

# Analysis of Water Sample of Sambhar Lake for Detection of Bacteriological Culture and their Antibiotic Resistance Pattern

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## Abstract

The present study was attempted to detect bacteriological culture in water samples of Sambhar lake of Rajasthan. Sambhar Lake is India's largest inland saline wetland as well as tourist destination, that is used not only for salt extraction but also an internationally acclaimed destination for thousands of migratory birds and water fowls. A total of 25 water samples were collected from different parts of lake and processed for bacterial isolation, eight bacterial isolates were obtained which were further characterized. The isolates were confirmed as Enterobacteriaceae members on the basis of growth on MacConkey agar, Eosin methylene blue agar and IMViC pattern. All isolates were lactose fermenter and one showed metallic sheen on eosin methylene blue agar while others were grown with dark brown mucoid and non-mucoid colonies. Further, all isolates were evaluated for antibiotic susceptibility pattern and it was found that 100% were resistant against penicillin-G while all were sensitive to co-trimoxazole. Variable sensitivity pattern was observed against chloramphenicol, imipenem and norfloxacin. All isolates were screened for various resistance genes. Looking to the presence of migratory bird in the lake, it is very important to study the bacterial flora of lake with special reference of sambhar lake with emphasis of detection of bacterial culture and genotyping of antibiotic resistance pattern.

**Key words:** Enterobacteriaceae MCA, EMB, Antibiotic Resistance and sambhar lake.

## Introduction

Although high salinity is generally considered lethal for most organisms, hypersaline environments contain large array of genetic diversity (Triado-Margarit & Casamayor, 2013) and are an excellent source of various new culturable microbes (Oren, 2002).

## Material and Method

Total of 25 water samples were collected from different parts of lake. Isolation and phenotypic characterization (cultural) of Enterobacteriaceae spp. were attempted by conventional methods as described by Cowan and Steel (1974) and Quinn *et al.*, (1994). Antibiogram was applied on different isolates using invitro disc diffusion techniques according to CLSI (2017) and performed on Mueller Hinton Agar plates and 15 discs of antibiotics of different groups were used.

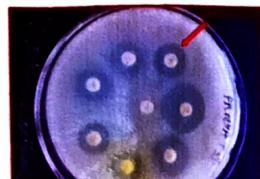
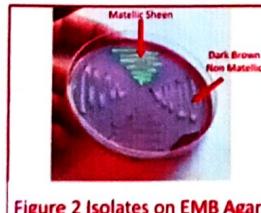
Table 1

Samples	25
Bacterial Isolates	08

Table 2

On MacConkey Agar	Bright pink colonies
On EMB Agar	One with Metallic sheen others with brown and pink colonies
On ESBL Agar	Purple and Blue colour

## Phenotypic Characterization



## Antibiotic Sensitivity Test

For antibiogram profiling total 15 antibiotics were used. all isolates were evaluated for antibiotic susceptibility pattern and it was found that 100% were resistant against penicillin-G while all were sensitive to co-trimoxazole. Variable sensitivity pattern was observed against chloramphenicol, imipenem and norfloxacin.

## Results

It was observed that not only all the isolates were resistant against penicillin-G but also observed that all the isolates were sensitive to co-trimoxazole. 87.5% of the isolates were sensitive to chloramphenicol and sulfafurazone whereas 62.5% of the isolates were resistant to cephalothin and cephalexin.

### Conclusion:-

Resistance was observed against penicillin and cephalosporin group antibiotics, presence of resistant microorganisms in the lake is an alarming sign for the health of migratory birds and probably indicates the environmental leakage that has resulted from imprudent use of antibiotics on the part of humans.

Table 3  
Antibiotic Sensitivity Pattern of Bacterial Isolates

S.NO.	ANTIBIOTIC	CODE	INTERPRETATIVE CRITERIA (S/I/R)	PERCENT		
				SENSITIVE	INTERMEDIATE	RESISTANT
1	Penicillin-G	P <sup>10</sup>	21/18-20/17	0	0	100
2	Nitrofurantoin	NIT	17/15-16/14	50	25	25
3	Cephalothin	CEP	15-21	12.5	25	62.5
4	Tobramycin	TOB <sup>10</sup>	15/13-14/12	62.5	12.5	25
5	Minocycline	MI	16/13-15/12	50	25	25
6	Ciprofloxacin	CIP <sup>5</sup>	21/16-20/15	50	37.5	12.5
7	Chloramphenicol	C <sup>30</sup>	18/13-17/12	87.5	12.5	0
8	Imipenem	IPM <sup>10</sup>	23/20-22/19	62.5	37.5	0
9	Norfloxacin	NX <sup>10</sup>	17/13-16/12	50	50	0
10	Sulphafurazone	SF <sup>300</sup>	17/13-16/12	87.5	12.5	0
11	Ceftriaxone	CTR	23/20-22/19	25	50	25
12	Cotrimoxazole	COT <sup>25</sup>	16/11-15/10	100	0	0
13	Gentamycin	GEN <sup>10</sup>	15/13-14/12	25	25	50
14	Cefamandole	FAM <sup>50</sup>	18/15-17/14	37.5	12.5	50
15	Cefalexin	CN <sup>30</sup>	14/-/14	37.5	0	62.5

## References

Oren A., (2002). Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology & Biotechnology*, (2002) 28, 56-63.

Triado-Margarit X and Casamayor E.O., (2013). High genetic diversity and novelty in planktonic protists inhabiting inland and coastal high salinity water bodies. *FEMS Microbiol Ecol* 85 (2013) 27-36.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R., (1994). *Clinical Veterinary Microbiology* (pp. 131). London: Mosby Elsevier.

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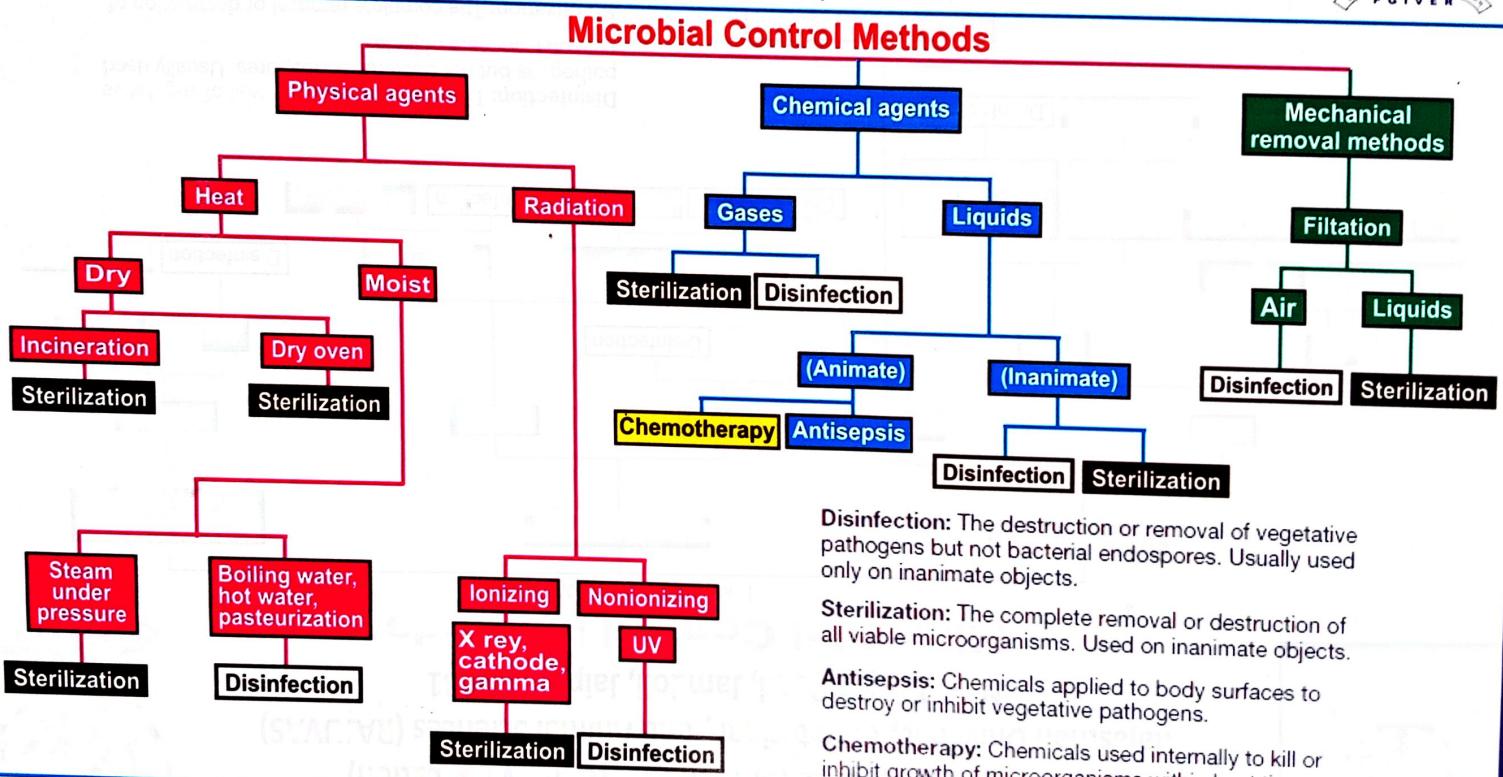
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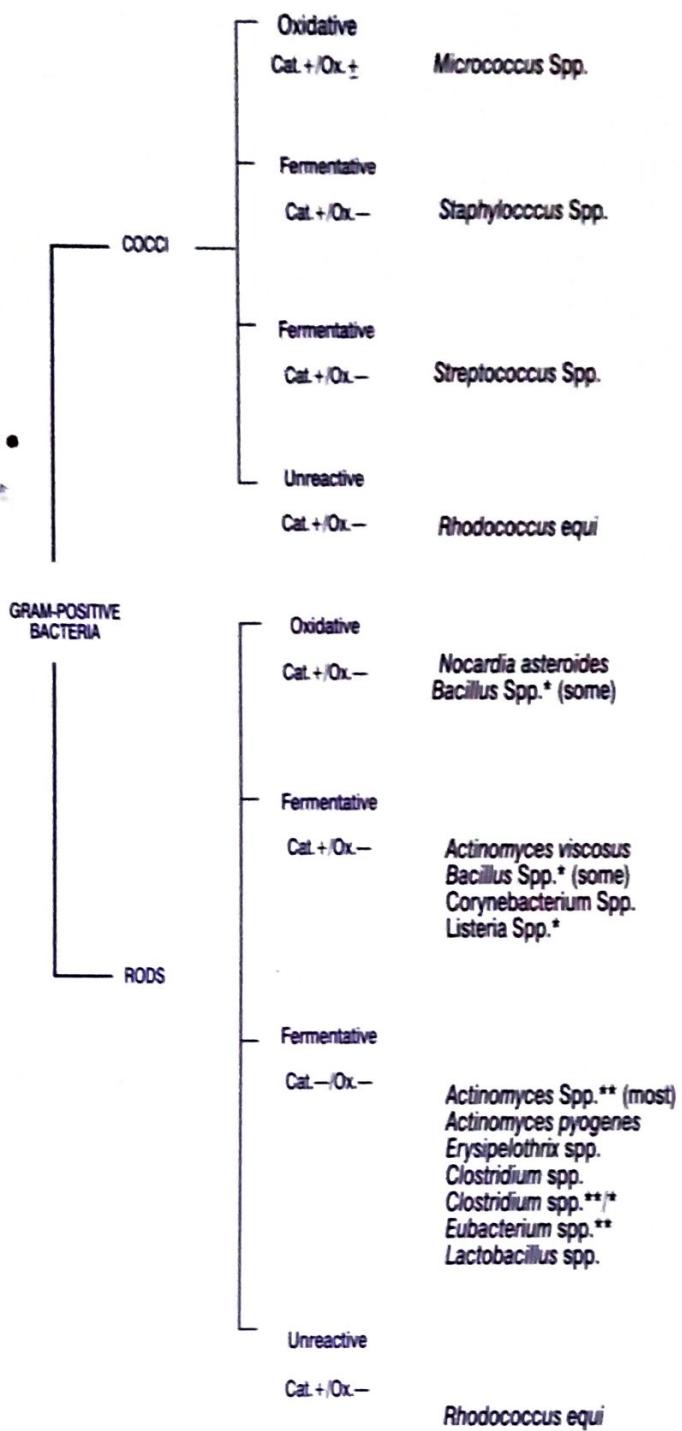
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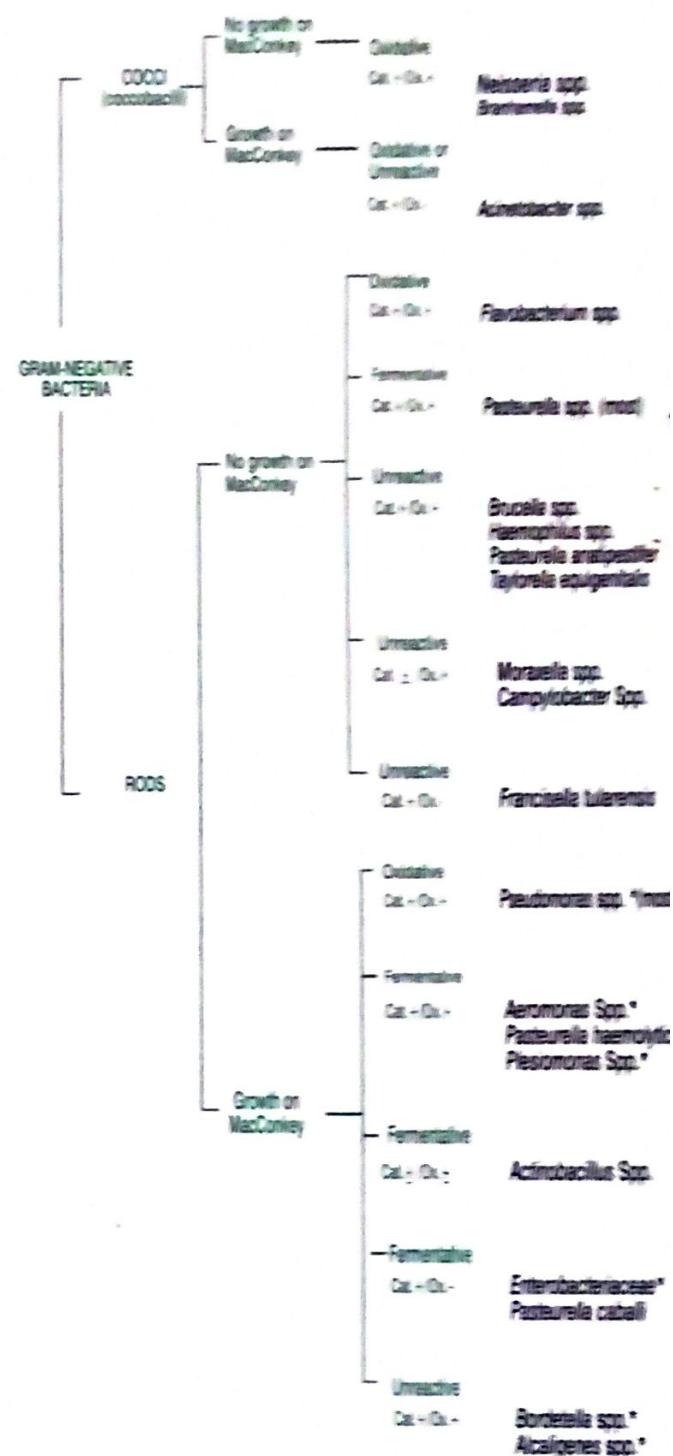


# Classification Of Bacteria

## Gram Positive



## Gram Negative



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## Difference Between Gram Positive and Gram Negative Cell Wall Structure

### Gram Positive

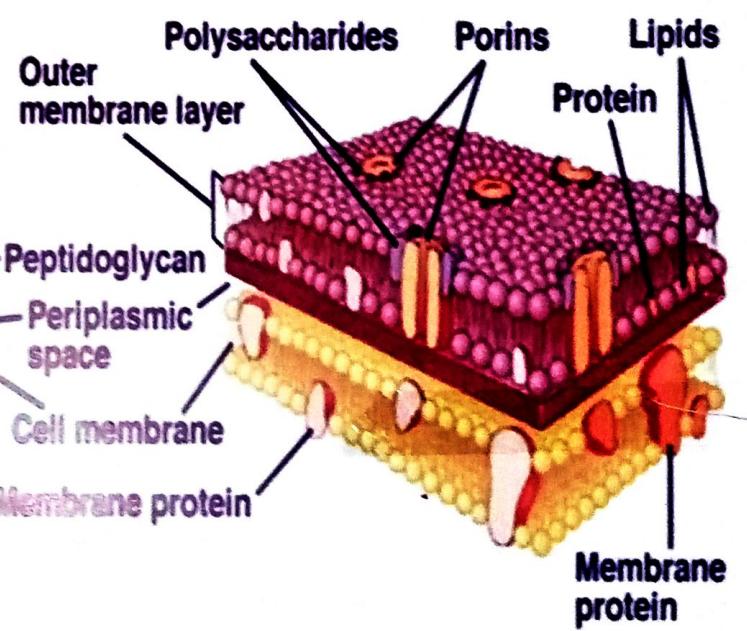
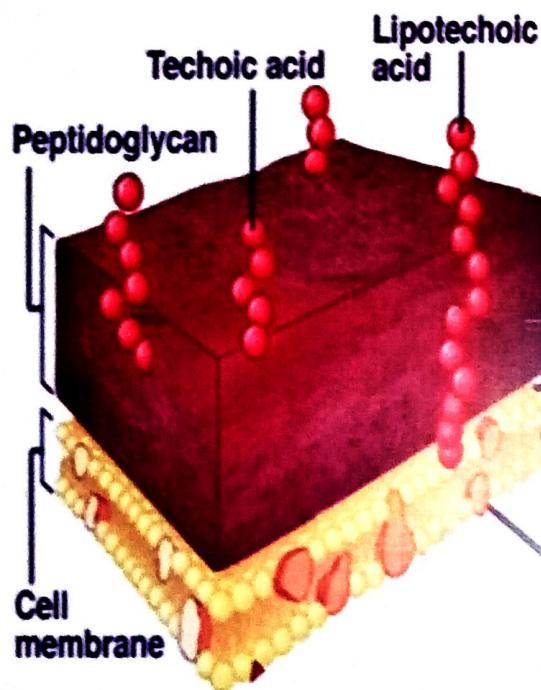
1. Thickness of wall is 150-200 Å°
2. Cell wall is single layered, smooth and in contact with the cell membrane
3. Lipid content in cell wall is 2-4%
4. Techoic acid is present in the cell wall
5. Peptidoglycan is 80% of cell wall
6. Muramic acid content of cell wall is more, 16-20% of dry weight
7. Periplasmic space is small or none
8. Outer membrane is absent
9. Cell wall is resistant to alkalis

Examples : *Bacillus subtilis*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, *Clostridium* etc.

### Gram Negative

1. Thickness of wall is 75-120 Å°
2. Cell wall is wavy and in contact with the cell membrane only at a few places
3. Lipid content in cell wall is 20-30%
4. Techoic acid is absent in the cell wall
5. Peptidoglycan is 2-12% of cell wall
6. Muramic acid content of cell wall is less, 2-5% of dry weight
7. Periplasmic space is present which contains enzymes for transport, degradation and synthesis
8. Outer membrane is present
9. Cell wall is sensitive to alkalis

Examples : *Escherichia coli*, *Rhizobium*, *Vibrio*, *Acetobacter* etc.





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# Characterization of *Staphylococcus aureus*



Mastitis affected teat of goat



Goat udder with mastitis

Fibrosed part of teat

Affected teats

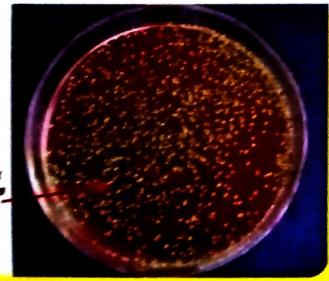


Mastitis in bitch

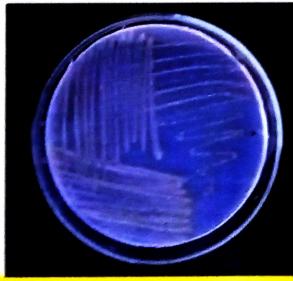


Mastitic milk samples

Pus formation



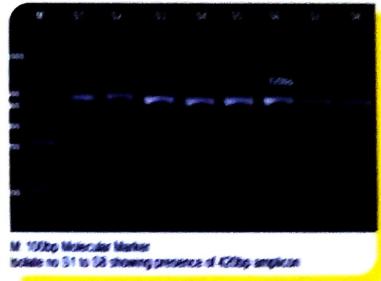
S. aureus on Mannitol Salt Agar



S. aureus on Nutrient Agar



Coagulase test of S. aureus

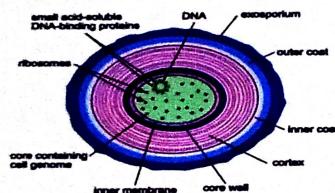


Genotyping of S. aureus by species specific primers through PCR

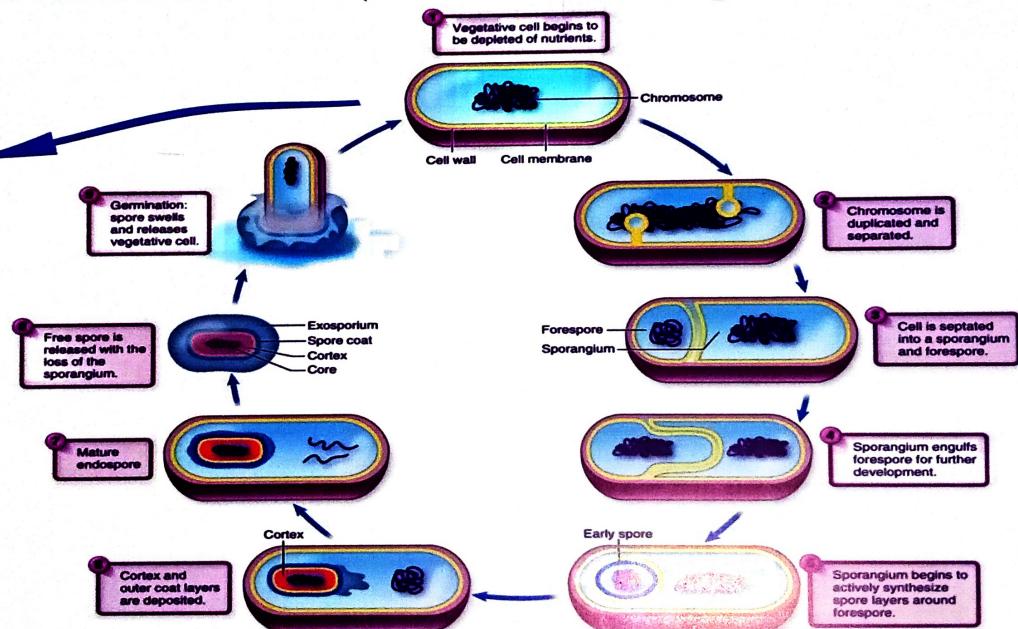
Positive



## Germination of Bacterial Spore



**Endospore**



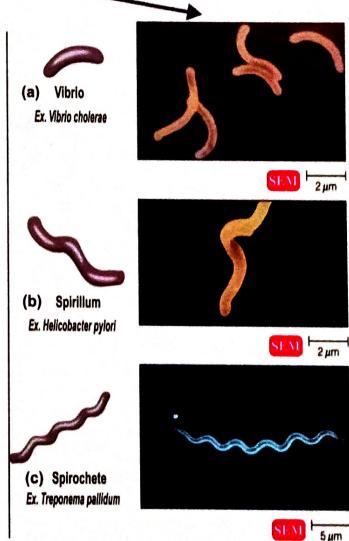
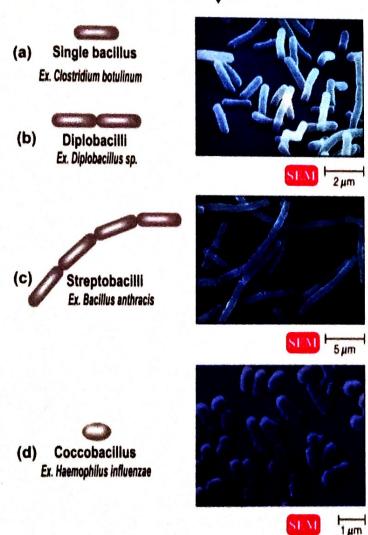
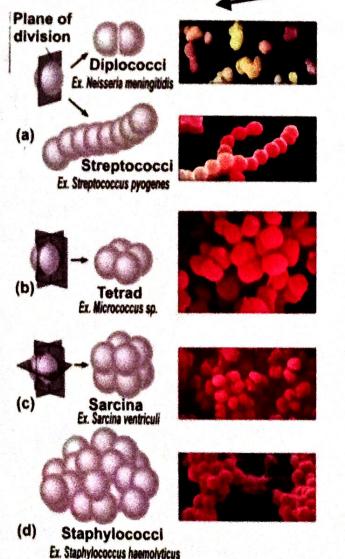


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## MORPHOLOGICAL CHARACTERISTIC OF BACTERIAL CELLS

### VARIOUS SHAPES AND ARRANGEMENT OF BACTERIAL CELLS



### MORPHOLOGY OF BACTERIAL COLONIES

Shape	Circular	Rhizoid	Irregular	Filamentous	Spindle
Margin	Entire	Undulate	Lobate	Curled	Rhizoid
Elevation	Flat	Raised	Convex	Pulvinate	Umbonate
Size	Punctiform	Small	Moderate	Moderate	Large
Texture	Smooth or rough				
Appearance	Glistening (shiny) or dull				
Pigmentation	Nonpigmented (e.g., cream, tan, white)	Pigmented (e.g., purple, red, yellow)			
Optical property	Opaque, translucent, transparent				

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Prince, Priya Darshika Shekhawat, Priyanka Garg, Punit Singh

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## Acid Fast Staining

### Procedure

Prepare and heat fix smear

Cover smear with carbol fuchsin

Heat the slide at bottom for 3-5 min.

Wash with distilled water

Acid alcohol

- Unit no large amounts of purple wash out (10-15 sec.)

- DO NOT OVER DECOLORIZE

Immediately Wash with distilled water

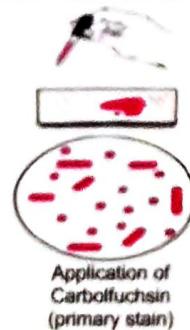
Methylene blue for 1 min.

Wash with distilled water

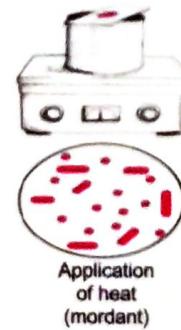
Blot Dry (bibulous paper)

Examination

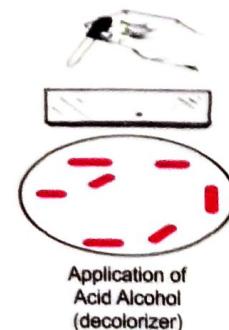
Primary Stain-1



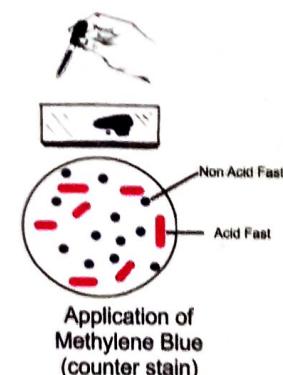
Mordant-2



Decolorizing Agent-3



Counter Stain-4

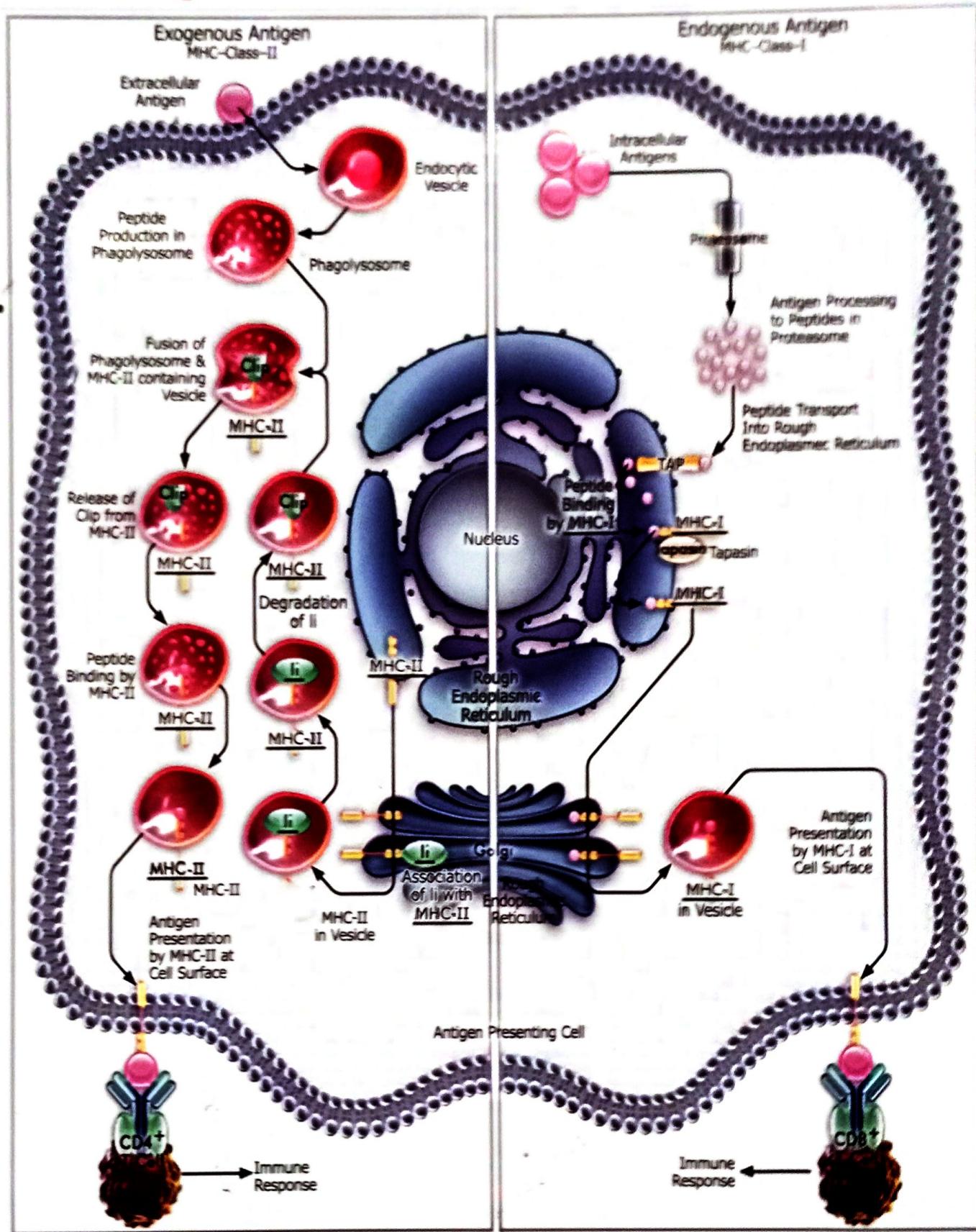


non-acid-fast bacteria- *Staphylococcus, streptococcus*

acid-fast bacteria- *Mycobacterium*



## Presentation and Processing of Antigen by Exogenous and Endogenous Pathway





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Characteristics	<b>Hypersensitivity Type</b>						
	Type - I	Type - II	Type - III	Type - IVa	Type - IVb	Type - IVc	Type - IVd
Name	Immediate/Anaphylactic Hypersensitivity	Antibody mediated / Cytotoxic Hypersensitivity	Immune complex mediated Hypersensitivity	Delayed Type Hypersensitivity	Delayed Type Hypersensitivity	Delayed Type Hypersensitivity	Delayed Type Hypersensitivity
Immune Reactent	IgE	IgG, IgM	IgG, IgM	IFN $\gamma$ , TNF $\alpha$ T $_{1,2}$ cells	IL-5, IL-4/IL-13 (T $_{1,2}$ cells)	Perforin/granzyme B (CTL)	CXCL-8, IL-17 GM-CSF (T-cells)
Antigen	Soluble antigen (exogenous)	Cell-bound antigen (Cell Surface)	Soluble antigen	Antigen Presented by cells or direct T-cell stimulation	Antigen Presented by cells or direct T-cell stimulation	Cell-associated antigen or direct T-cell stimulation	Soluble antigen presented by cells or direct T-cell stimulation
Effector	Mast cell activation	FcR+ cells (phagocytes, NK cells)	FcR+ cells complement	Macrophage activation	Eosinophils	T-cells	Neutrophils
Transferred with	Antibody	Antibody	Antibody	T-cells	T-cells	T-cells	T-cells
Examples	Allergic asthma, allergic rhinitis, anaphylaxis	Drug-induced thrombocytopenia, haemolytic anaemia of newborn (e.g., penicillin)	Serum sickness, arthus reaction	Tuberculin reaction, contact dermatitis (with IVc)	Chronic asthma, chronic allergic rhinitis maculopapular exanthema with eosinophilia	Contact dermatitis Maculopapular and bullous exanthema hepatitis	AGEP Behcet's disease
Therapy	Steroids	Steroids	Steroids	Steroids	Steroids	Steroids	Steroids

**BATCH - 2015 Pawan Kumar (SR), Omprakash**



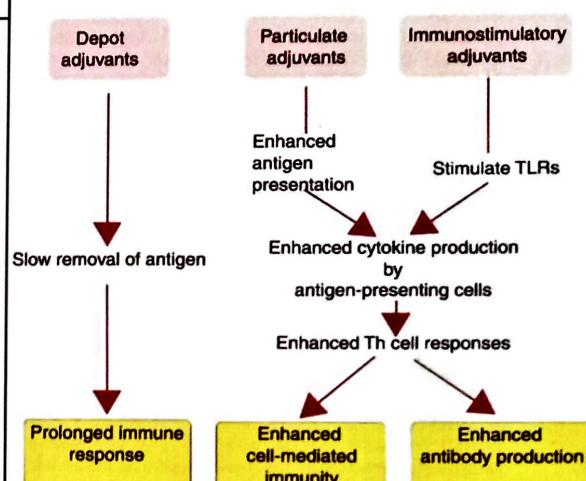
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## Different types of Adjuvants and their mode of actions

Adjuvant are substances that enhances the body's immune response to an antigen.

Type	Adjuvant	Mode of Action
Aluminum salts	Aluminum phosphate Aluminum hydroxide Alum	Slow-release antigen depot
Water-in-oil emulsions	Freund's Incomplete adjuvant	Slow-release antigen depot
Bacterial Fractions	Anaerobic corynebacteria BCG Muramyl dipeptide Derdetella pertussis Lipopolysaccharide	Macrophage stimulator Macrophage stimulator Macrophage stimulator Lymphocyte stimulator Macrophage stimulator
Surface active agents	Saponin Lysolccithin Plutonic detergents	Stimulates antigen processing Stimulates antigen processing Stimulates antigen processing
Complex carbohydrates	Acemannan Glucans Dexinan sulfate	Macrophage stimulator Macrophage stimulator Macrophage stimulator
Mixed adjuvants	Freund's complete adjuvant	Macrophage and T cell stimulation



## Mode of Action of Adjuvant

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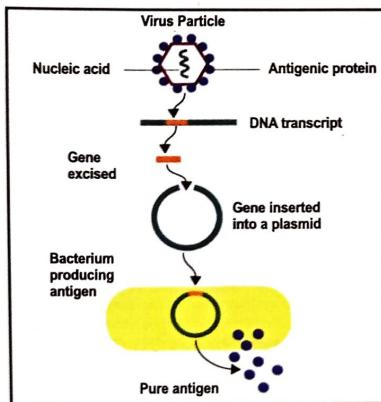


## Recombinant Vaccine Production

### Antigens Generated by Gene Cloning (Category I)

Vaccines that contain inactivated recombinant organisms or purified antigens derived from recombinant organisms

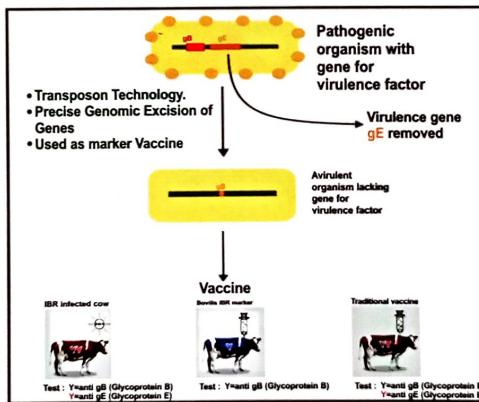
Example : Feline Leukemia Virus Vaccine



### Genetically Attenuated Organisms (Category II)

Vaccines containing Live organisms that contain gene deletions or Heterologous marker genes

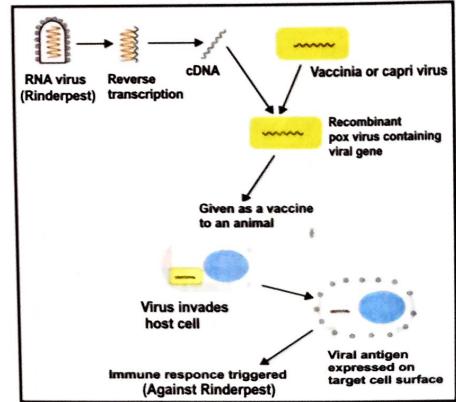
Example : Herpes Virus Vaccine



### Live Recombinant Organisms (Category III)

Vaccines that contain Live expression vectors expressing heterologous genes for immunizing antigens or other stimulants

Example : Rinderpest virus vaccine



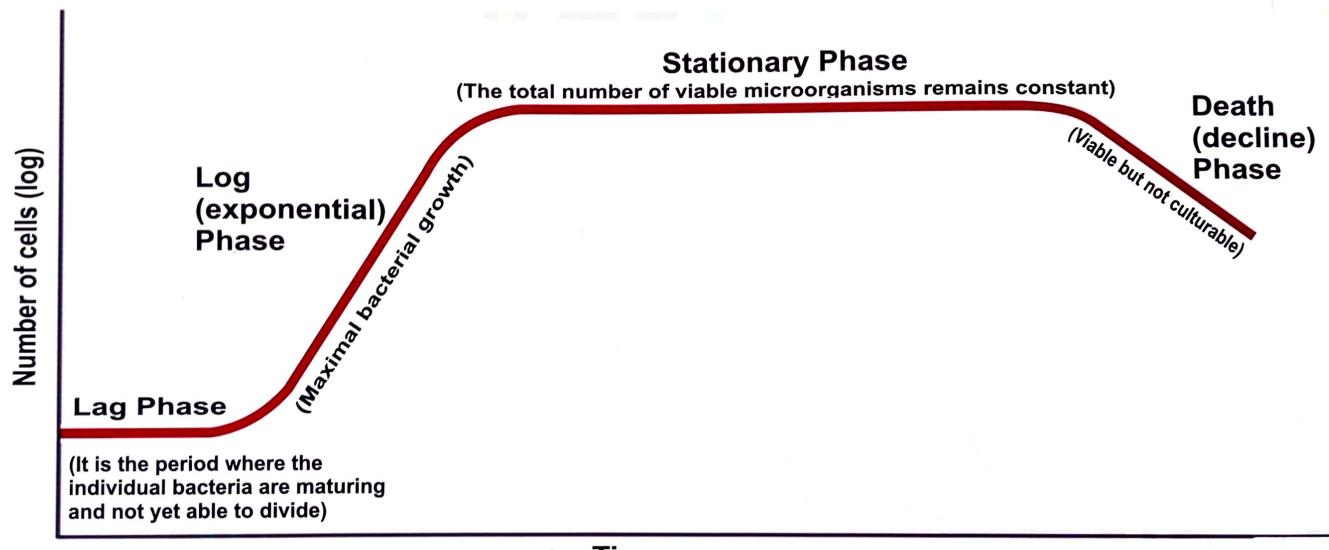
BATCH - 2015 Nikhil Kumar Sharma, Neeraj Bhaskar



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## BACTERIAL CELL GROWTH CURVE



Let  $N$  = the initial population number

$N_0$  = the population at time  $t$

$n$  = the number of generations in time  $t$

For populations reproducing by binary fission

$$N_0 = N \times N_0$$

Solving for  $n$ , the number of generations, where all logarithms are to the base 10

$$\log N_0 = \log N + n \cdot \log 2,$$

$$n = \log N_0 - \log N / \log 2 = \log N_0 - \log N / 0.301$$

The growth rate constant ( $k$ ) is the number of generations per unit time ( $n/t$ ).

$$\text{Thus } k = n/t = \log N_0 - \log N / 0.301t$$

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**Karan Mahar, Lokesh Meena**



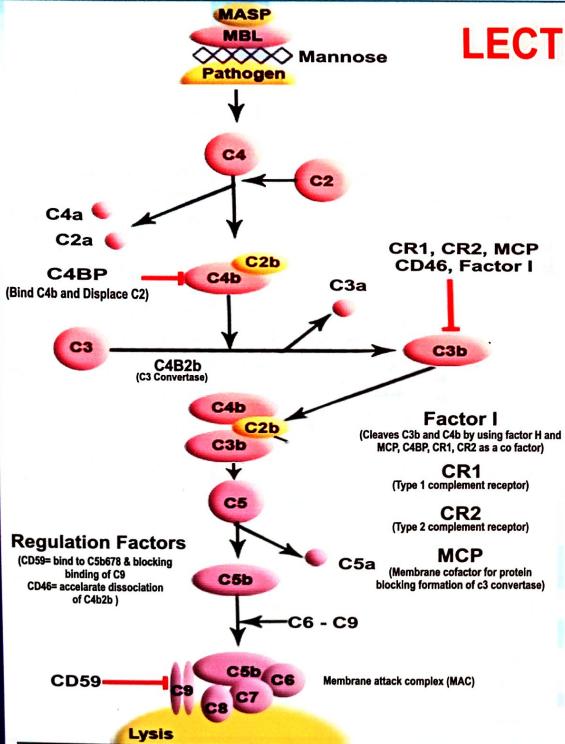
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### LECTIN PATHWAY OF COMPLEMENT SYSTEM



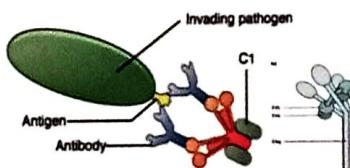
- Mannose Binding Lecting (MBL) bind foreign surface (bacteria virus fungi)
- MBL associated proteases (MASP-1+2) bind MBL
- MASP activete C4 & C2 union form the C4b2b complex
- The C4b2b complex activates C3 which binds to the membrane
- Complement proteins C5-C9 bind to C3 & form a hole in the microbial membrane
- Killing of the organism by membrane attack complex (MAC)

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Dinesh Kumar, Ganpat Ram

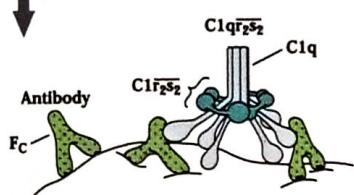


## Classical Pathway of Complement System

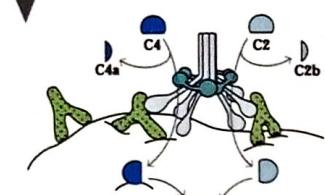


C1 binds to an antigen-antibody complex on an invading pathogen

Causes C1 r<sub>2</sub>s<sub>2</sub> to dissociate from C1q



C1q binds antigen-bound antibody. C1r activates auto-catalytically and activates the second C1r; both activate C1s



C1s cleaves C4 and C2. Cleaving C4 exposes the binding site for C2. C4 binds the surface near C1 and C2 binds C4, forming C3 convertase

C4b-binding protein (C4bBP)  
CR1 & MCP  
Block formation of C3 convertase

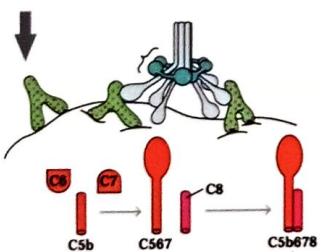
DAE or CD55  
Accelerates dissociation of C4b2a

C3 convertase hydrolyzes many C3 molecules. Some combine with C3 convertase to form C5 convertase

Factor - I  
C4b2a3b  
C5 convertase  
Cleaves C4b or C3b using C4bBP, CR1, factor - H, DAE, or MCP as cofactor

The C3 b component of C5 convertase binds C5, permitting C4b2a to cleave C5

### C5 convertase



C5b binds C6, initiating the formation of the membrane-attack complex

S-protein  
Prevents insertion C5b67 into cell membrane

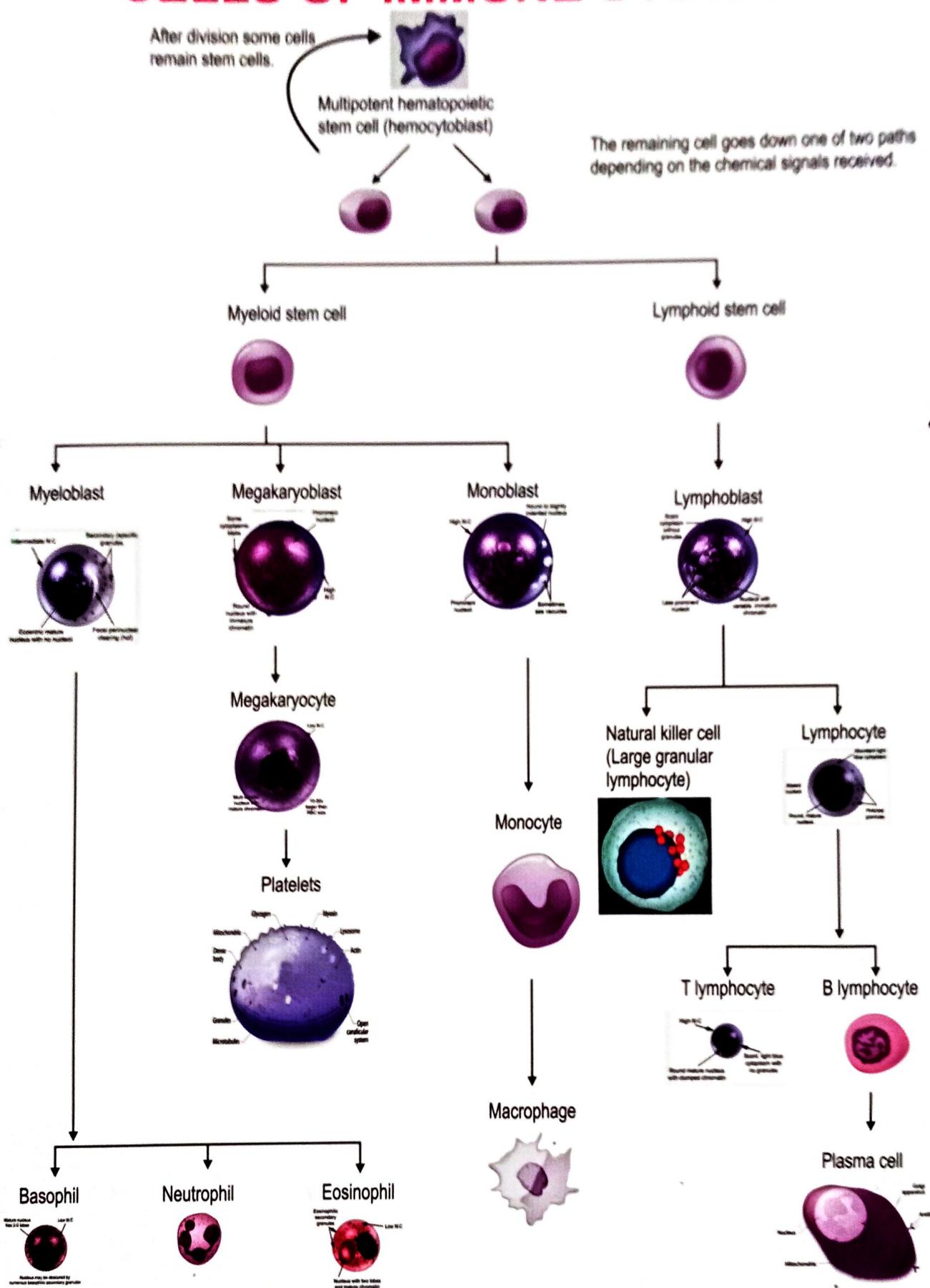
HRF & MIRL  
Blocking binding of C9 with C5b678

Formation of complete membrane attack complex consist of poly-C9, result in cell disruption

Abbreviations-DAE=Decay accelerating factor, HRF =Homologous Restriction factor, MIRL=Membrane inhibitor of reactive lysis



## CELLS OF IMMUNE SYSTEM

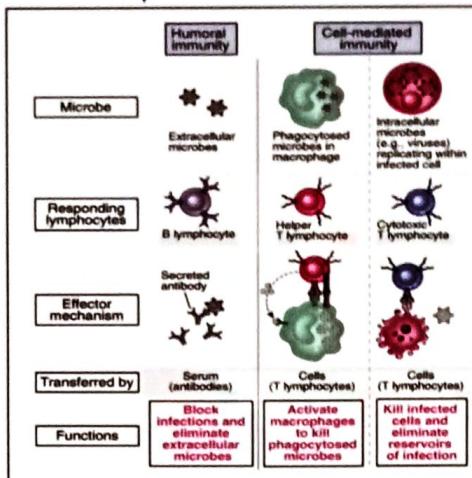
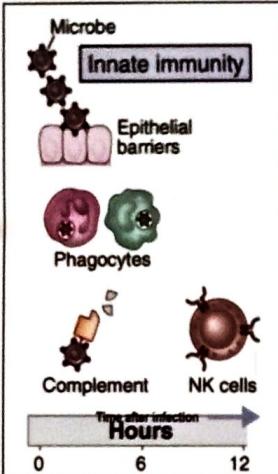




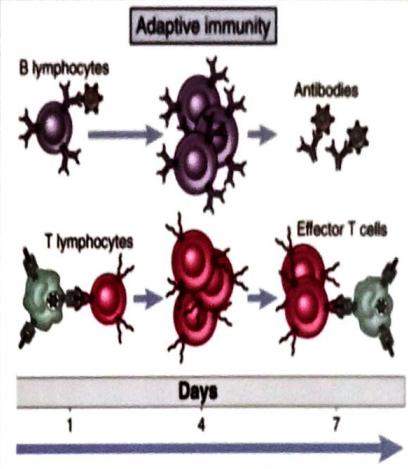
## TYPES OF IMMUNITY

### INNATE (inborn) Genetic factors

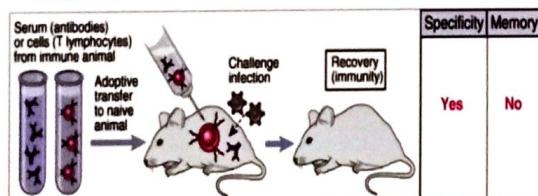
(Skin, Tears, Low pH of Stomach Normal Flora of Gut)



### ACQUIRED (adaptive)



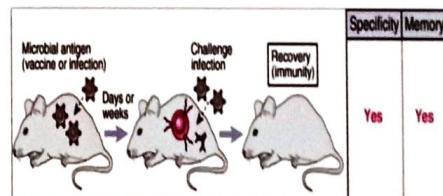
### PASSIVE Ready-made antibodies



**NATURAL**  
Maternal antibodies

**ARTIFICIAL**  
Antibodies from other sources

### ACTIVE Own antibodies



**NATURAL**  
Exposure to infectious agent

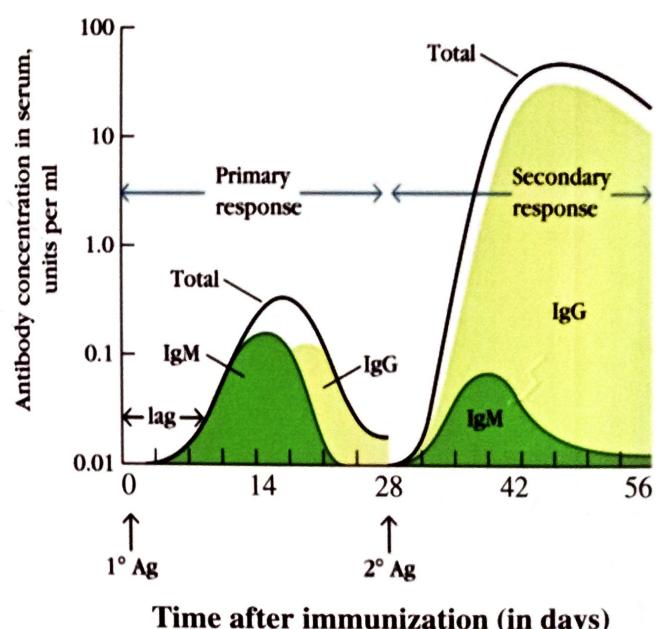
**ARTIFICIAL**  
Immunization

**Active and Passive Immunity :** Active immunity is conferred by a host response to a microbe or microbial antigen, whereas passive immunity is conferred by adoptive transfer of antibodies or T lymphocytes specific for the microbe. Both forms of immunity provide resistance to infection and are specific for microbial antigens, but only active immune responses generate immunologic memory.



## Comparison of Primary And Secondary Immune Response

Attribute	Primary response	Secondary response
Antigen type	Both T dependent and T independent	Only T dependent
Responding cells	Naïve B or T cells	Memory B or T cells
Lag period	Longer (4- 7 days)	Shorter (1- 3 days)
Peak response	Occurs in 7- 10 days	Occurs in 3- 5 days
Magnitude	Low	High (100- 1000x)
Antibody isotype	IgM predominates	IgG predominates
Antibody affinity	Lower	Higher
Exposure To Antigen	Primary Contact with Antigen Secondary Contact with Antigen & subsequent exposure of the same antigen	Secondary Contact with Antigen & subsequent exposure of the same antigen
Antibody Level	Declines rapidly	Remain high for longer period





## Important Characteristics of Immunoglobulins of Domestic Animals

	IgG	IgM	IgA	IgE	IgD
Properties					
Molecular Weight	180,000	900,000	360,000	190,000	180,000
Sub Units	Monomer	Pentamer	Diamer	Monomer	Monomer
Heavy Chain	$\gamma$	$\mu$	$\alpha$	$\epsilon$	$\delta$
Sedimentation Coefficient	7 S	19 S	7S to 11 S	8 S	7 S
Carbohydrate Percentage	3	12	7	12	12
Mainly Synthesized in	Spleen and lymph nodes	Spleen, bone marrow and lymph nodes	Intestinal and respiratory tracts	Intestinal and respiratory tracts	Spleen and lymph nodes
Percentage of Total Serum Antibody	80%	5-10%	10-15%	Less than 0.1%	0.2%
Complement Fixation	Yes	Yes	No	No	No
Antigen Binding Sites	2	10	4	2	2
Biological Property	Major Ig in Serum. Provides the Majority of antibody based immunity against invading pathogens. Moderate complement fixer (IgG3) can cross placenta	First response antibody, Expressed on the surface of B cells and in secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated immunity before there is sufficient IgG.	Most produced Ig. Found in mucosal areas, such as the gut, respiratory and urogenital tract, and prevents their colonization by pathogens. Resistant to digestion and is secreted in milk.	Binds to allergens and triggers histamine release from mast cells and is involved in allergy. Also protects against parasitic worms.	Function unclear. works with IgM in B-cell development mostly B cell bound

- IgA more abundant in colostrum of Cattle and Buffalo while in milk of these animal IgG is abundant.
- IgG more abundant in colostrum of Mare, Sow and Human while in milk of these IgA is abundant.

# Department Of Veterinary Microbiology and Biotechnology

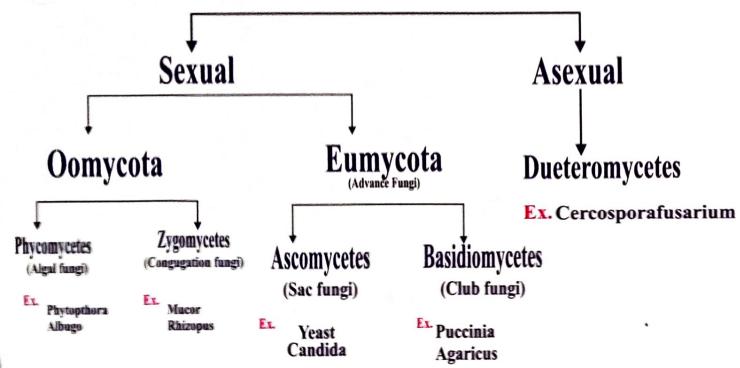


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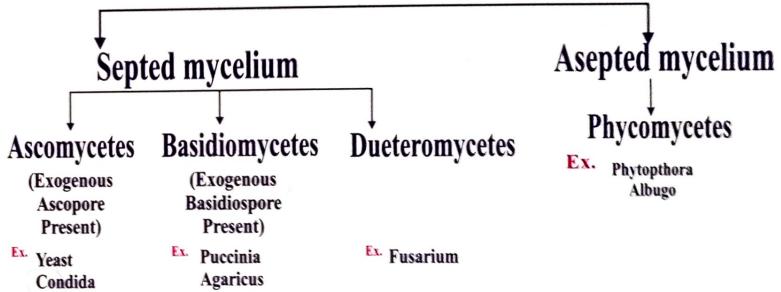


## Classification of fungi

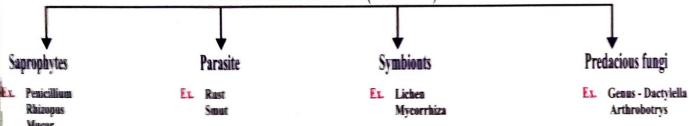
### 1. on the basis of Reproduction (MYCOTA)



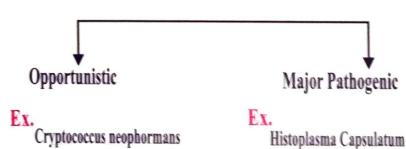
### 2. on the basis of Septa (MYCOTA)



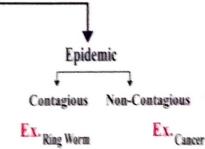
### 3. On the basis of Nutrition (MYCOTA)



### 4. On the Basis of Virulence



### 5. On the Basis of Geographical



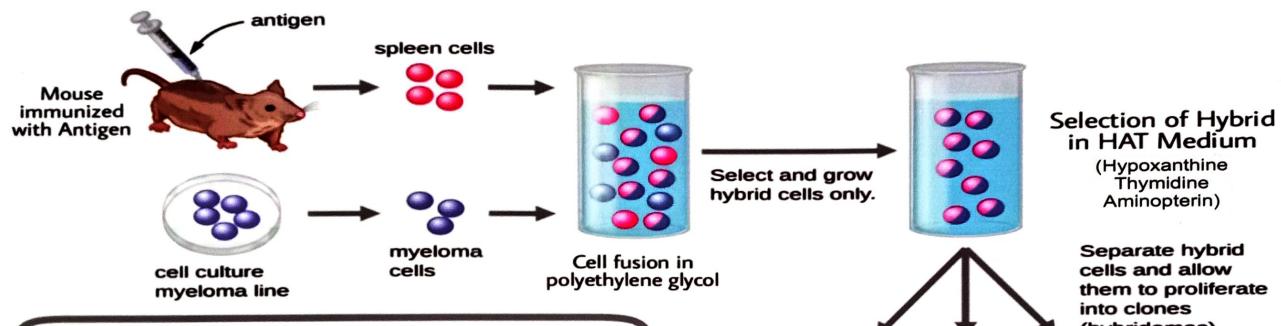
**BATCH - 2015** Dinesh Kumar, Gaurav Agrawal, Ganpat Ram Bhati, Devendra Kumar



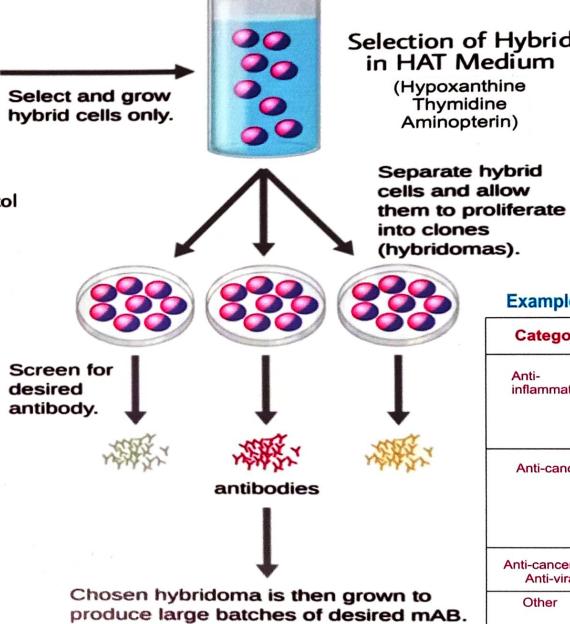
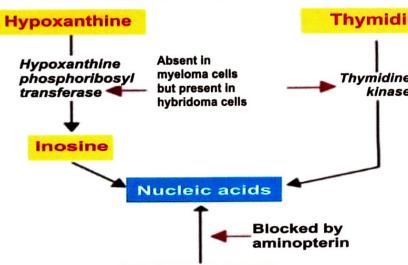
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## MONOCLONAL ANTIBODY PRODUCTION



The pathway of purine synthesis and mechanism of action of the HAT Medium



Example of Monoclonal Antibodies

Category	Type	Application
Anti-inflammatory	Infliximab Adalimumab	Rheumatoid arthritis Crohn's disease ulcerative colitis
Anti-cancer	Rituximab Cetuximab	Non-hodgkin's lymphoma Squamous cell carcinomas, colorectal carcinoma
Anti-cancer and Anti-viral	Bavituximab	Cancer, hepatitis C infection
Other	Palivizumab	RSV infections in children

BATCH - 2015 Brijendra Kumar Jat, Chandra Prakash Hindoniya

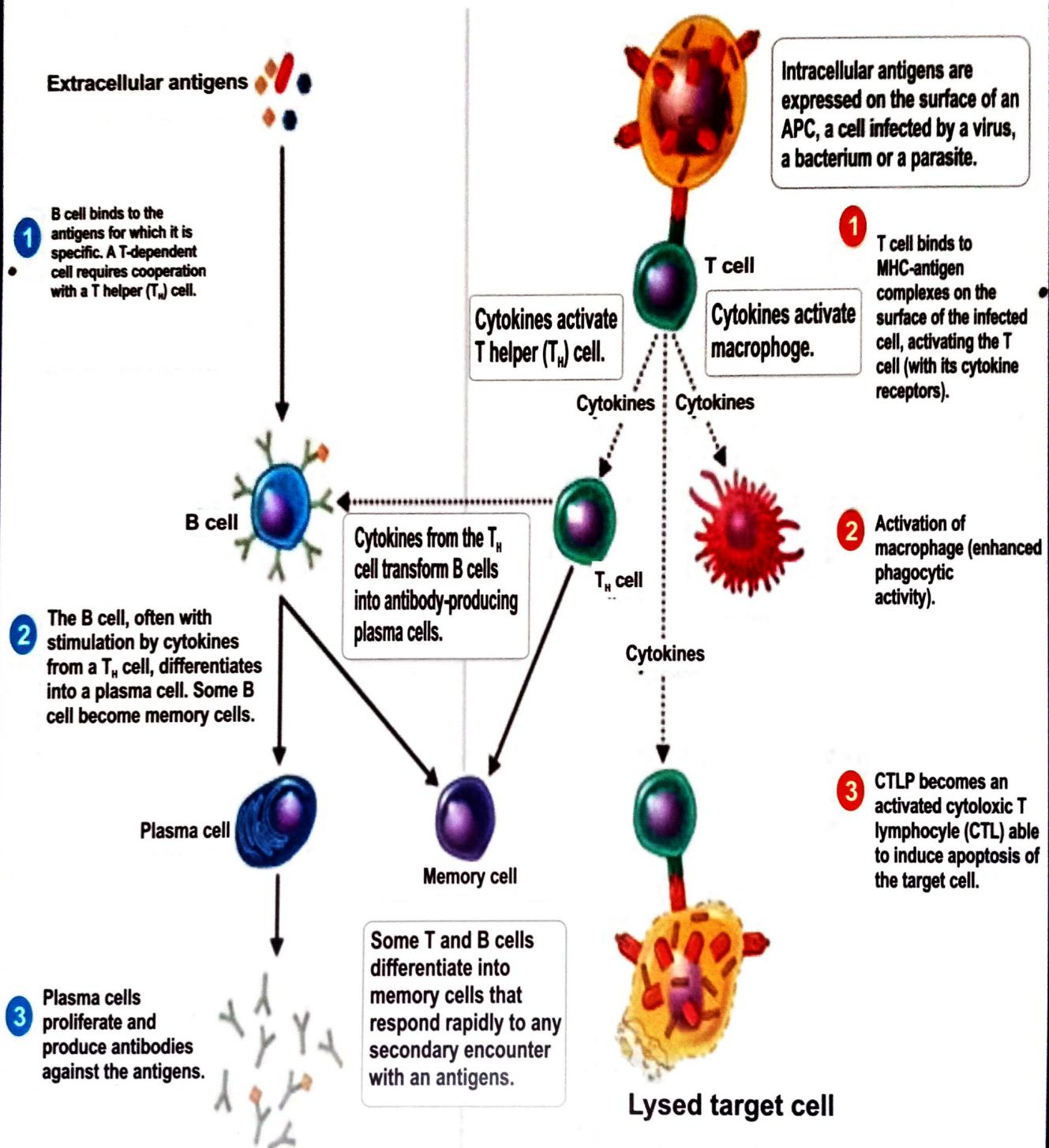


## **Humoral (antibody-mediated) immune system**

**Control of freely circulating pathogens**

## **Cellular (cell-mediated) immune system**

### **Control of intracellular pathogens**





## Acute Respiratory Tract Infection & Abscess In Camel

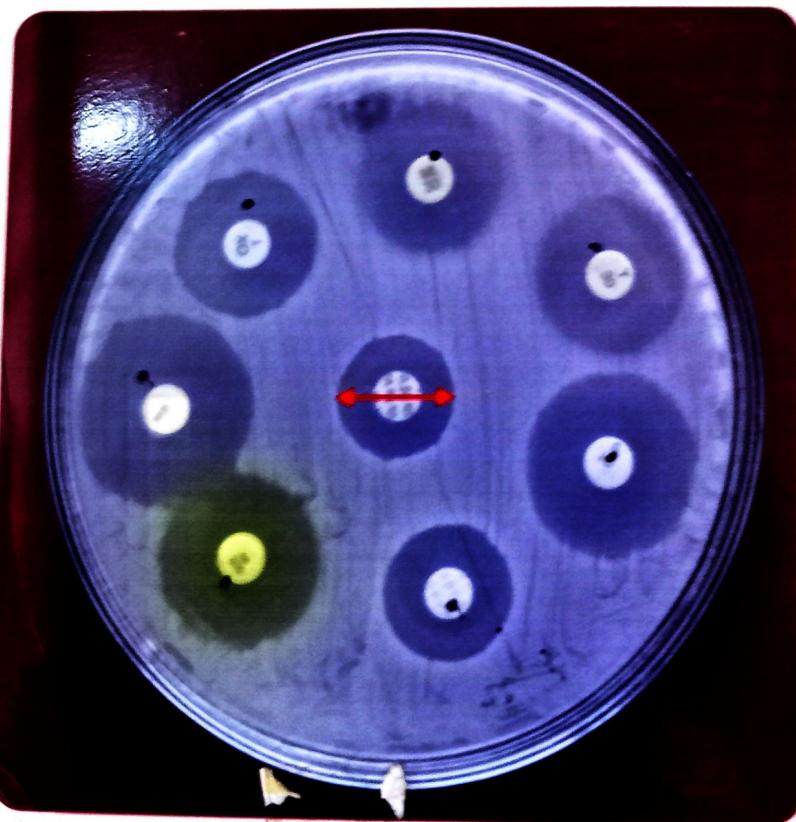


Severe nasal discharge



Pus Discharge From Abscess

### Antibiotic sensitivity test



Measure diameter of  
zone of inhibition

↓  
Compare with standard  
chart as described  
by CLSI

↓  
Resulted as sensitive,  
intermediate and  
resistant



## **Pyocyanin Pigmentation Of *Pseudomonas aeruginosa***



**Pigmented nutrient agar slant**



**Pigmented colonies on cetrimide agar**



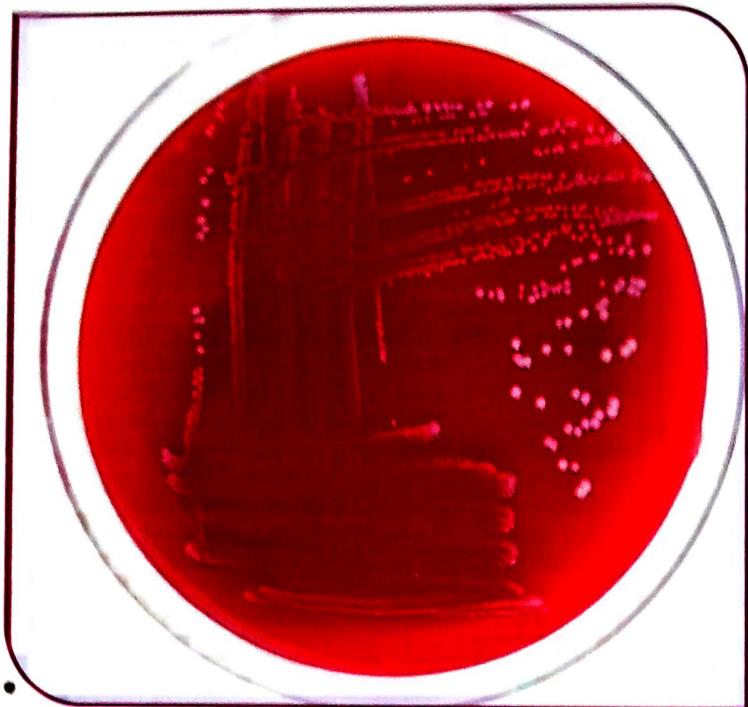
**Negative reaction**

**Positive reaction  
with gas  
and acid production**

**Sugar fermentation reaction of bacterial pathogen**



## BACTERIAL PATHOGEN ON 5% SHEEP BLOOD AGAR



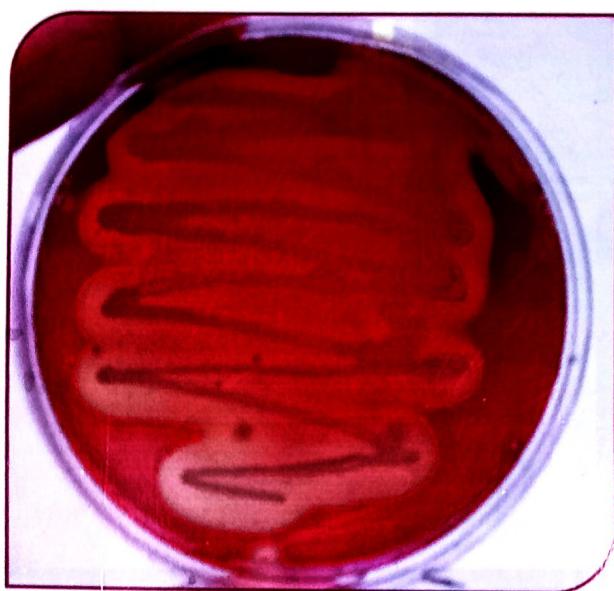
Non hemolytic grey white colonies  
of *Klebsiella pneumoniae*



*Staphylococcus aureus*

Incomplete haemolysis  
(beta haemolysin)

Complete haemolysis  
(alpha haemolysin)



Complete haemolysis  
(beta haemolysis ) Streptococci



Incomplete or greenish haemolysis  
(alpha haemolysis), streptococci



## BACTERIAL PATHOGEN ON EOSIN METHYLENE BLUE AGAR

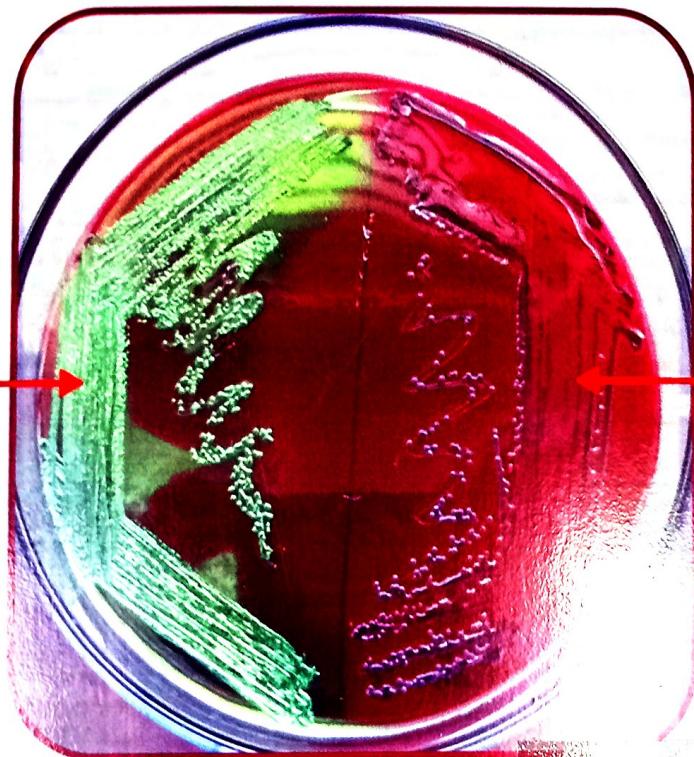
Dark colored nonmetallic  
mucoid colonies  
(*K. pneumoniae*)



## EOSIN METHYLENE BLUE AGAR AS DIFFERENTIAL MEDIA

Metallic green sheen  
showing coliform  
(*E. coli*)

Nonmetallic sheen  
showing coliforms  
(*K. pneumoniae*)





## Gram's Staining

### Procedure

Prepare and heat fix smear



Crystal Violet for 1 minute



Wash with distilled water



Gram's iodine for 1 minute



Wash with distilled water



95% ethyl alcohol

- Unit no large amounts of purple wash out (10-15 seconds)
- DO NOT OVER DECOLORIZE



IMMEDIATELY Wash with distilled water



Safranin for 1 minute



Wash with distilled water

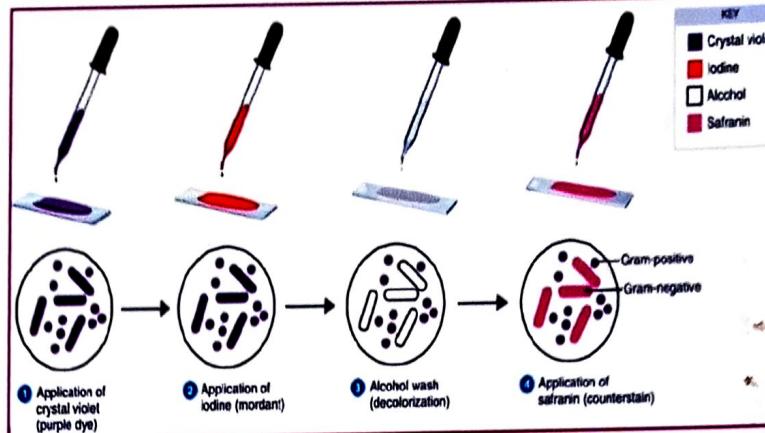


Blot Dry (bibulous paper)



Examination

### Primary Stain - 1

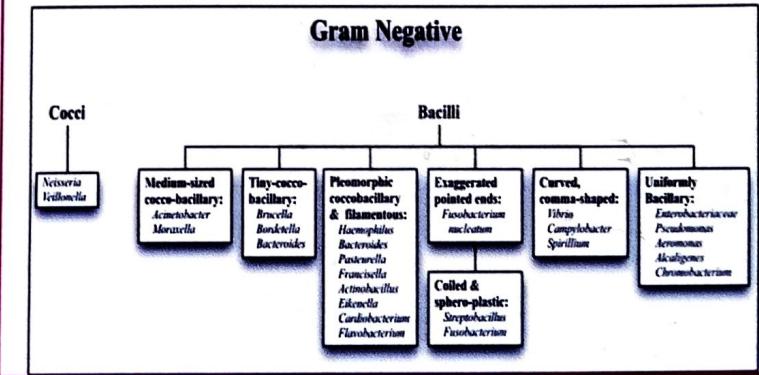
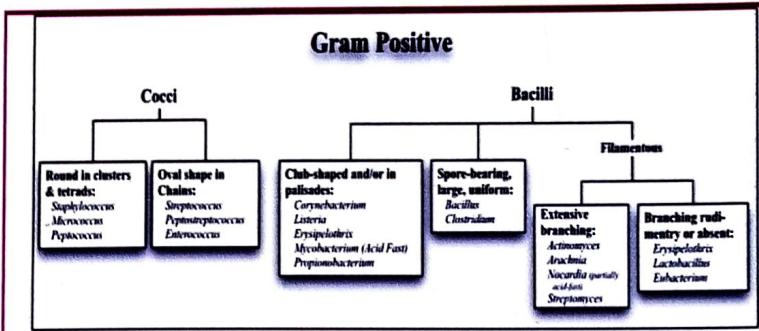


### Mordant - 2

### Decolorizing Agent - 3

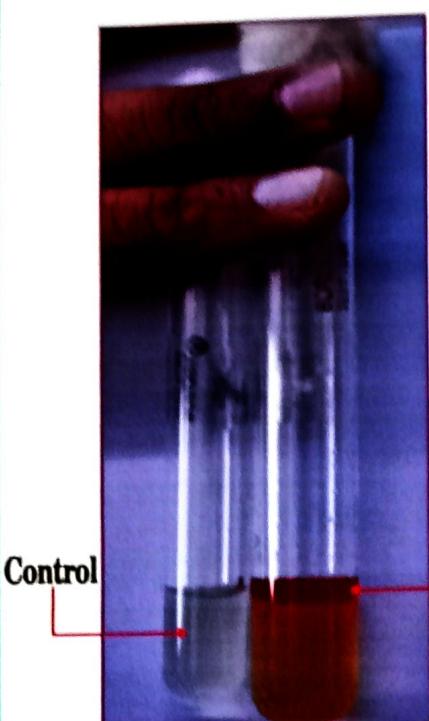
### Counter stain - 4

## DIFFERENTIATION VIA GRAM'S STAINS AND CELL MORPHOLOGY





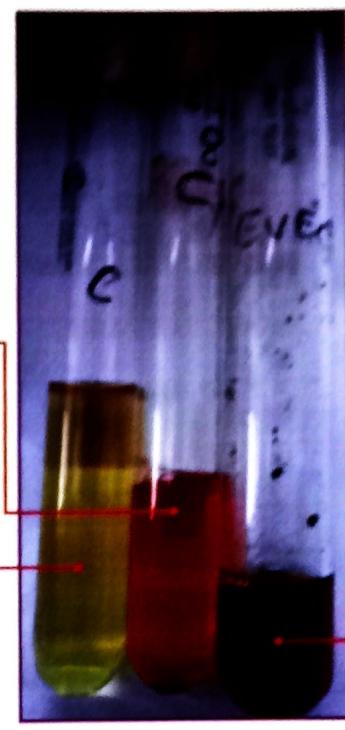
## IMViC PATTERN OF BACTERIAL PATHOGEN



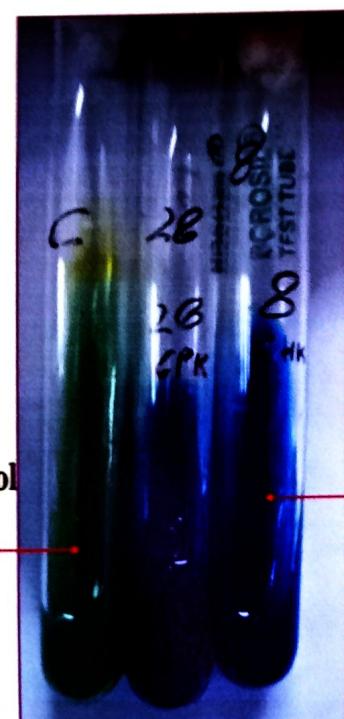
*Indole test*



*Methyl red test*



*Voges-Proskauer test*



*Citrate test*

## DIFFERENTIATION VIA IMViC PATTERN

	<i>Escherichia coli</i>	<i>Salmonella</i> serotypes	<i>Yersinia</i> species	<i>Proteus</i> species	<i>Enterobacter</i> spp.	<i>Klebsiella</i> pneumoniae
Clinical importance	Major pathogen	Major pathogens	Major pathogens	Opportunistic pathogens	Opportunistic pathogen	Opportunistic pathogen
Cultural characteristics	Some strains haemolytic	-	-	Swarming growth <sup>a</sup>	Mucoid	Mucoid
Motility at 30°C	Motile	Motile	Motile <sup>b</sup>	Motile	Motile	Non-motile
Lactose fermentation	+	-	-	-	+	+
IMViC tests						
Indole production	+	-	+	+	-	-
Methyl red test	+	+	+	+	-	-
Voges-Proskauer test	-	-	-	+	+	+
Citrate utilization test	-	+	-	+	+	+
H <sub>2</sub> S production in TSI agar	-	+	-	+	-	-
Lysine decarboxylase	+	+	-	-	+	+
Urease activity	-	-	+	+	-	+

a - when cultured on non-inhibitory medium

b - except *Y. pestis*

c - *P. vulgaris* +; *P. mirabilis* -

v - reaction varies with individual species

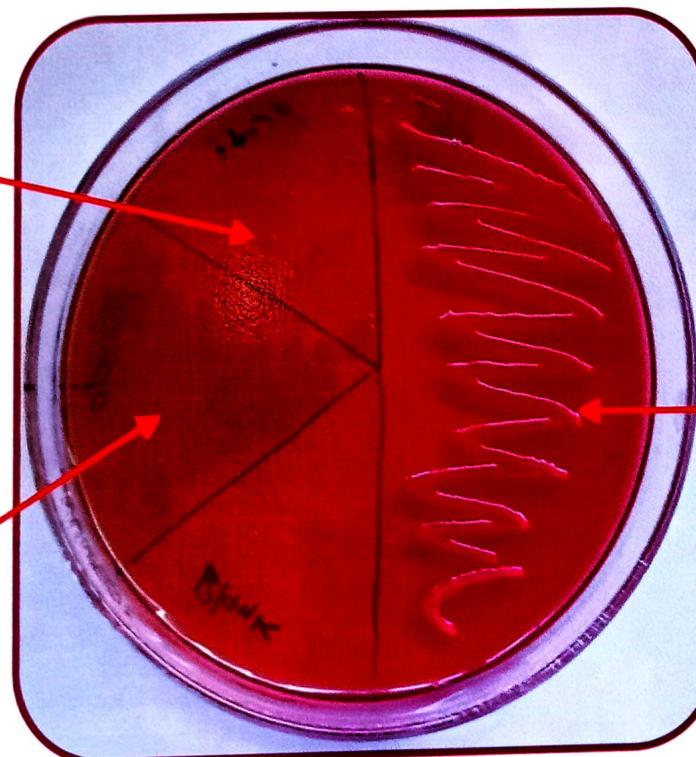


## Bacterial pathogen on Mac-Conkey Agar



Lactose fermenting pink  
mucoid colonies  
*(K. pneumoniae)*

## Mac-Conkey Agar as differential media



Lactose fermenting pink  
non mucoid colonies  
*E. coli*

Lactose non  
fermenting yellow  
growth *Pseudomonas* spp.

Lactose fermenting  
pink mucoid  
growth *Enterobacter* spp.

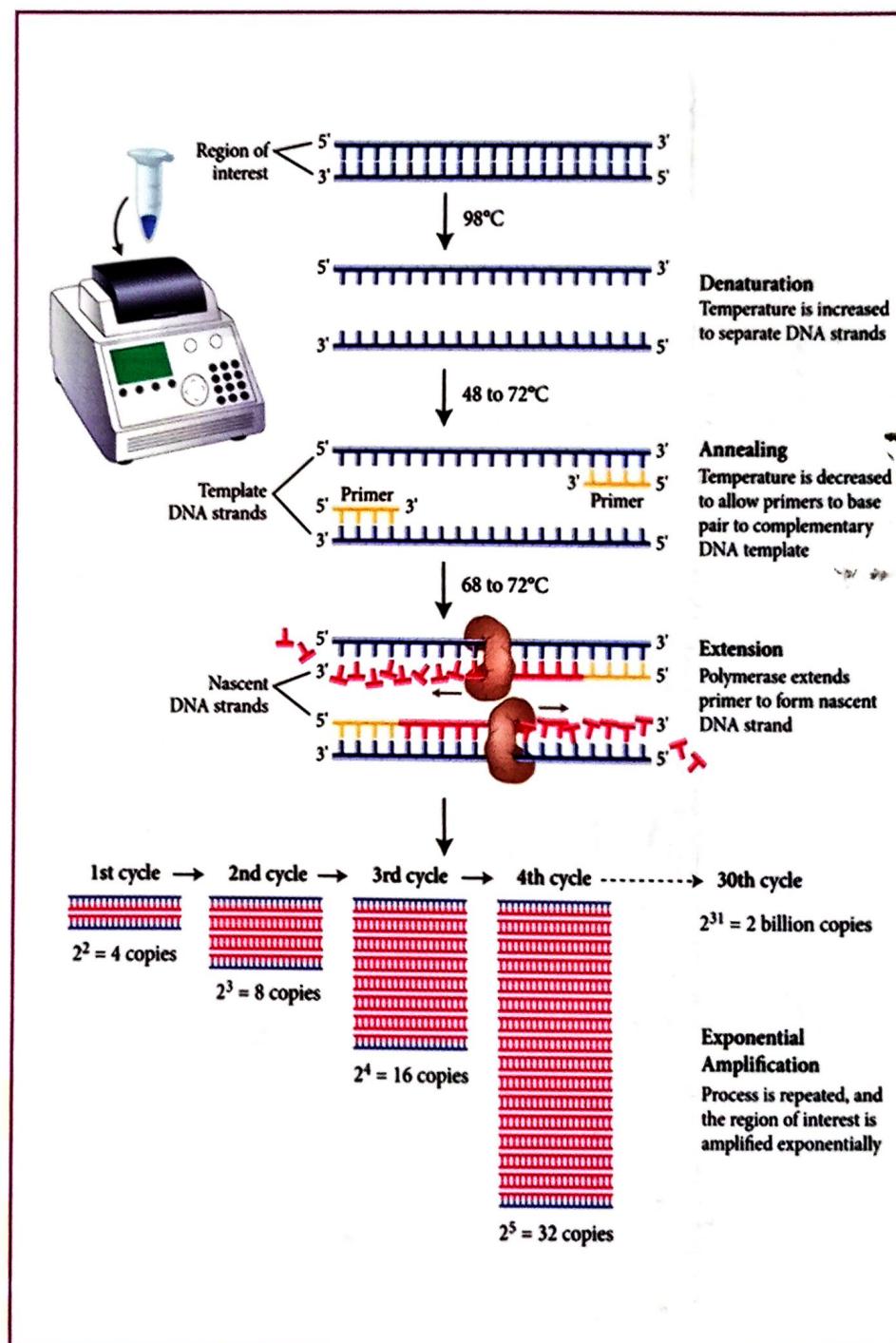


## Purpose

The polymerase chain reaction (PCR) is a powerful technique that exponentially amplifies a small amount of deoxyribonucleic acid (DNA) into a large amount by repeating a simple three step process: denaturation, annealing and synthesis. PCR is frequently employed to detect pathogens, contamination in tissue cell culture (laboratory) and is used in genetic monitoring of animals.

## Applications

- Medical application: Genetic testing: For the presence of disease, mutations, oncogenes and tissue typing for organ transplantation.
- Genetic detection of infectious disease of animals viz. PPR, FMD, Animal Pox, Brucellosis & Tuberculosis etc.
- Genetic monitoring and surveillance of disease organism of domestic and wild animals.
- Forensic application: Genetic fingerprinting of any individual and parental testing.
- Research application:
  - ▶ Use for generating hybridization probes for Southern or Northern blot hybridization.
  - ▶ Assist in DNA sequencing, DNA cloning, generate mutations, gene expression and preparation of genetic mapping.
  - ▶ Retrieval of DNA from highly degraded template DNA from ancient sources viz. bone of Neanderthals or from frozen tissue of Mammoths.



### Advantage

- PCR has high sensitivity due to exponential amplification of the template DNA.
- PCR is highly specific due to the specificity of primer annealing.
- The PCR technique can be completed in one working day, providing rapid results.

### Disadvantages

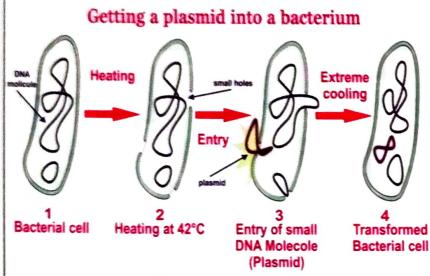
- Minute amounts of contamination can lead to false positive results.
- The PCR technique is expensive.
- Inhibitors of the PCR reaction can lead to false negative results.

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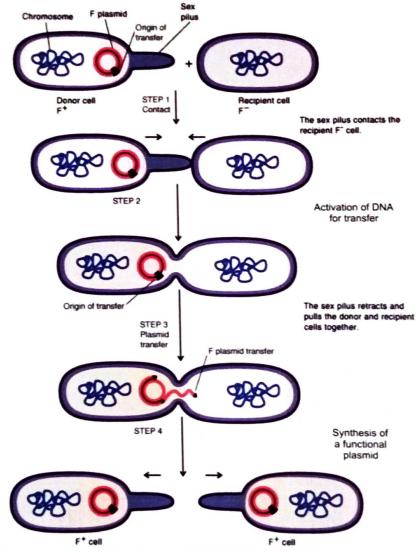
## Transmission of Genetic Material in Bacteria

### Bacteria Transformation

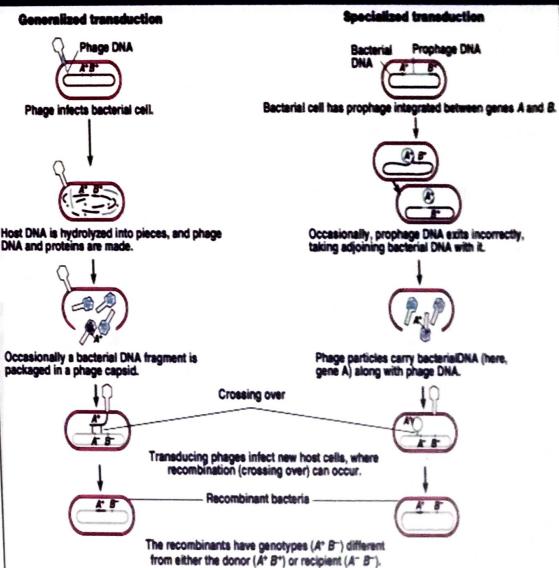


Transformation of plasmid / other small DNA molecule from one bacterial cell (donor) to another (recipient) bacterial cell

### Bacterial Conjugation



### Bacterial Transduction



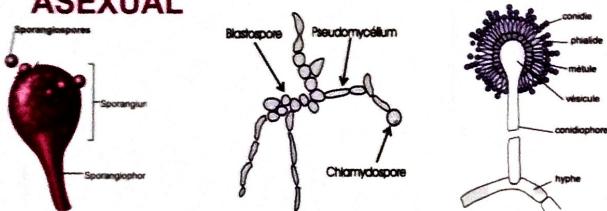
**BATCH - 2015** Rajkumar Baghel, Rajkumar Meena, Rajdeep, Rajendra Prasad

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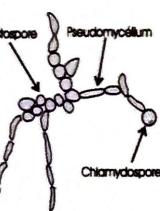


## REPRODUCTION OF FUNGI

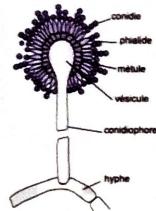
### ASEXUAL



**1. Sporangiospore**  
Ex. *Mucor & rhizopus*



**2. Blastospore**  
Ex. *Candida albicans*



**3. Conidium**  
Ex. *Penicillium & Aspergillus*



**4. Chlamydospore**  
Ex. *Candida & Panus*



**5. Arthrospore**  
Ex. *Zygomycota & Mitosporic*

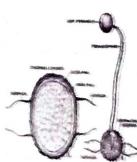
### SEXUAL

*It involves three step :-*

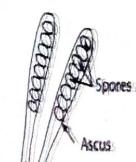
- 1. Plasmogamy** → It is the union of protoplast of reproductive hyphae.
- 2. Karyogamy** → The fusion of two nuclei which take place in the next phase.
- 3. Meiosis** → By meiosis producing four haploid sexual spore from diploid nuclei.

Plasmogamic copulation:
1. Isogamy : Ex. Ophiotrichum
2. Anisogamy : Ex. <i>Genus Allomyces</i>
3. Oogamy : Ex. Pythium
→ Gametangial Contact :
Ex. <i>Pencillium</i>
→ Gametangial copulation :
Ex. <i>Mucor &amp; Rhizopus</i>
→ Somaticy :
Ex. Members of Ascomycetes & Basidiomycetes
→ Spermatization :

The sexual spores produced in fungi are of three types:-



**1. Zygospore**  
Ex. *Mucor & Rhizopus*

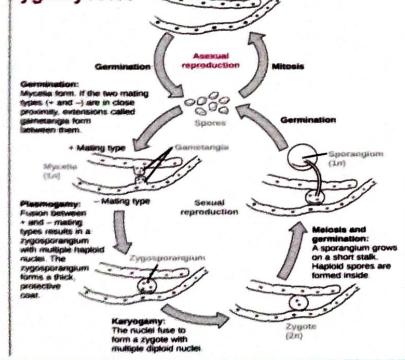


**2. Ascospore**  
Ex. *Aspergillus*

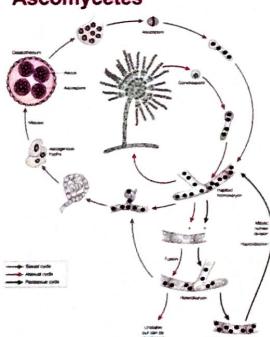


**3. Basidiospore**  
Ex. *Agaricus*

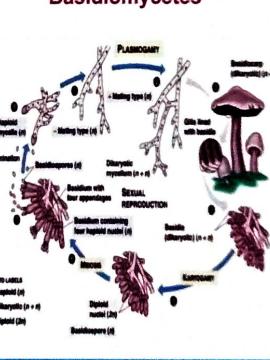
### Zygomycetes



### Ascomycetes



### Basidiomycetes



BATCH - 2015

**Ranveer Singh, Rohit Damor, Rohit Yadav**

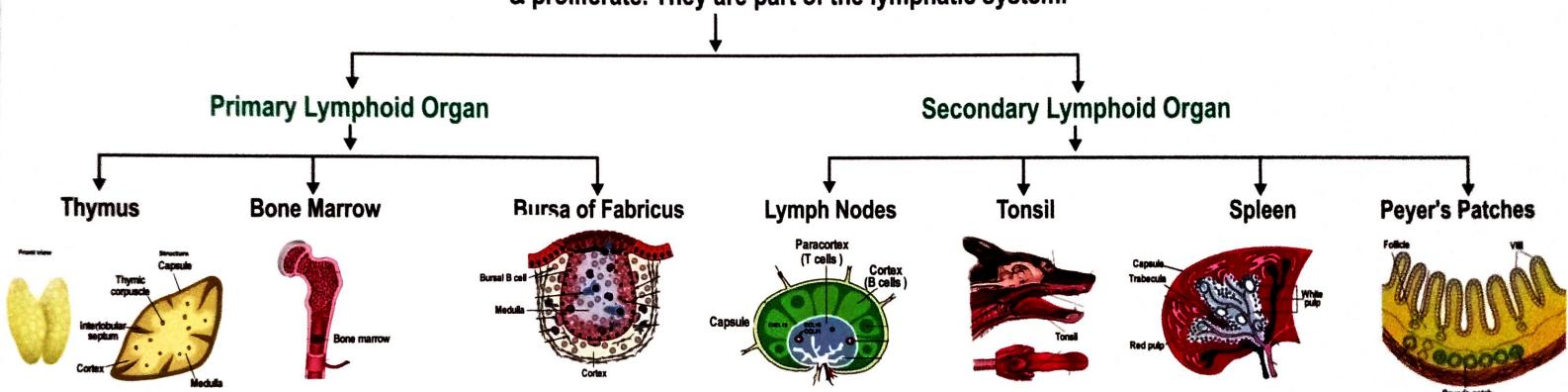


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## CLASSIFICATION OF LYMPHOID ORGANS

**LYMPHOID ORGANS :** Lymphoid organs are certain organs respectively, parts of tissues, in which lymphocyte can differentiate & proliferate. They are part of the lymphatic system.



Characteristics	Primary Lymphoid Organ	Secondary Lymphoid Organ
1. Origin	Endoderm	Mesoderm
2. Time of development	Early in embryonic life.	Late in foetal life.
3. Persistence	Involutes after puberty	Persists through adult life.
4. Effect of removal	Loss of lymphocytes & immune response	Minimal or no effects
5. Response to Antigen	Not dependent	Fully reactive
6. Examples	Bone Marrow, thymus, bursa of Febricus	Spleen, Tonsil, Lymph Nodes, Peyer's Patches

**BATCH - 2015 Rohit Damor, Rohit Yadav**

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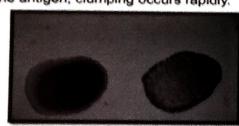
### Types Of Agglutination Reaction

#### Tube Agglutination Test

- Also known as the standard agglutination test or serum agglutination test (SAT)
  - Test serum is diluted in a series of tubes (doubling dilutions)
  - Constant defined amount of antigen is then added to each tube and tubes incubated for 20-37°C
  - Particular antigen clumps at the bottom of the test tube
  - Test is read at 50% agglutination
  - Quantitative
  - Example : Brucellosis screening, Widal Testing
- In this case, the titre is 1/40

#### Plate Agglutination Test

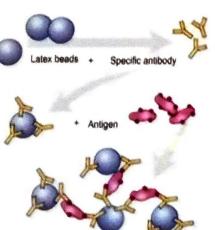
- This is rapid Qualitative agglutination test
- Here, a coloured antigen is used which facilitates the reading of the test
- Coloured antigen (*Brucella abortus*, *Salmonella pullorum*)
- It is commonly used as a screening test for the diagnosis of a disease and to determine immune status of a herd.
- If the test serum or blood contains specific antibodies to the antigen, clumping occurs rapidly.



#### Indirect Agglutination Test

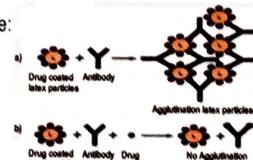
#### Latex Co-agglutination Test

- Antibody molecules can be bound to each latex beads
- It will increase the potential number of exposed antigen-binding sites.
- When an antigen is present in test specimen, it may bind to the latex bead thus forming visible cross-linked aggregates.



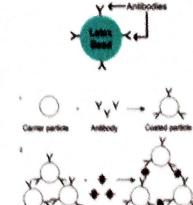
#### Passive Haemagglutination Test

- An agglutination reaction that employs particles that are coated with antigens not normally found in the cell surfaces
- Particle carriers include:
  - Red blood cells
  - Polystyrene latex
  - Bentonite
  - Charcoal



#### Reversed Passive Haemagglutination Test

- In reverse passive agglutination, antibody rather than antigen is attached to a carrier particle.
- The antibody must still be reactive and is joined in such a manner that the active sites are facing outward
- This type of testing is often used to detect microbial antigens
- Latex particle coated with Ab (known) + serum looking for particular Ag
- If Ag present, then visible agglutination is observed

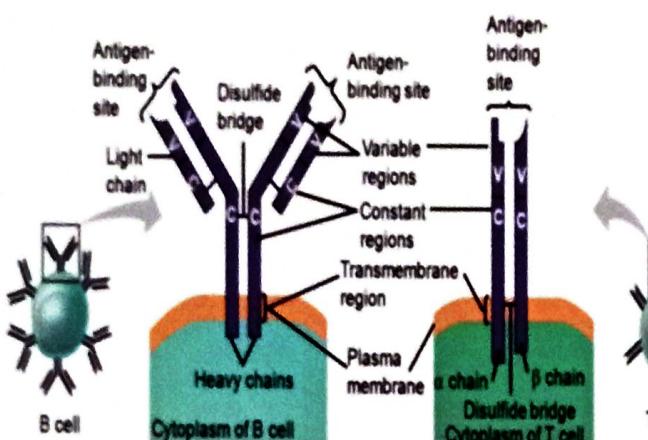


TCH - 2015

**Rajdeep, Rajkumar Meena**

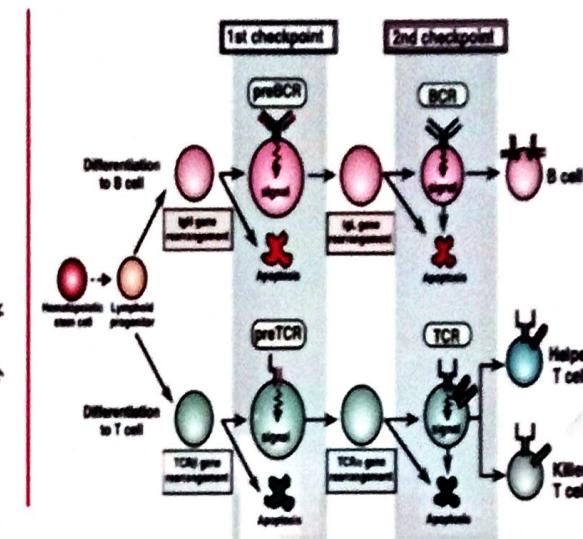


## DIFFERENCE BETWEEN T & B-CELLS



- (a) A B cell receptor consists of two identical heavy chains and two identical light chains linked by several disulfide bridges.
- 

- (b) A T cell receptor consists of one α chain and one β chain linked by a disulfide bridge.

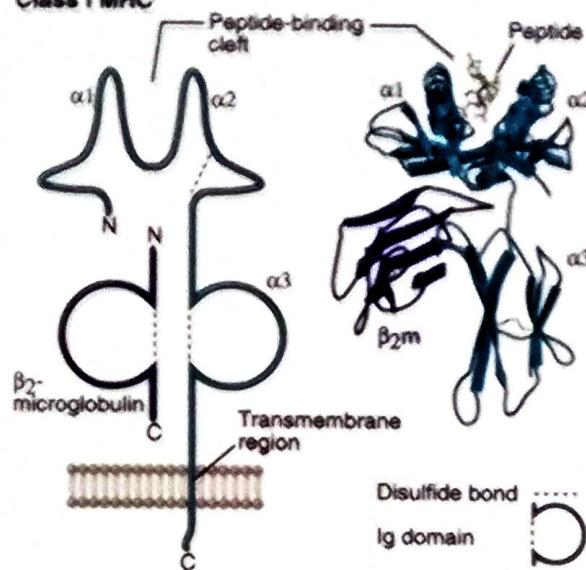


Sr. No.	Features	B cells	T cells
1.	<b>Origin</b>	Bone marrow	Bone marrow
2.	<b>Mature with</b>	Bursa, Bone marrow, Payer's patches	Thymus
3.	<b>Distribution</b>	Lymph node cortex, Splenic follicles	Lymph node -paracortex, Spleen -periarteriolar sheath
4.	<b>Circulate</b>	No	Yes
5.	<b>Life span</b>	Short (few days to few weeks)	Long (more than 1 year usually 6 months to 10 years)
6.	<b>Surface immunoglobulin</b>	Present (IgM, IgD, IgG)	Absent
7.	<b>Immunity type</b>	Humoral	Cell mediated
8.	<b>Secreted products</b>	Immunoglobulins	Cytokines
9.	<b>Response to mitogens</b>	Pokeweed, Lipopolysaccharides	Phytohaemagglutinin (PHA), Concanavalin-A, BCG Vaccine, Pokeweed
10.	<b>EAC Rosette formation</b>	Yes [ B cell bind to sheep RBC coated with antibody and complement due to (C3 receptor or CR2 on B cell surface)].	No
11.	<b>E or SRBC rosette formation</b>	No	Yes (T cell bind to sheep RBC due to CD2 antigen on T cell surface)
12.	<b>Production pathway</b>	Short	Long
13.	<b>Antigen uptake</b>	Can take up unprocessed antigen	Only processed antigen
14.	<b>Diversity</b>	No diversity in function	Diverse in function

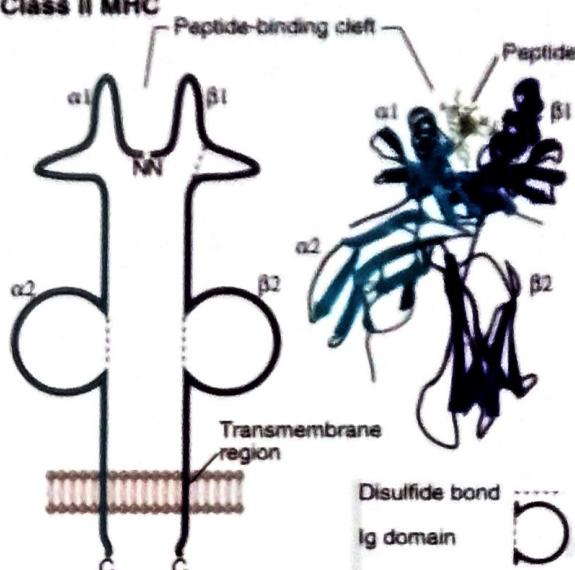


## Comparison of MHC Molecules

Class I MHC



Class II MHC



S.No.	Features	Class I MHC Molecule	Class II MHC Molecule
1.	Composition of stable peptide MHC complex	Polymorphic α chain, β <sub>2</sub> microglobulin, peptide	Polymorphic α and β chains, peptide
2.	Peptide-binding groove	α <sub>1</sub> /α <sub>2</sub>	α <sub>1</sub> /β <sub>1</sub>
3.	Nature of peptide-binding cleft	Closed at both ends	Open at both ends
4.	General size of bound peptides	8-10 amino acids	13-18 amino acids
5.	Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide	Anchor residues distributed along the length of the peptide
6.	Nature of bound peptide	Extended structure in which both ends interact with MHC cleft but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of MHC cleft
7.	Location	All nucleated cells	APC (dendritic cell, macrophage, B-lymphocyte)
8.	Responsive T cells	CD8 <sup>+</sup> T cells(Cytotoxic T cells)	CD4 <sup>+</sup> T cells(T helper)
9.	Antigen presentation	Endogenous antigen	Exogenous antigen
10.	Immunity against	Intracellular pathogen	Extracellular pathogen
11.	Source of protein antigens	Mainly cytosolic protein (usually synthesized in the cell; may enter cytosol from phagosomes) also nuclear and membrane proteins	Endosomal and lysosomal proteins (mostly internalized from extracellular environment)
12.	Enzymes responsible for peptide loading of MHC	Cytosolic proteasomes	Endosomal and lysosomal proteases (e.g. cathepsins)
13.	Site of peptide loading of MHC	Endoplasmic reticulum	Specialized vesicular compartment (class II vesicle, MHC class II compartment)
14.	Molecules involved in transport of peptides and loading of MHC molecules	Chaperones, Transport associated with antigen processing (TAP) in endoplasmic reticulum	Chaperones in endoplasmic reticulum, invariant chain in endoplasmic reticulum and class II vesicle, MHC class II compartment, Golgi apparatus

### **Class III MHC Molecules**

**Class III MHC molecules include several proteins with immune functions: components of the complement system(such as C2,C4 and B factor) cytokines(such as TNF-α,LTA and LTB) and heat shock proteins.**