercaria - Metacercaria

Definitive Host

- T. saginata
- Diphyllobothrium
- Hymenolepis
- Dipylidium.

Intermediate Host

- Echinococcus
- Sparganium
- Coenurus

Both Definitive & intermediate - T. solium.

Paratenic Host

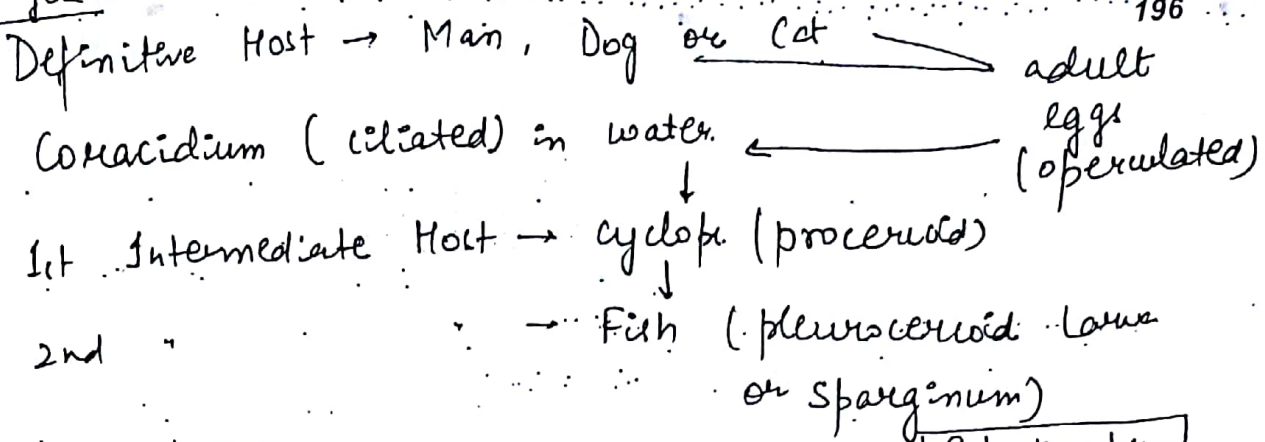
- no development of parasite in this host

- 1) Prawn → Angiostrongylus cantonesis
- 2) Big Fish → pleurocercoid larva of D. latum
- 3) Fish → Gnathostoma spinigerum
- 4) Man → pleurocercoid larva of sparganium Q

# DIPHYLLOBOOTHRIUM LATUM

Life cycle

196



infective form

## Pathogenicity:

Asymptomatic Infection - (M/C)

Abdominal Pain

Pernicious Anaemia (absorption of B<sub>12</sub>)

## LARGEST CYSTODE

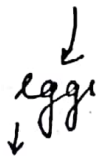
Δ - Eggs 45-700 (upto 100 μm)

Brown = Operculum at one end.  
Knob at another

Eggs of Diphyllobothrium, Fasciola + Fasciolopsis  
> 100 μm, operculated, indistinguishable.

## SPARGANUM

Definitive host → Dog, Cat → adult.



Coracidium in water





1st intermediate host - Cyclops (procercoid Larva)  
→ infective form ← (M/C)

197

2nd intermediate host → fish, reptiles, amphibians [pleurocercoid Larva]  
infective form

Man - pleurocercoid Larva (paratenic form).

## TAENIA

### INTESTINAL TAENIASIS

Infective form - Cysticercus (single scolex)

T. Solium

(Pork)

2.5 m

T. Saginata

(Beef)

10 m.

Asymptomatic  
Malabsorption

Δ - scolex → hooks 13 No. in rostellum ⇒ T. Solium

Proglottide

eggs

Bile stained  
strands



### NEUROCYSTICERCOSIS

Infective form - eggs (contaminated vegetables)  
of T. solium

↓  
Onchosphere

M/C

↓  
Cysticercosis

Brain - epilepsy

Absolute Criteria

↓  
Cysticercus in tissue.

.. by funduscopy

.. by Radiology (MC)



If cysticercus not seen, then Other Criteria :-

- a) other radiological signs
- b) Ab detection by ELISA
- c) Clinical evidence
- d) Epidemiological Indicators

COENURUS (T. multiceps or T. serialis)

- Multiple scolices
- Coenuri of T. multiceps → found in eye + Brain
- T. serialis → subcutaneous tissue.

ECHINOCOCCUS (HYDATID CYST)

Ectocyst (Outer cuticular layer)

acellular

Laminated hyaline membrane

appears as white of hard boiled egg.

Endocyst (Inner germinal layer)

cellular

vital layer of cyst

gives rise to brood capsules + scolices.

Secretes the specific hydatid fluid - forms outer layer

E. URANULOSUS → Hydatid Cyst

199

E. MULTILOCULARIS - Alveolar Cyst  
(Cyst Metastases)

E. VOGELI - Polycystic Ds

Dog Tapeworm [Definitive Host]

Δ - 1) Casoni Test (anaphylaxis)

2) Ab detection by ELISA

3) CT scan IOC

Screening for Echinococcus  
for E. multilocularis

## DIPYLIDIUM

Infective Form = flea harboring cysticercoids [Dog, Cat, Man]  
↓  
Solid Cyst = scolex

M/c in children

Asymptomatic M/c

## HYMENOLEPIS

Infective Form - egg → cysticercoid → Adults.

One Host (NO intermediate Host) ♀

Δ - Eggs - 30-40µm

Non-Bile Stained

6 spicules = Knobs = HEXACANTH

} Similar to  
egg of  
Taenia

# TREMATODES

Q The infective stage of trematode causing swimmer itch is Cercaria.

Eggs as infective form 200

Hymenolepis

Echinococcus

T. multiceps

serialis

T. solium (NCC)

## SCHISTOSOMA

Separate sexes

Male - gynaecophoric canal

~~Open~~ Non-operculated

No Rediae

Inf. form → Cercaria



penetrates skin

Life cycle

## OTHERS

Hermafroditic

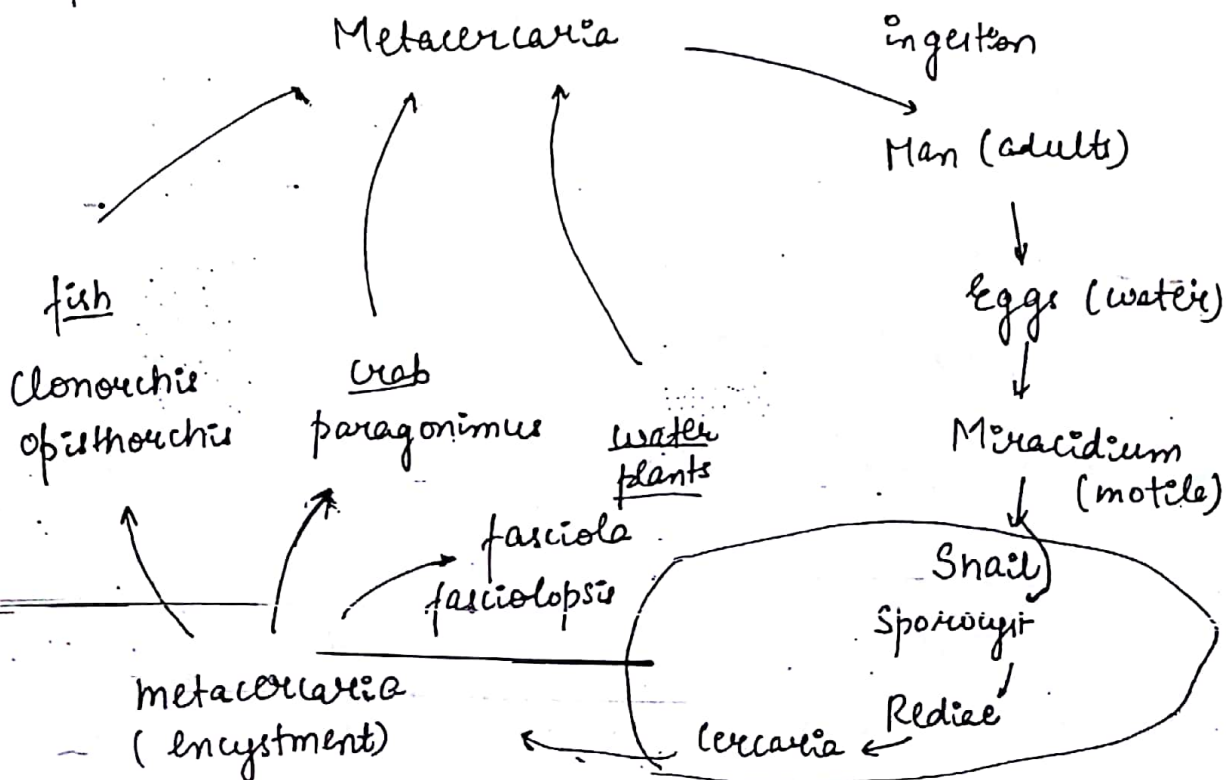
Operculated

Rediae

Metacercaria



Ingestion





All Trematodes are oviparous

201

Hepatomegaly → egg granuloma

Katayama Syndrome → oviposition, a serum sickness like illness.

Fibrosis (Symmer's)  
due to Th1 response

Swimmer's itch [cercarial dermatitis]  
in *S. mansoni*, *S. japonicum*.

*S. Haematobium*

Egg - 100-150  $\mu\text{m}$ , non-operculated

*S. Haematobium* - terminal spine

Ca Bladder.

*S. Mansoni* - Lateral spine

*S. Japonicum* - spine inconspicuous

CLONORCHIS OPISTHOCIS

Only egg of Trematode < 100  $\mu\text{m}$  = 15-30  $\mu\text{m}$   
operculated  $\bar{c}$  shoulder.

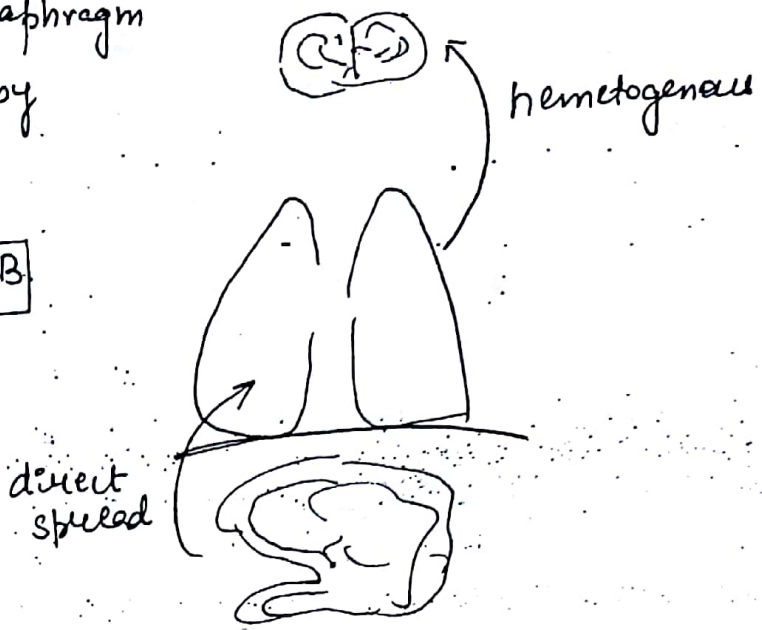
Cholangiocarcinoma

PARAGONIMIAS

# PARAGONIMUS

It directly pierces Diaphragm  
But spread to brain by  
haematogenous spread

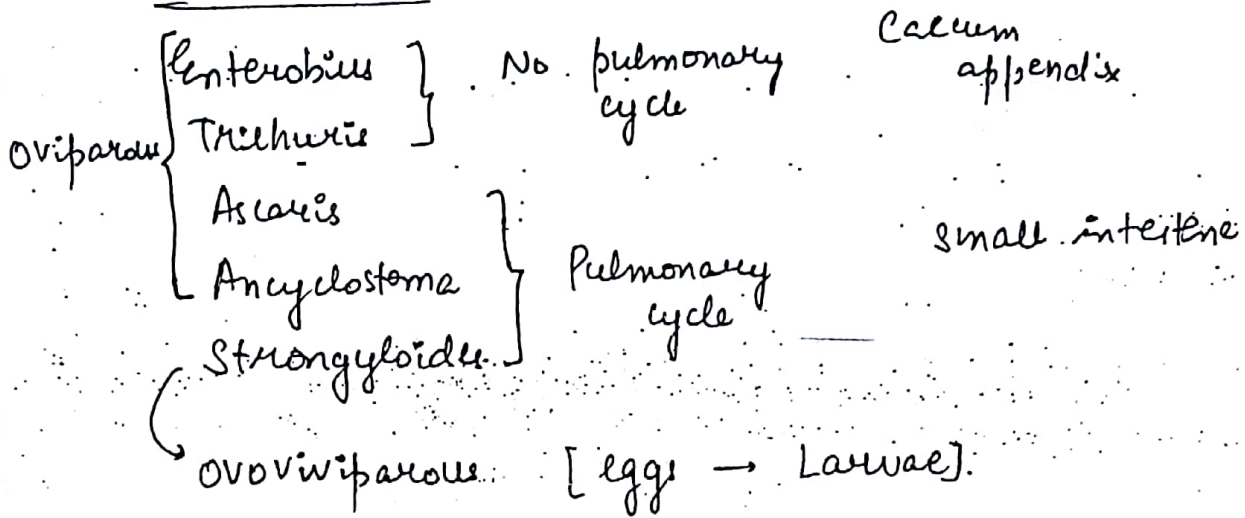
Paragonimus Mimic P.TB



Δ - Golden Brown eggs in the sputum.

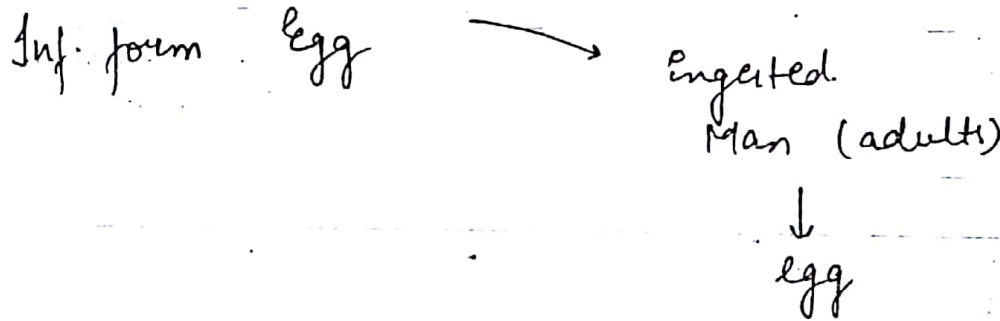
# NEMATODES

## INTESTINALS



Larvae In Stool → Strongyloides <sup>oo</sup>

## ENTEROBIUS — (Pin worm) —



Adults — Pointed / sharp ends → Pruritus M/c symptom

Δ Scotch tape (NIH swab)  
↓  
Perianal region.

Egg :- 70 μm  
Non Bile stained  
Plano-convex Δ

# TRICHURIIS (Whip worm)

Asymptomatic Infection

Anaemia

Rectal Prolapse.

Δ - eggs in stool.

70-80 μm

Bile stained

Barrel shaped

Bipolar plugs.

## ASCARIS

Infective Form - egg = rhabditiform larvae



Larvae

(penetrates into circulation)



Heart

↓  
Lungs

↓  
Epiglottis

(swallowed)

small Intestine  
(adults)



Adults - Fecundity ↑ (No. of eggs laid/worm/day)  
(2.4 lakhs)

Cause intestinal obstruction.

Larvae → Loeffler's syndrome

Δ - adults (male are shorter & have curved ends)

egg

50-60 μm

Bile stained

Rugosity





Non-Human Ascaris worms

Toxocara canis M/c

205

" cati



Visceral Larva migrans

## ANKYLOSTOMA

Infective form - Filariiform Larvae

↓  
penetration of skin

Adults - 0.2 mL of blood/worm/day

Anaemia

Larva - Loeffler's syndrome -

△ eggs - 50-60µm  
Non bile stained  
& blastomeres



## Non-Human Ankylostoma

A. Braziliensi → Cut. Larva Migrans  
M/c "creeping eruptions"

## STRONGYLOIDES

Inf. form - filariiform Larvae  
↓  
penetration of skin

Parthenogenic female - lay eggs to out males  
fertilised

~~Indirect development in soil → ♂ = ♀~~

Dermatitis LARVA CURRENS - larvae migrate @ 10cm/hour.

Δ ① Bermann Funnel technique

② culture by Harde - More Filter paper technique / agar plates.

Filariform Larvae → sharp @ side



Rhabditiform Larvae - Blunt 1 end

TISSUE NEMATODES

Filarial worms      Trichinella spiralis

FILARIAL WORMS

Inf. form → 3<sup>rd</sup> stage Larva  
↓ mosquito  
Tissue

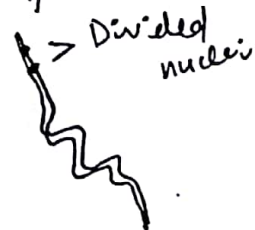
Signs + symptoms → due to adult  
except ONCHOCERCA.

Δ - Microfilariae in blood.  
except onchocerca

BRUUIA MALAYI

Fragmented Nucleus in tail end of microfilariae

Nucleus - Blue  
Cytoplasm - Pink



W. BANCROFTI

Nuclear material do not extend to the tip

LOA - LOA

Nuclear material extends upto loa loa

## MANZONELLA

Unsheathed.

Nuclear material upto tip

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## ONCHOCERCA

Simulium (deer fly)

↓  
Inf. form

Larva

↓  
adults in tissue nodules  
over bony prominences.

also causes → RIVER BLINDNESS

Δ. adults in tissue

culture of larvae from skin snips

## ANGIOSTRONGYLUS CANTONENSIS (rat lungworm)

eosinophilic meningitis

Infective form - Ingestion of 3<sup>rd</sup> stage in mollusks.

visceral larva migrans in Brain - M/c cause is this.

## Angiostrongylus costaricensis

Abdominal angiostrongyliasis M/c.

Symptoms mimic appendicitis.

## ANISAKIASIS

Anisaki simplex 1 ~~Pseudoterranova~~ *terranova* *decipiens*

3<sup>rd</sup> stage Larva in fish.

Surgical Resection.

# Gnathostoma spinigerum

3<sup>rd</sup> stage Larva in fish or poultry

Eosinophilic meningoencephalitis

Regulatory cutaneous swellings of the eye & visceral organs.

Surgical Resection.

## TRICHOSTRONGYLUS

Infection - Ingestion of Larva (vegetable)

Ingest far less blood than hookworms

Asymptomatic (MC)

Heavy infections - anaemia + eosinophilia

Stool exam

eggs resemble hookworm eggs but are larger.  
(85 by 115  $\mu$ m)

## TRICHINELLA SPIRALIS

Infected form - Encysted Larvae in pork or polar bear

↓  
adults in intestinal mucosa

↓  
migration + encysted larvae  
cause signs + symptoms

Δ - Eosinophilia

↑ CPK

Ab detection

M/s Biopsy (at the tendon insertion)



Lemon Sign (nurse cells)

Bachman Intradermal Test

209

M/s involved → EOM, Biceps, Jaw, Diaphragm.

Larval Load - < 10 larvae/gm of tissue  
↓  
asymptomatic

> 50

→ fatal.

Egg Load → Chandler's Index

7300 → Major Public Health problem  
seen in Hookworm

### AUTOINFECTION

C - Cryptosporidium, Capillaria philippinensis

H - H. Nana

E - Enterobius

S - Strongyloides

T - Tenia Solium

# MYCOLOGY

210

Cell Wall - Chitin  
Mannan  
Glucans

Cell Membrane - Ergosterol  
 $\beta$ -glucan assay - all fungi except Cryptococcus.

SDA  
isolates  $\rightarrow$  Lactophenol  
cotton Blue.

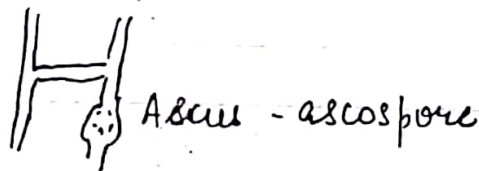
Calcofluor white  $\rightarrow$  fluorescent

Classification. (Sexual reproduction)

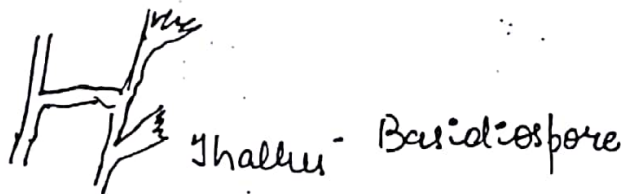
Zygomycete



Ascomycete



Basidiospore



Deuteromycete No sexual spore

'Fungi Imperfecti'

Morphology

Yeast - Cryptococcus

Yeast like - Candida

Dimorphic -  $\left\{ \begin{array}{l} 37^{\circ}\text{C} \\ 25^{\circ}\text{C} \end{array} \right.$  Yeast      Endemic  
Mould

Ph I Sporothrix schenckii - Himalachal. [Rose Gardener's D]

Penicillium marneffii - Manipur. 211

Histoplasma → Eastern } North America

Blastomycosis

Coccidiomycosis → western }

Paracoccidiomycosis → South American

Moulds

Reit

## OPPORTUNISTIC FUNGAL INFECTIONS

M/c - Candida

### CANDIDA

Endogenous

↓  
CMI ↓ → mucocutaneous

Neutrophil ↓ → invasion

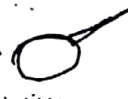
↓  
True + Pseudohyphae

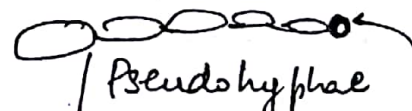
True Hyphae → apical elongation when candida is grown in serum for 2 hours  
[Raynaud Braun phenomenon]

Pseudohyphae → failure of daughter buds to separate

↓  
Seen in Corn meal Agar

[Nutritionally deficient media]

Germ Tube →  HYPHAE

 Pseudohyphae

constrictions

Chlamydoconidia

Candida Albicans

Non albicans

Germ Tube +

Chlamydoconidia +

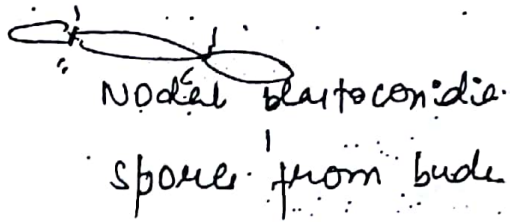
Chlamydoconidia

8-1/2

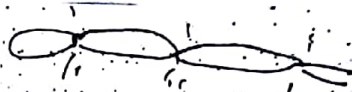
C. Dubliniensis - non-albicans = can produce  
germ tube, chlamydoconidia

### NON-ALBICANS

C. Tropicalis



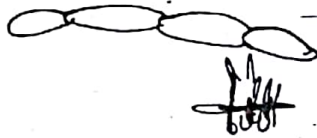
C. parapsilosis



C. Glabrata  
(mucoid)

No pseudohyphae

C. Krusei



### CRYPTOCOCCUS

It causes infect<sup>n</sup> in HIV pt.

Virulence factors:

- (1) Sialic acid
- (2) melanin
- (3) Urease
- (4) superoxide dismutase
- (5) Mannitol fermentation
- (6) capsule
- (7) Mating Types

1° Infection ⇒ LUNGS → then Brain.



Δ culture in Niger Seed Agar → Brown mould colonies  
India Ink.

213

Aq Detection by Latex Agglutination.

IgA + IgG<sub>2</sub> ⇒ protective against capsule

~~OBITU~~ ZYCOMYLETES

obtuse ∠



Nodal - Rhizoids



Absidia  
Internodal rhizoids

Mucor - No rhizoids

Angio invasive → Rhinocerebral mucormycosis  
↑ mortality

R/F - Diabetes ketoacidosis

Desferrioxamine R

Δ - Profuse growth → Lid thrower.

ASEPTATE IRREGULAR BROAD, ribbon like hyphae  
Branching at OBTUSE ANGLE.

ASPERGILLUS

Fumigatus → Invasive (↑ neutrophil)

Flavus → Keratitis

Niger → Otitis

ACUTE ANGLE DICHOTOMOUS BRANCHING

'V' forms



## PENICILLIUM MARNEFFI

Umbellated lesion. → like molluscum contagiosum  
seen in HIV pt

Δ - Septate yeast → Binary Fission

Red Pigments



Broom stick

## PNEUMOCYSTIS JIROVECI

cause Interstitial Pneumonia (Non productive cough)

AIDS - induced sputum

HIV - BAL

Δ - can't be cultured

Gomori methamine silver → cyst wall

Toluidine Blue →

Giemsa → sporozoites (8 in NO)

Best IFA → Best Technique

## Fungal Infection In Immunocompetent

Dermatophytes (MIC)

↓  
Keratinophilic

	Macroconidia	Microconidia
Trichophyton (skin, hair, nail)	pencil shaped few	plenty 215
Microporum (skin, hair)	spindle (boat) plenty	few
Epidermophyton (skin, nail)	clavate (club)	No
Mentagrophytes (Trichophyton)	→ Hair Perforation (+) Urease (-)	—

T. VERSICOLOR

Malassezia globosa (M/C) , furfur  
↓  
Lipophilic

Δ culture - SDA ± olive oil  
Spagetti x Meat ball

TPN Rx → Invasive  
(± lipids)

SPOROTHRIX SCHENCKII

Thorn prick 'Rose Gardener's Ds'  
Lymphatic spread → series of ulcers

Δ - Cigar shaped yeast  
Rosette like conidia in SDA  
Splendore Hoeppli phenomenon  
(asteroid)

→ Cutaneous zygomycete

→ Blastomyces

## CHROMOBLASTOMYCOSIS

Coloured fungi (Pigmented)

216

Δ - Sclerotic Body → Brown septate Yeast appearing like "copper coin".

## 'BOOMERANG CONIDIA' CURVULARIA

Dermatitides

1) Curvularia

2) Alternaria

3) Bipolaris

4) Cladophialophora

5) Exophiala

6) Fonsecaea

7) Madurella

8) Scedosporium

9) Scytalidium

10) Wangiella etc.

CF - verrucose cauliflower like lesion.

## MYCETOMA

CF

Swelling

sinus

Granuloma

## RHINOSPORIDIUM SEEBERI

- PROTOZOA

- Polyps R/F → Pond Bathing

- Δ - 10% KOH → spherules ̄ endoscope




# SYSTEMIC FUNGI

Infective form  $\Rightarrow$  Arthroconidia  $\rightarrow$  spores in Hyphae 217

$\Downarrow$   
Pulmonary Lesions  
TB like

$\Delta$  - Blastomycosis  $\rightarrow$  Broad Based budding

Paracoccidioidomycosis  $\rightarrow$  multiple budding yeast

Martinez / Pilot wheel 

Coccidioidomycosis  $\rightarrow$

Valley Fever

Desert Rheumatism

Barrel shaped arthroconidia



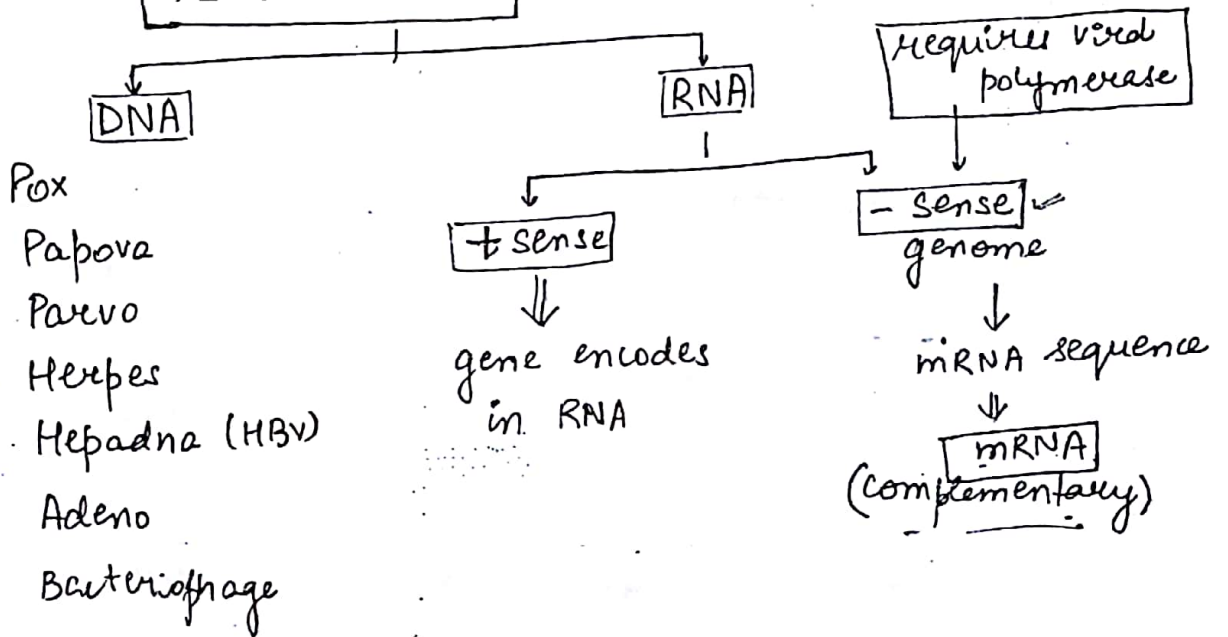
Spherules (tissue)

Histoplasma Capsulatum  
found in Bats

$\rightarrow$  Tubercular ~~like~~ projections  
on 'spores'.



## VIROLOGY



Non-Enveloped Virus

⇒ released by Lysis

DNA

RNA

Parvo

Astro

Adeno

Picornia

Papova

Reo

Calci

HAV

HEV

SP Cancel. Ho. RAE

enveloped virus released by budding.

Segmented RNA viruses

Genetic re-assortment occurs.

They can show genetic shift

B - Bunya virus (3 segment)

I - Influenza virus (8 segments)

R - Rota virus (11 segment)

A - Arena virus (2 segment)

DNA VIRUSES

PARVO B19

Smallest 20nm

affinity to immature RBC

Immuno competent child.

(5<sup>th</sup> DS) Erythema Infectiosum



Ab excess

↓  
Immune complex

↓  
Vascular Damage

← (slapped cheek)

Immuno competent Adult - Ab excess → polyarthralgia

Sickle cell → Ab response → Aplastic (Transient) crisis

Immunocompromised - No Ab response → Pure Red Cell Aplasia (PRCA) 219

♀ → fetal liver, spleen, kidney ⇒ Hydrops Fetalis.

Δ - Ab detection

EPCR

Quantitative PCR (Real Time PCR) (Best)

↓  
Taq man assay.

**PAPOMA**

POLIOMA = JC virus → Progressive Multifocal leucoencephalopathy (PML)

BK virus → Kidney Infection

PAPILLOMA =

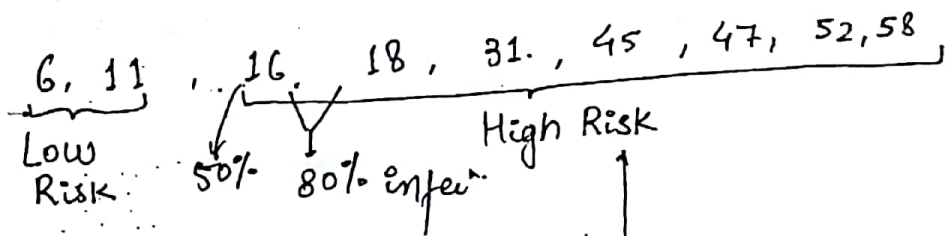
↓ warts

Ca Cx → **E protein** → virulence

↓  
Suppress the tumour

Suppressor genes

**L protein** → serotyping



VACCINE → Cervarix (16, 18)

Gardasil (6, 11, 16, 18)

PrI Gardasil 9

Δ of Ca Cx = Koilocyte → cells = pyknotic nucleus + perinuclear halo



**Pox**

Molluscum Contagiosum

↓  
Pearly white umbilicated nodular lesions  
self limiting  
direct contact

Δ - Molluscum Body (IB)

Bollinger Body → fowl-pox } Inclusion Body  
Guarniere Body → vaccinia }

DNA virus → Intracellular except Pox  
RNA " → Intracytoplasmic except influenza, HIV

**HERPES**

HHV 1 } α virus  
HHV 2 } ↓  
HHV 3 } epithelial cell

HHV 5 } β virus  
HHV 6 } ↓  
HHV 7 } Glands

HHV 4 } γ virus (oncogenic)  
HHV 8 } ↓  
Lymphocytes

HHV1  
Stomatitis  
Keratitis  
Encephalitis  
[Temporal → frontal]  
Less

HHV2  
Genital infec  
Aseptic meningitis  
More virulent

Anti HSV → non protective

due to trivial exposure

1° Infection → Latency → Reactivation  
↓  
EPISOME (integration of viral DNA in host chromosome)



A - ~~classical~~ Tzanck Smear → Giemsa

↓  
Multinucleate Giant cell

221

Eosinophilic IB. (intra nuclear)



↓  
**Cowdry A**

② Ab detection

③ PCR → IOC

**HHV 3** - V-Z virus

Chickenpox → Lesions in crops

Lifelong Immunity in case of Chickenpox

Shingles → Reactivation from dorsal n/v root ganglion.  
of Trigeminal & Sacral n/v.

⇓  
affect T<sub>3</sub> to L<sub>2</sub> U/L

Trigeminal, Facial  
(Ophthalmic Div.)

Ramsay Hunt Syndrome.

Ant 2/3<sup>rd</sup> of Tongue

middle ear lesions

Bell's Palsy

Congenital V-Z Syndrome

Scarring of lesion (skin)

Hypoplasia of limbs

Chorioretinitis

Δ - Tzanck

Ab detection

PCR



## HHV-5 CMV

223

enlargement of cells

ubiquitous

secreted in all body secretions



Retinitis in HIV ⊕ < 50 CD4

Inclusion Body Ds - children.

Transplantation

Δ ⊕ Owl eye I.B.

⊙ Ag Detect

⊙ PCR

HHV-6 - 6<sup>th</sup> Ds - ROSEOLA INFANTUM  
or  
Erythema Subitum

HH7 → ↓ CD4 in HIV

HH8 → Kaposi's Sarcoma

## ADENO VIRUS

Apical Fibrils

"space objects"



serotype

8, 19, 37

→ Epidemic Keratoconjunctivitis

11, 21

→ Acute haemorrhagic  
cystitis

40, 41

→ Infantile Gastroenteritis

1, 2, 3, 5

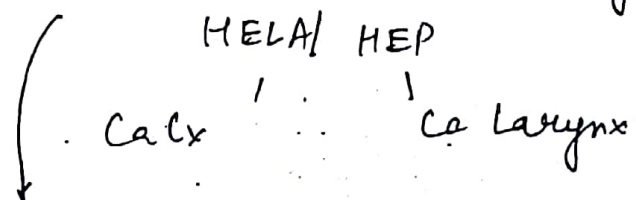
→ Resp. Infections.

Vaccine ∴ Live Non-attenuated Vaccine  
↳ Administered through oral route

Δ - ① Ab detection

② Culture in cell lines (human origin)

②



Barophilic I.B. [Cowdry B]  
(intracellular)

**RNA VIRUSES**

Infantile Gastroenteritis.

**Rota Virus** M/c → ds RNA

vomiting followed by diarrhoea

family - ~~Ra~~ Reo

11 segments.

Vaccine - VPG + VP7.

↓  
Intussusception.

Δ - VPG Ag detection by ELISA in stool

**CALCI**

— Noro (Norwalk) → M/c in adults + children  
Sapo

**ASTRO**

**Toga**

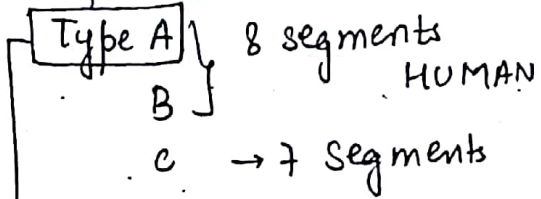
Adeno Type 40, 41 - DNA Virus. (only)

Δ - can't be cultured in  
EM.

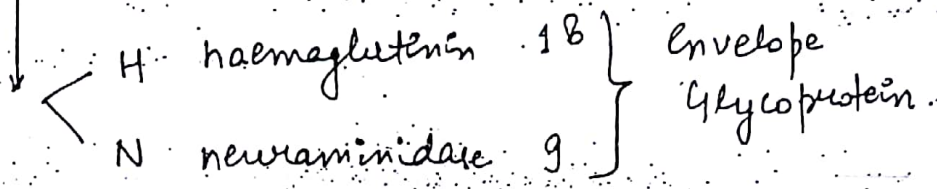
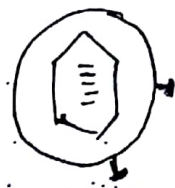


# ORTHOMYXO

## Influenza



Based on nucleocapsid protein.

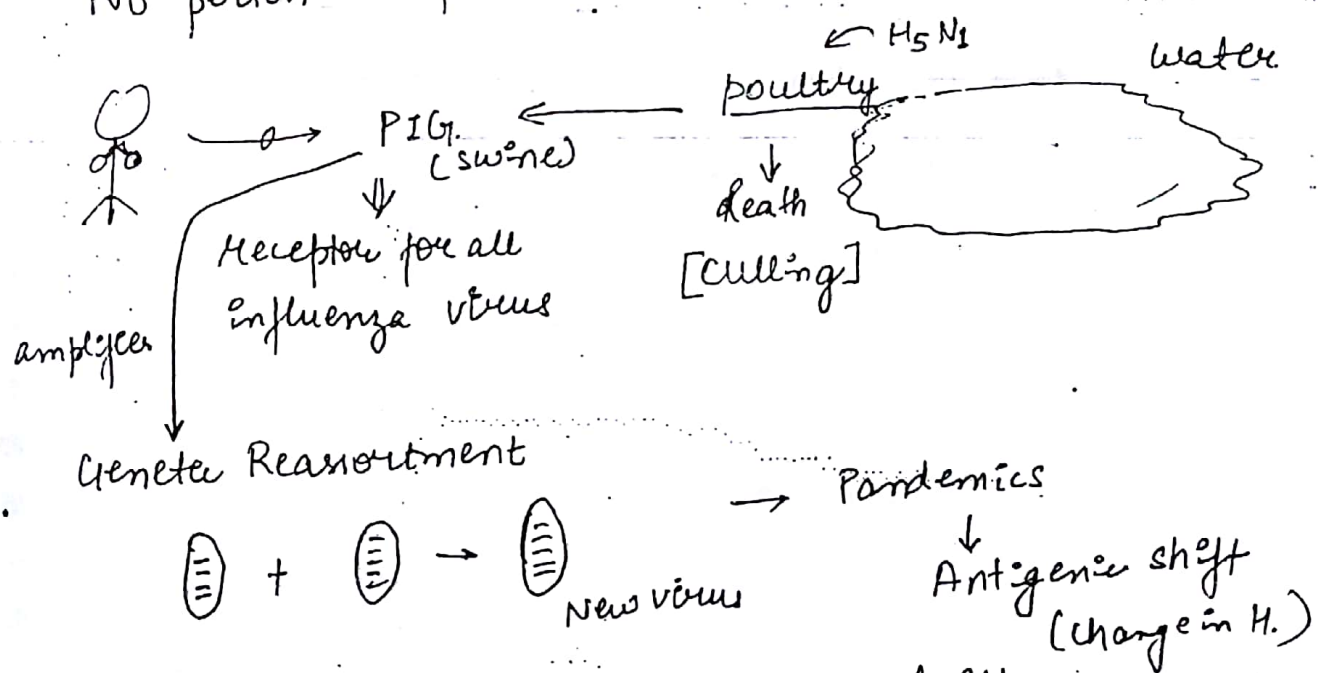


Δ - RT PCR.  
 sample - nasopharyngeal swab.

## AVIAN INFLUENZA

Reservoir. H5N1

↑ virulent  
 NO person to person Transmission.



Mutation → epidemics → antigenic drift.

- H<sub>1</sub>N<sub>1</sub> → 1918 Spanish flu
- H<sub>2</sub>N<sub>2</sub> → 1957 Asian
- H<sub>3</sub>N<sub>2</sub> → ~~1968~~ 1968 Hong Kong

Pig → Reservoir.

Von Magnus phenomenon

↑ titre (haemagglutinin)

↓ infectivity

Δ - RT PCR.

PARAMYXO

RSV → Bronchiolitis

Mumps → Parotitis - aseptic meningitis M/C  
orchitis

Vaccine - Jeryllynne strain.

Measles → Fever + Rash (IP - 14 days)

↓  
Behind ears (Ht)

KOPLIK'S SPOT → Opp. 2<sup>nd</sup> Molar (lower)

M/C complication - otitis media

M/C CNS " - Post measles encephalitis  
(autoimmune)  
in 1 year.

SSPE - Rare complication

↓ 5-25 years.

( due to defective virus. (spongiform encephalitis)

Prion proteins

Δ - Ab detection (after 7 days of onset of rash)

PCR (nasopharyngeal swab)  
in 5 days.

# Vaccine - Edmonstan Zagreb

## RUBELLA

German Measle

Vaccine → RA 27/3

Adults → Exanthematous Rash / Keratitis.

### Congenital Rubella Syndrome

Cataract

Deafness

heart Defects

Δ - Ab detect<sup>n</sup>

⊙  
+ 1st Trimester - is exposed to her friend suspected of Rubella - what next?

susceptibility

If friend have IgM +ve,  
then check for IgG in ⊙  
IgG +ve → no worry  
IgG -ve → come for next  
month  
IgM +ve → abortion.

a) IgG

b) IgG, IgM

c) IgG

d) -

⊙ → diagnose

IgM

IgM.

## ARBOVIRUS

arthropod virus

Flav: Virus

Bunya "

Toga "

→ mosquito + ticks.

## Ticks

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✓ Powassan

✓ Russian spring summer

✓ Kyasanur forest ds

✓ CCHF (Crimean Congo HF) haemorrhagic fever

## DENGUE

5 serotypes

DF

→ DHF/DSS

Ab enhancement

Immune complex

\* Factors affecting Haemorrhage :-

1) Repeat infection  $\bar{c}$  different serotype

2) Sequence of infection. Type 1 followed by Type 2

3) Serotype 2

4) White

5) < 12 yrs.

6) Female

→ Malnutrition → protective

Δ - IgM Capture ELISA

NS1 Ag (in 5 days)

## ZIKA

\* Microcephaly in newborn.

JE → pig → amplifying host

Δ - Ab detection



Vaccine - SA-14-14-2 (Live) - 1 dose

229

↓  
tissue culture

CCHF (Crimean Congo Haemorrhage Fever)

- Case reported in Gujarat, Rajasthan.

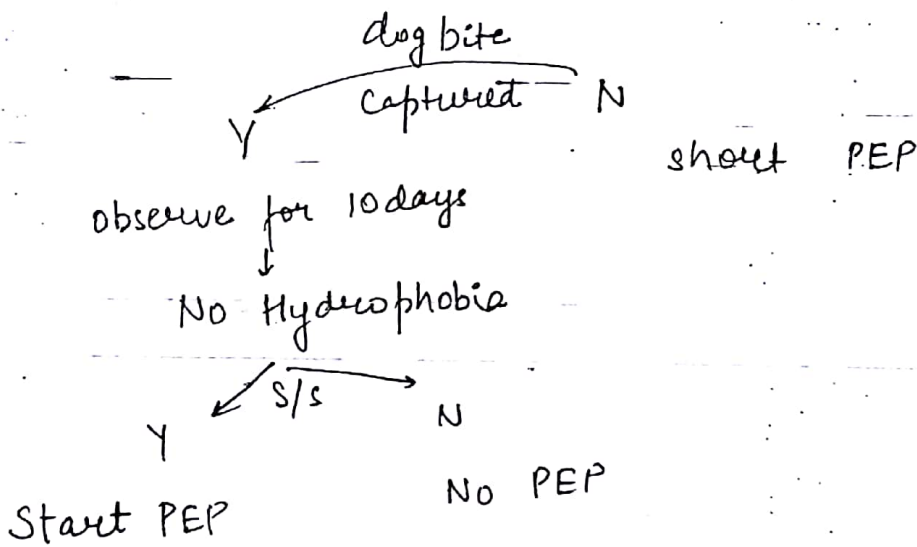
Δ - Ab detection

✓ PCR

Rhabdovirus

Bullet shaped

3 mm/hr or 230 mm/day [Rate of spread].



↓  
antemortem (pt) → skin from  
nape of neck.

↓  
Immunofluorescence

Post mortem (behead dog) → Brain.

↓  
Sellar stain

↓  
Negri Body → + Hippocampus  
Cerebellum

AFP

Δ - stool culture (2 samples)

↓  
sequencing → Type 1 or 3 or VDPV

HEPATITIS

**HAV** (Picornavirus)

feco-oral route → acute infection

↓  
fulminant in adults  
outbreak

Δ - only hepatitis virus cultured in cell lines

IgM Anti-HAV

↓  
one serotype & 4 genotype

**HEV** (Hepe virus)

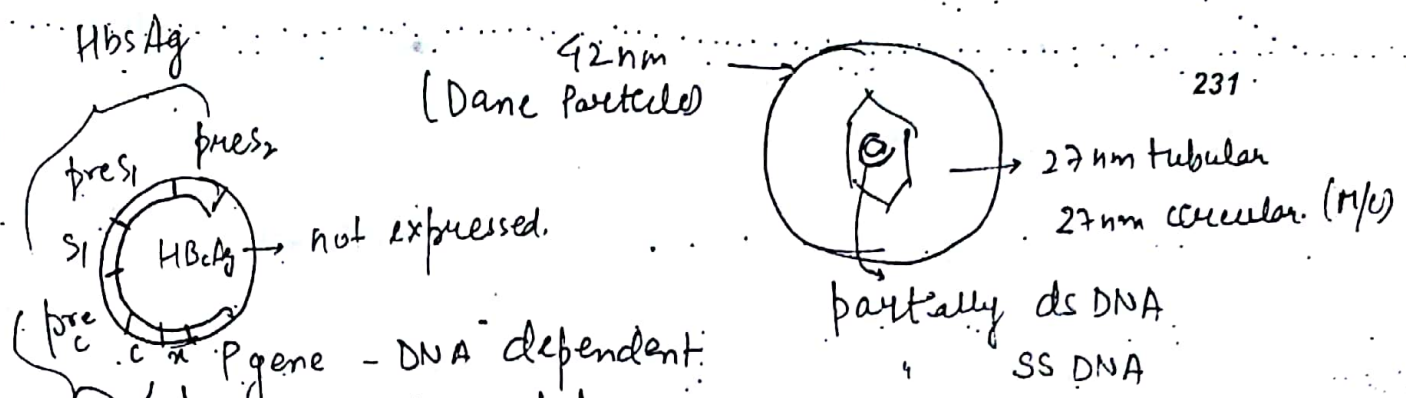
feco-oral route → acute infection

↓  
fulminant in pregnancy  
outbreak

Blood Transmission (rarely documented)

Δ - IgM anti HEV

**HBV** (Hepadnavirus)



P gene - DNA dependent DNA polymerase completes the strand.

Reverse Transcriptase

RNA Template

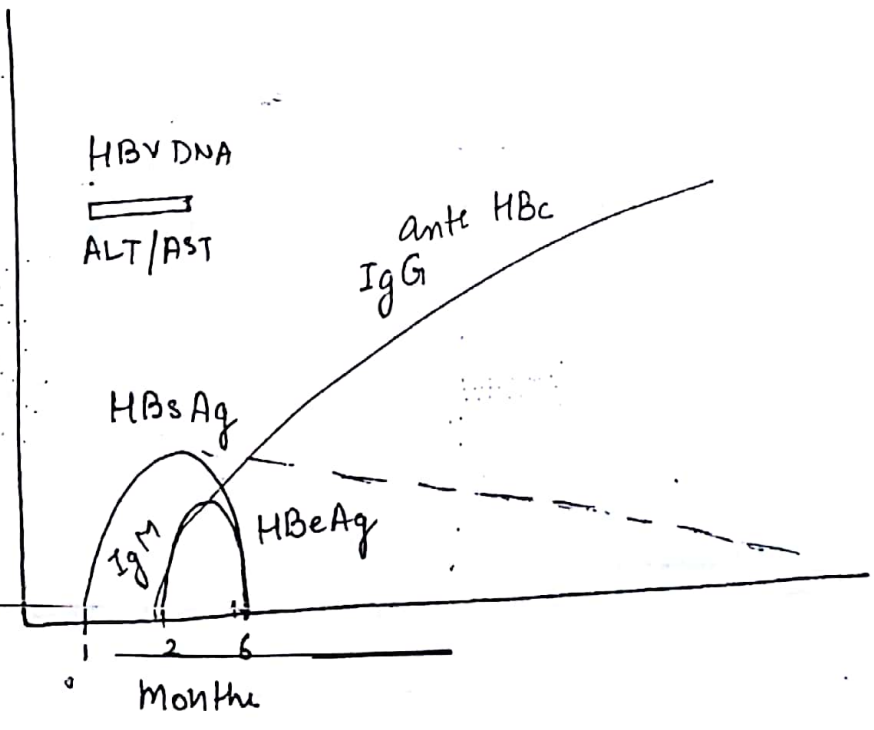
HBcAg protein synthesis (Viral replication)

Many DNA transcripts

Viral Load Testing (HBV DNA)

HbxAg

in liver hepatocyte leads to HCC



Acute Hep B infect in adult  $\rightarrow$  usually resolve 232

Vertical transmission  $\rightarrow$  chronicity  $\uparrow$

(M/c)

Best Marker of Acute Hep B  $\rightarrow$  IgM Ante HBc

Chronic active supercarrier  $\rightarrow$  Infections  $\cdot$  Require Rx

$\downarrow$   
IgG ante HBc  $\cdot$  HBV DNA

Chronic persistent  $\rightarrow$  IgG ante HBc  $\oplus$  HBV DNA  $\ominus$

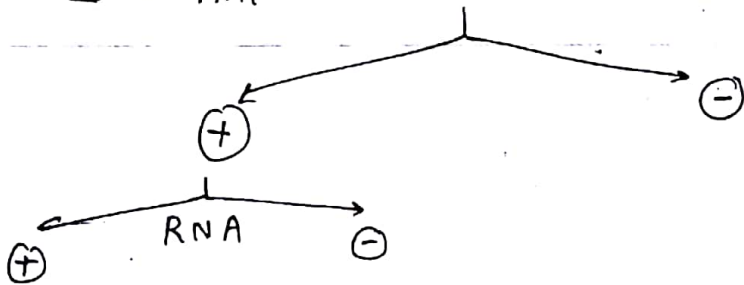
HCV flavivirus.

Chronic infect  $\uparrow\uparrow$

acute  $\rightarrow$  No signs/symptoms.

unsafe inject  $\rightarrow$  (M/c)

$\Delta$  - Ante HCV



Rx

Sofosbuvir + daclatasvir

3 months

genotyping not Req

Rebavirin + IFN  $\rightarrow$  genotyping

Index Type 3  $> 1$



Co-infection → IgM Anti HBe

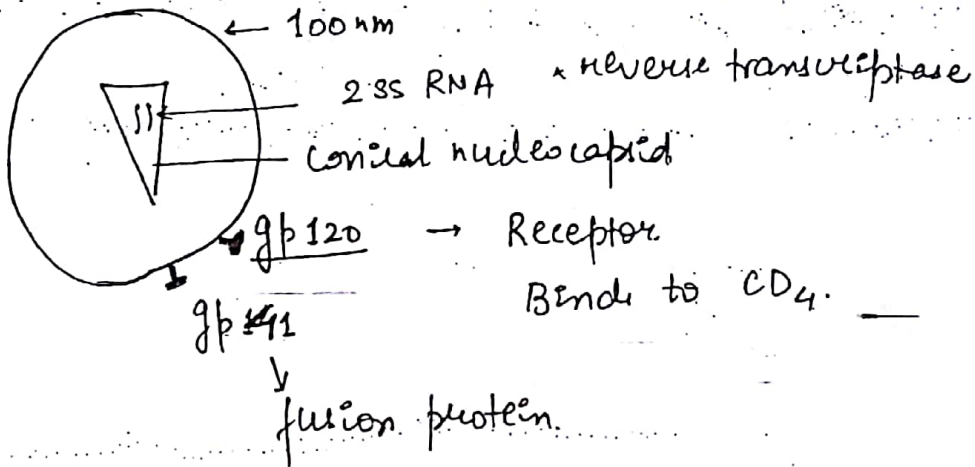
Super infection → IgG anti HBe

fulminant

↑ incidence of fulminancy - 20%

H/c fulminancy → HBV

HIV



Co-receptor on the host cell

(CCR5)

CXCR4

(R5 virus)

(X4 virus)

M trophic

T Trophic

↓  
Monocytes/  
macrophages

↓  
Lymphocyte

↓  
Resistance

HIV 1

HIV 2

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M N O  
Major Human

West Africa  
Intense (R) to NNRTI

Subtypes - C - India

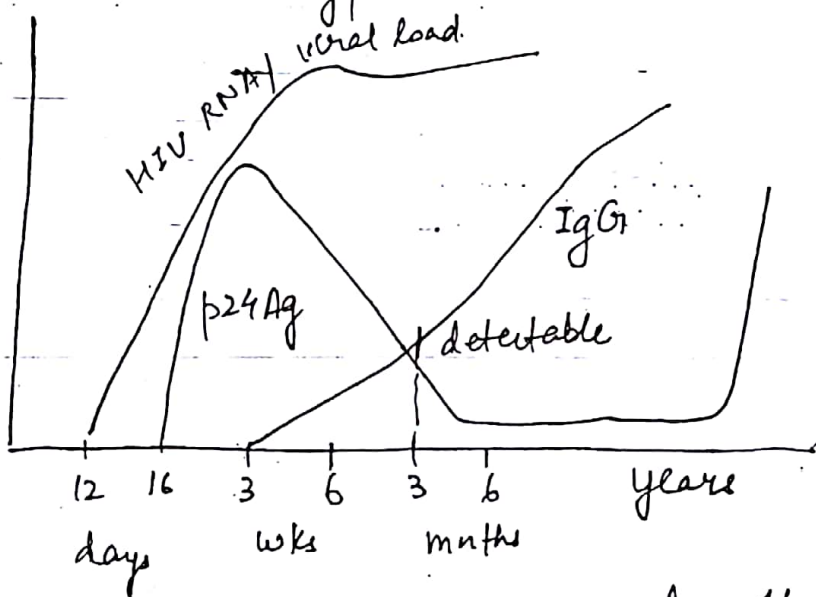
Env gene is conserved between HIV 1 & 2

gag & pol share 90% homology

env Ag used for diagnosis

gp 120, gp 41 - HIV 1

gp 36 - HIV 2



Recent H/o exposure p24 Ag → day 16 to 3 months

Δ of HIV → Ab detection

Most sensitive → 4th generation ELISA

detects both p24 Ag, HIV Ab

↓ 3 tests

Confirmation → Western Blot

Δ in children - DNA PCR Q.

"dried blood spot"

Disease Monitoring

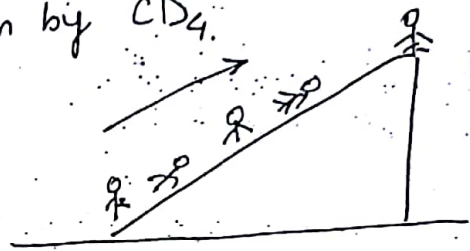
CD4 → opportunistic Inf<sup>n</sup>, treatment

Viral Load → Prognosis

Present Immune status given by CD4.

Rapid progressor (sybers)

slow "

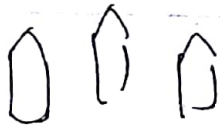


SEROLOGY

Ag - Ab Rxn.



False -ve Rx



If Ab excess ⇒ PROZONE

If Ag excess. POST ZONE



zone of equivalence

Serological Tests → dilution (2 fold serial) of serum

Titre → Highest Dilution at ± Rxn is seen.

PPT<sup>n</sup>

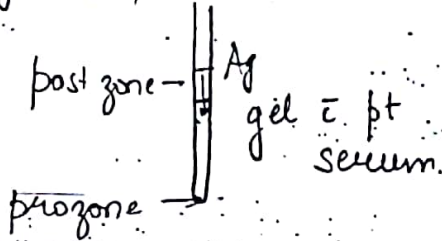
Agglutination

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Soluble Ag binds to  $\bar{c}$

Ab to form soluble ppt

Single diffusion in one dimension Oudin procedure



Double diffusion in one dimension Ouchley Fulthrop procedure

Single diffusion in two dimensions Radial immunodiffusion

Double-diffusion in two dimensions Ouchterlony procedure  
eg. Elek gel's electrophoresis

Quantitative  $\rightarrow$  Rocket Electrophoresis

Agglutination

Particulate Ag binds to Ab to form visible clumps

$\downarrow$

Passive Agglutination  $\rightarrow$  Particles to detect Ab

Reverse " "  $\rightarrow$  particles to detect Ag



# Complement Fixation

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Detects Ab.

ELISA is used nowadays → as it detects Ag + Ab

ELISA large no. of samples ~~test~~ tested at 1 time

Direct ELISA

detects Ag  
specific

Indirect ELISA

detects Ab  
sensitive

Competitive ELISA

— detects Ab → highly specific

Capture ELISA

detects isotype of Ab →

using monoclonal Ab → isotypes are captured it

eg. Dengue IgM.



mAb for IgM.

add Dengue Ag + Ab + antigen

↓ now add substrate  
+ enzyme

colour change

# Immunofluorescence

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FITC dye

fluorescein isothiocyanate.

Chemiluminescence (CLIA)

↳ light emitting particles.

10-100 times more sensitive ELISA.

## Obligate Intracellular Parasite

CRV CM PTL

Chlamydia

Rickettsia ( Ehrlichia +  
anaplasma)

viruses

Coxiella Burnetii

Cryptosporidium parvum

Mycobacterium leprae

Plasmodium sp.

Pneumocystis jirovecii

Toxoplasma gondii

Trypanosoma cruzi

Leishmania Donovanii

## Facultative Intracellular Parasite

MBBS CRV for NHL

Mycobacterium

Bartonella henselae

Bruceella

Salmonella Typhi

Cryptococcus neoformans

Rhodococcus equi

Yersinia

Francisella tularensis

Nocardia

N. meningitidis

Histoplasma capsulatum

Listeria monocytogenes

Legionella