STERILISATION

Sterilization is a process in which all viable life forms are either killed or removed.

Aseptic technique: Procedure that exclude the excess of viable microorganisms into the products.

D-Value or Decimal Reduction Time: Time in minutes at any defined temperature to destroy 90% of viable organism.

Z-value or Thermal Destruction Time: Number of degree of temperature change to produce a tenfold change in D-value.

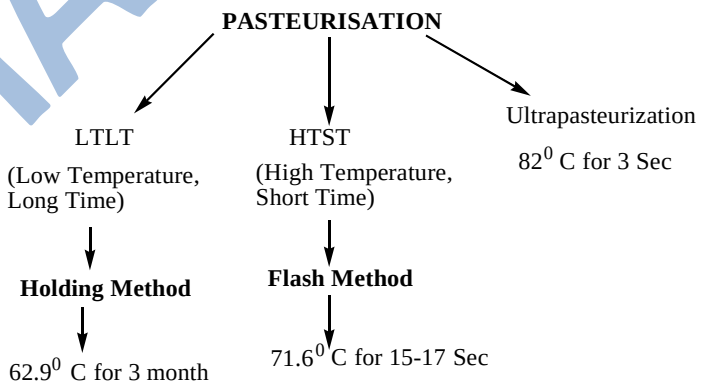
Holding and time for sterilization:

Wet heat:

118^0-121^0 C for 15-20 minute ´ Pressure 10 lb
124^0-127^0 C for 15 minute ´ Pressure 15 lb
126^0-129^0 C for 10 minute ´ Pressure 20 lb
134^0-138^0 C for 3 minute ´ Pressure 30 lb

Dry heat:

180^0 C ´ 30 minute
170^0 C ´ 1 hour
160^0 C ´ 2 hour
140^0 C ´ 3 hour



Tyndallization

Heating of material at **80^0 C for 3 successive days** for **20 minutes**.
Spores killed by **propiolactone, formaldehyde**
Ethylene oxide should be used with CO_2 or fluorocarbon due to its inflammability.
HEPA filters ´ Pore size ´ **0.3μm**.
Heated with bactericides at **80-90^0 C for ophthalmic preparations**, vaccines.
Preservative: **Benzalkonium chloride** ´ Not given in CSF and Spinal fluid.

Sterilization method	**Mechanism**	**Application**	**Validation**
Dry heat	Oxidation of proteins	Glassware, porcelain and metal equipments. Fats, oils, powder.	**Physical –** Temperature Recording Charts **Chemical –** Browne's the Bowie Dick heat sensitive tapes. Spores of *Bacillus Subtilis* and *Clostridium Sporogens*
Moist heat	Denaturation and coagulation of proteins	Aqueous solution and suspension. Surgical dressings, plastic and rubber closures. Metal instruments, Glass apparatus.	*Bacillus Stearethamoph* *Bacillus cogulans*
Ethylene oxide **Gaseous Sterilisation**	Alkylation of –SH, -NH, COOH, OH group of proteins	Surface sterilsation of powders, syringes, needles, catheters. Geiger Muller Counter	Chemical – Reyce Sac *B. subtilis* *Var. Niger*
Formaldehyde	Alkylating agent (Same as above)	Fumigation of empty rooms	
UV-rays (Non-ionizing radiations)	Nuclear protein damage by UV of 253.5 nm	Treatment of air in sterile areas and hospitals and thin layer of water.	**Chemical –** Dorimeters *Bacillus pumilus* *Bacillus Sphaerians*
Ionizing radiations γ rays	Denaturation of enzymes, DNA by excitation, ionization free radical formation	Plastic syringes, Catheters, Hypodermic needles, Catgut	*Micrococus radiofena*
Filteration **(Dessicators destroys the spore)**	Retention of bacteria	Thermolabile liquids and solutions, Antisera	Physical – **Bubble point pressure test** ***Pseudomonas diminut*** ***Serrata marcesens***

TEST FOR STERILITY

Test for sterility are based upon the principle if microorganisms are placed in a medium which provides nutritive material and water and kept at favorable temperature, the organism will grow and their pressure indicated by **Turbidity method**.

Culture media

1. **Selective media:** Permits only one type of microorganisms to grow and other type kills.

In Bile salts ´ Gram –ve to grow

In Thyoglycolate ´ Anaerobic bacteria to grow

2. **Differential media:** Not kills the microorganisms only there is different coloring. If we add cellulose then Bacteria which contain cellulose then white color of cellulose disappear.

3. **Simple media:** No idea about composition

Nutrient agar ´ Solid
Nutrient Broth ´ liquid
¾ **1.5% agar is used in culture media.**

4. **Synthetic/complex media:** Exact composition

Yeast extracts ´ Source of **vitamin B**
Beef extract ´ Source of **water soluble vitamins**
Silica ´ Used for **autotrophic bacteria**
Sodium Thioglycollate or Cysteine ´ for anaerobic bacteria

For Endospore ´ Malachite Green is used.
Archaebacteria ´ found under **high temperature, pH, Salt Concentration**
Glycolipids present in cell wall instead of peptidoglycan.

Method of culturing:

(i) Serial dilution method
(ii) Streaking method
(iii) By use of micromanipulator

(i) Roll tube technique: For culturing the anaerobic bacteria

(ii) Pore plate (For dilution agar medium is used) **or Spread plate technique** (Diluted with water or buffer)

Disadvantages

Mesophils and Psychrophils does not grow.

Maintainance and preservation method:

1. **Periodic culture**
2. **Subculturing**
3. **Cryopreservation:** Reduces metabolic activity. Liquid N_2 at -196^0 C, ethylene glycol, DMSO. Reduces metabolic activity.
4. **Freeze drying (Lyophillization):**

Vacuum generate

For lactobacillus

Mineral oil forms barrier and **O_2 supply is reduced**.

Metabolic activity is reduced.

Toxins

Exotoxin	Endotoxin
Released in culture media because of metabolic products.	Integral part of body.
Proteinaceous	Lipopolysacchrides
Due to proteinaceous nature at high temperature, exotoxin denatures	They can not be denaturated.
Dangerous: Neurotoxic, cardiotoxic	Mostly pyrogenic, Pyrogenic activity due to lipid A portion.
Easiy filterable, water soluble	Not filterable

Clostridium Prefinges
Clostridium Welichi
Clostridium Septicum
] Gas Gangrene

Methods of Cell Count

1. **Direct method**:

Petroff's Hauser counting chamber

Electronic particle chamber

2. **Indirect Method**

Colony count

Memrane filter count

Capsule stain ´ Muir's stain

Loeffeter stain ´ Simple stain

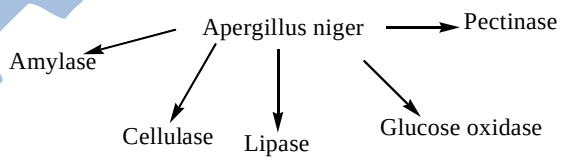
Volutin Containing ´ Albert's stain organism

On the basis of Cell Mass

1. **Direct method**:
   By direct weighing
   By measurement of N_2 of cell.
   Turbidometry

ANTIBIOTIC SOURCE

Bacitracin	Bacillus Subtilis
Polymyxin	Bacillus polymxa
Oxytetracycline	Streptomyces rimosus
Paramomycin	
Tetracycline	Streptomyces aureofacines
Chlortetracycline	
Demeclecycline	
Amphotericin B	Stretomyces nodosus
Chloramphenicol	Streptomyces venezule
Streptomycin	Streptomyces griseus
Neomycin	Streptomyces fradiae
Erythromycin	Streptomyces erythraeus
Bleomycin	Streptomyces verticulis
Framycetin	Stretomyces lavendulae
Tabramycin	Streptomyces tenebrarus
Kanamycin	Streptomyces Panamyceticus
Gentamicin	Micromonospora purpura
Greisiofulvin	Penicillium griseofulvin (Fungi)
Penicillin	Penicillium notatum (Fungi)
Cephalathin	Cephalosporium

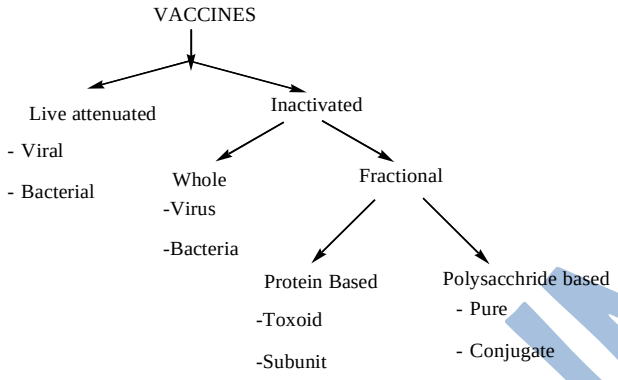


Leuconostoc mesentroides ´ Dextran Sucrose
Bacillus Subtilis ´ Penicillinase
Asperligus oryza ´ Protease
Streptococus pyrogens ´ Streptodornase
Pseudomonas Species ´ Vitamin B_{12}
Comeybacterium ´ Lysine
Glutamicum ´ Glutamic acid
Ashbya gossypii (fungi) ´ Riboflavin B_{12}

Episomes are genetic element attached to Bacterial Chromosome.

VACCINES

Vaccines have the pathogen like characteristics but not true pathogen characteristics to activate the Immune system without causing life threatening diseases.



Vaccines have:

Antigenic material (live attenuated, killed etc.)
Stabilizers (**Monosodium Glutamate**, **2 Phenoxy ethanol**)
Adjuvants (Increases immune response)
Preservatives (Prevents fungal and Bacterial growth). E.g. **Antibiotics, formalin, thiomersal**

Live attenuated vaccines are attenuated form of the wild Bacterium or Virus.
Must replicate to be effective.
Immune response similar to natural infection.
Usually effective with one dose.
E.g.

Viral	Bacterial
Measles, Mumps, Rubella, Vaccinia Varicella zoster, yellow fever, reovirus (Ds RNA) Intranasal Influenza, oral polio	BCG, Oral typhoid

Inactivated Vaccines – Can not replicate
Whole cell Vaccines – Not effective as live vaccine

Viral Polio, Hepatitis A, Rabies, Influenza - Less interference with live antibodies than live vaccines.
Bacetrial Dextrus, typhoid cholera, plague – Immune response mostly humoral

Functional Vaccine	Pure Polysacchride vaccine
Subunit: Hepatitis B, Influenza, Anthrax, Lyme, Pertusis.	- Not consistently immunogenic in children younger than 2 years of Age. - No booster response. - Antibody with less functional activity.
Toxoid: Diptheria, tetanus	-Immunogenicity improved by conjugate.

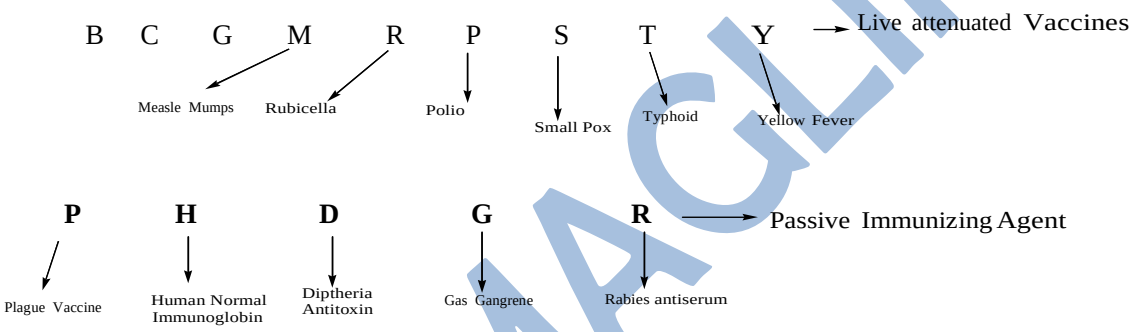
PURE POLYSACCHRIDE VACCINES

Pneumonococcal
Meningococcal
Salmonella Typhi

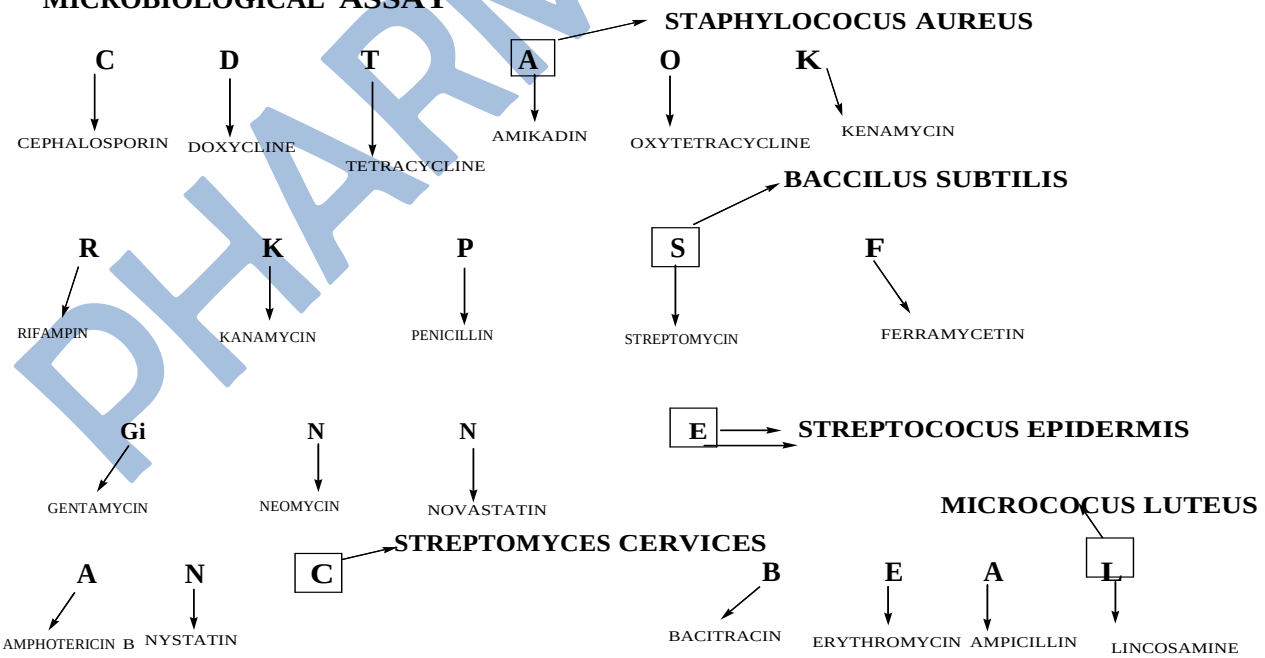
CONJUGATE POLYSACCHRIDE VACCINES

Haemophilus Influenza type B
Pneumonococcal
Meningococcal

SOME TECHNIQUES



MICROBIOLOGICAL ASSAY



BACITRACIN IS GIVEN TOPICALLY

MICROBILOGICAL ASSAYS

Antibiotic	Assay Method	Test Organism
Cephalosporin	B	Staphylococcus aureus
Doxycline	B	Staphylococcus aureus
Tetracycline	B	Staphylococcus aureus
Amikacin	B	Staphylococcus aureus
Oxytetracycine	B	Staphylococcus aureus
Kanamycin	B	Staphylococcus aureus
Rifampin	A	Bacillus Subtilis
Kanamycin	B	Bacillus Subtilis
Penicillin	B	Bacillus Subtilis
Streptomycin	A	Bacillus Subtilis
Gentamycin	A	Streptococus epidermis
Neomycin	A	Streptococus epidermis
Novosin	A	Streptococus epidermis
Amphetericin	A	Streptomyces cerevisiae
Nystatin	A	Streptomyces cerevisiae
Bacitracin	A	Micrococus luteus
Erythromycin	A	Micrococus luteus
Ampicillin	A	Micrococus luteus
Lincosamide	A	Micrococus luteus
Bleomycin	A	Mycobacterium semgmatis
Carbencillin	A	Pseudomonas aeruginosa

KANAMYCIN SULPHATE
TRAMYCETIN
BACILLUS SUBTILIS
BACILLUS PULMILIS

TETRACYCLIN
OXYTETRACYCLINE
Bacillus cereus
Staphylococus aureus

POLYMYXIN B - Bortedella bronchioseptica

Microbiological assay involve the measurement of **relative potency** of compounds by determining amount of compound required to produce an suitable effect on suitable microorganism under standard condition.

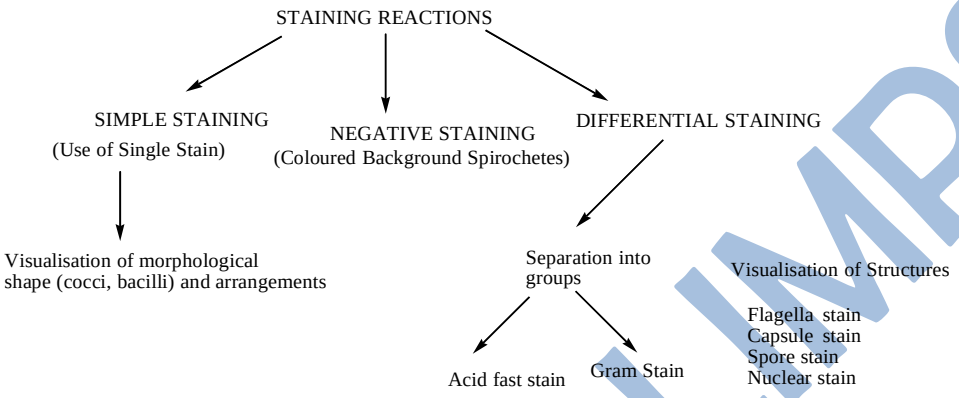
It is based upon comparison of inhibition of growth of bacteria by test concentration with standard concentration of the antibiotic.

Method (A)/Cylinder Plate/ Cup Plate method:

In this method, antibiotic solution is filled into a vertical cylinder or cavity cut through a solidified agar plate. The antibiotic diffuses throughout the agar layer and inhibits the growth of added microorganism resulting in a circular area or zone of inhibition around the cavity. Zone of inhibition is produced by test concentration is compared with standard concentration of antibiotic.

Method B/Turbidimetric/Tube assay method:

In this method, the fluid media containing antibiotic is inoculated with specified microorganism and incubated, the growth of microbial culture results in turbidity. The turbidity produced by the test concentration of antibiotic is compared with that produced by standard concentration of antibiotic.



Simple staining

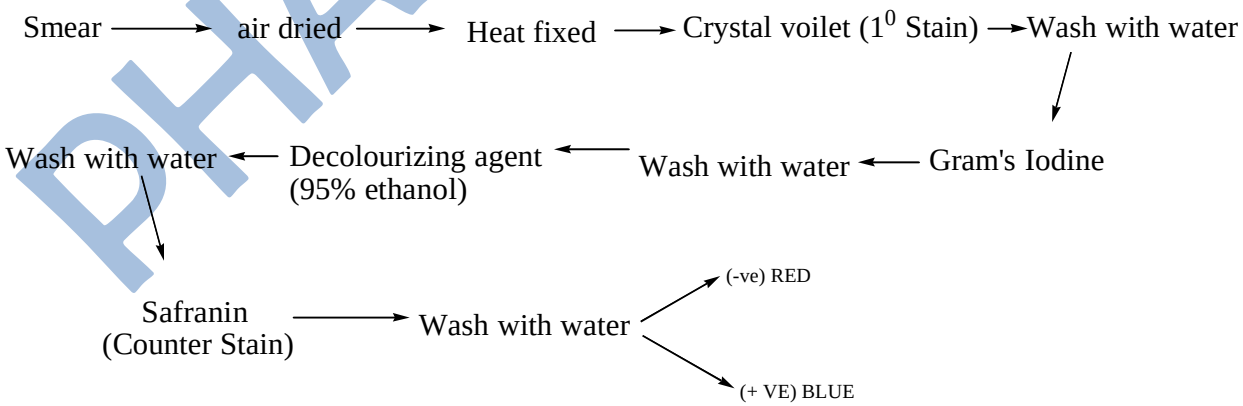
Smear → air dried → Heat Fixed → Basic Stain (Methylene blue) → Wash with water → Observed under microsope

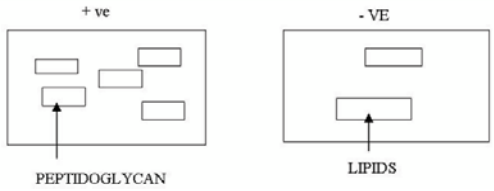
AIDS is caused by HTLV – III.

Negative Staining:

Acidic stain such as **eosin or nigrosin** is used. These stains donot penetrate the cells because of negative charge on the surface. The unstained cell can be observed in their natural size and shape against coloured background. In this staining, Smear is not heat fixed.

Gram Staining:



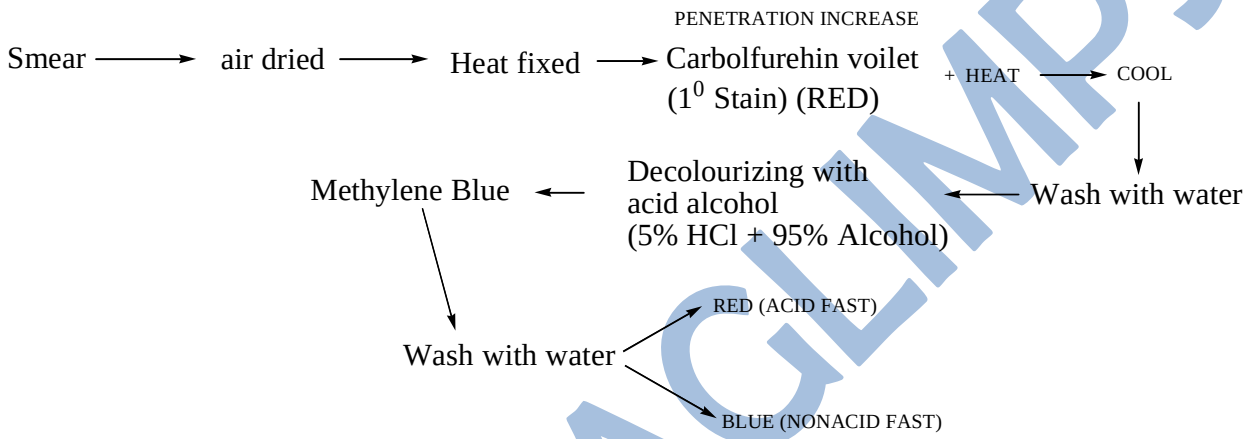


Due to ethanol, lipid extracts. So pore formation takes place. So 2^0 dye enters and gives Red/Pink Colour.

Iodine acts as moderant (to form complex so dye cannot leaks out)

Acid Fast/Zeil-Nelson Staining:

Used for mycobacterium species ´ Waxes like substance present ´ Mycolic acid



ACID FAST STAINING IS DUE TO MYCOLIC ACID

Cell wall of Bacteria is made up of **Peptidoglycan** (meurin). It is composed of **N-acetyl muramic acid, N-acetyl Glucosamine.**

In Gram + ve ´ 80-90%.

In Gram –ve ´ 10-20%

Mycoplasma, PPLO ´ Lacks cell wall

In Gram +ve – Techoic acid present

In Gram –Ve – Techoic acid absent

Spores mostly produced by *Bacillus clostridium*. Spores are composed of **dipicolic acid** and their Ca^{2+} salts.

1 microorganism gives only one endospore but 1 microorganism gives many exospores. Exospores are environment resistant. Endospores are present inside bacteria and release after death of bacteria. **Fungi produces exospores**.

Capsule/Pilli are made up of **Glycopolysacchrides** or **Glycoproteins** and antigenic in nature.

Pilli causes Haemoglutination Reaction (Clumping of RBC).

Chaperons are the proteins involved in folding of protein during unfavourable condition.

Phase contrast microscope for live organisms.

Hanging drop method: To find out motility or find out flagella present or not.

Scanning Electron microscopy: Surface properties/Topography of microorganisms

In electron microscopy, the specimen have made to be conductive. So **Osmium tetrachloride** or **Gold** used for coating so conducting occurs.

Bacteria are –ve charged cell wall: So for visualization of morphological shape of microorganism. So +ve charged Basic dye used Methylene blue, crystal violet.

Negative staining ´ Background is **colored**. Negrosin, Indian Ink (-ve charged) & **used for spirochetes**.

Giemesa's stain is used for staining of malarial parasites.

Toxoids are made up of Exotoxins.

Rickettsia is Gram –ve, Plasmorphic, small cocal bacilli but do not grow in non-living media.

IDENTIFICATION OF MICROBES ON THE BASIS OF THEIR NUTRITIONAL REQUIREMENTS

Type	Energy Source	Carbon Source	Examples
Photoautotrophs	Light	CO_2	Phosphosynthetic bacteria, cyanobacteria, algae
Photoheterotroph	Light	Organic compound	Purple non-sulphur and Green non-sulfur bacteria
Chemoautotroph	Electrons from inorganic comounds	CO_2	H,S, Fe and Nitrifying bacteria
Chemoheterotroph	Electrons	organic compounds	Mostly bacteria, Fungi, Protozoans.

OBLIGATE AEROBES: Requires O_2 for Growth

OBLIGATE ANAEROBES: Do not required O_2. E.g. Clostridium species.

FACULTATIVE AEROBES: Mainly anaerobes

FACULTATIVE ANAEROBES: Mainly aerobes (E.coli, clostridium sporogenes)

MICROAEROPHILE: Require very low concentration of oxygen.

Anaerobes have absence of **3 enzymes** which are present in aerobes.

(i) **Superoxide demutase**: Eliminate Superoxide radical.

(ii) **Catalase:** Breakdown of H_2O_2 ´ $H_2O + O_2$

(iii) **Peroxidase:** H_2O_2 + Reduced substance ´ H_2O + Oxidised substance

So free radicals produced in anaerobes in O_2 environment. So anaerobes dies.

¾ Optimum pH for bacterial growth is 6.5-7.5.

¾ Bacteria can't tolerate salt but **fungi tolerates salt**.

Psychrophiles: Below 25^0 C (vibrio species)

Mesophiles: 25-45^0C

Thermophiles: Optimum 55-65^0C. Thermophilus.

Acidophilic: Tolerate high acidic condition (Lactobacilli)

Basophilic: Tolerate Alkaline condition (*Vibrio cholera*e)

Toxoids are manufactured from Bacterial Toxins:

Some bacterial pathogens such as diphtheria, tetanus produces exotoxins. Diptheria, tetanus toxoids are made by purifying the bacterial exotoxin and then inactivating the toxin with formalin to form toxoid.

Bacterial Polysaccharides capsule are used as vaccines:

Virulence of some pathogenic bacteria depends upon antiphagocytic properties of their hydrophilic polysaccharide capsule. Coating of capsule with antibodies greatly increases the ability of macrophages and Neutrophils to phagocytose pathogens.

E.g. Neisseria meningitides, Streptococus pneumonia

Inactivated whole virus vaccine:

The outer virion coat should be left intact but replicative function should be destroyed. Non-replicating virus contains much more antigens than live vaccines that are able to replicate in the host. Preparation of killed vaccines may take the route of heat or chemicals. Chemical used include formaldehyde, β-propiolactone. Excessive treatment can destroy immunogenicity.

Subunit Vaccine:

It is now possible to produce non-replicating vaccines by identifying the **peptide sites** responsible for the major antigenic site of viral antigens from which highly purified subunit vaccines can be produced. **Increased purification may lead** to **loss of immunogenicity**. So this may necessitate coupling to an immunogenic carrier protein or adjuvant such as aluminium salts. E.g. Vaccines for Influenza A and B.

Recombinant Vector Vaccine:

In this, uses attenuated virus or bacteria as vector to express major antigens of virulent pathogens like proteins of capsid. Attenuated organism replicate with the host and expresses gene product of the pathogen vaccinia virus, adenovirus, certain strains of *Streptococous* induces both type of immune response.

Conjugate Vaccine:

Virulence of some pathogenic bacteria depends on polysaccharides can be used as vaccines, but these capsular polysaccharides antigens are coupled with to protein carrier to give conjugate vaccines which then stimulate T-cell as T-cell activation only needs proteins, generally small peptides but above component is polysaccharides *H.influenza.*

DNA Vaccines:

Plasmid DNA encoding antigenic protein injected directly into muscle of recipient. Encoded protein antigen is expressed in muscle activation humoral and cell mediated resistance.

IMMUNOLOGICAL PRODUCTS

Name	Nature	Category
BCG Vaccine (Bacillus Calmette Guerin) Freeze dried	Live attenuated bacterial vaccine of a strain of Bacillus of Calmetted and Guerin	Active Immunizing agent
Cholera	Killed bacterial vaccine having strain of **Inaba** and **Ogama**	Active
Diptheria antitoxin	Antitoxic globulin or its derivative obtained by purification of hyperimmune serum or Plasma of healthy horses and capable of neutralizing toxin produced by Corybacterium diptheriae. Liquid or freeze dried powder.	Passive
Diptheria and tetanus vaccine toxoid (adsorbed)	Purified formal prepared by neutralizing toxins of *C. diptheriae* and Clostridium tetani with formalin adsorbed on $Al(OH)_3$ or Aluminium phosphate or calcium phosphate. Sterile suspension, whitish turbid liquid	Active
Diptheria, Tetanus Pertusis Vaccine (Adsorbed)	Purified diphtheria and tetanus formal toxoid plus killed Bortedella pertusis adsorbed on aluminium or calcium	Active

	phosphate.	
Hepatitis B Virus Vaccine (Inactivated)	Non infectious, formalin inactivated, adjuvant viral vaccine derived from surface antigen of Hepatitis B-virus. Harvested and Purified from plasma of Human Hepatitis B carriers.	Both Active
Recombinant	Non infectious viral vaccine containing the purified hepatitis B surface antigen produced by genetically enginnered yeast cell carrying the gene for antigen absorbed on aluminium hydroxide.	
Human normal Immunoglobulin	Sterile solution or freeze dried products containing Immunoglobulin mainly IgG, obtained from plasma, serum or frozen placenta from healthy donor.	Passive
Gas gangrene Antitoxin -Odematiens -Perfinges -Septicum -Mixed	Specific antitoxic globulins obtained by purification of Hyperimmune serum of Horses and have specific activity of neutralizing the α-toxin formed by -clostridium oedematiens -Clostridium prefringes -Clostridium Septicum - Mixture of all the above	Active
Japanese encephalitis vaccine	Freeze dried product containing inactivated, Japanese encephalitis vaccine Produced by Inoculating virus intracerebrally in Healthy mice, harvesting and inactivating by formalin.	Active
Measles Vaccine	Freeze dried product containing live attenuated measles virus grown in culture of chick embryo	Active
Plaque vaccine	Sterilized suspension of killed Yeserinia pestis	Passive
Rabies antiserum	Antirabies globulin derived from serum or plasma of hyperimmune horses.	Passive
Rabies Vaccine Human (cell culture)	Freeze dried product by culturing, strains of rabies virus. Harvested Inactivated by β-propionlactone.	Active
Rabies Vaccine Human (Neural tissue)	Sterile suspension of inactivated rabies virus in brain tissue prepared by injecting intracerebrally into sheep, rat, mice, rabbit. Harvesting and Inactivated by Phenol and β-propiolactone.	Active
(a) Shick Test Toxin	Sterile filterate containing diphtheria toxin, obtained from culture of toxigenic. Strains of cornybacterium diptheriae in liquid	Dermal reactivity Indicator (Diagnostic and susceptibility of diphtheria)

(b) Shick control	media. Shick toxin heated at a temperature 70-80^{0}C for not less than 5 minutes.	
Scorpion Venom Antiserum	Sterile liquid or freeze dried product containing antitoxic Globulin or their derivative capable of neutralizing venom of scorpion. Obtained from serum or plasma of healthy horses immunized against *Scorpion venom.*	
Snake venom antiserum	Sterile liquid or freeze dried product containing antitoxic. Globulin or their derivative capable of neutralizing the venom of one or species of snake obtained from plasma or serum of healthy horses immunized against specific species.	

BIOCONVERSIONS

It is a **chemical modification of organic compound** by modification of organic compound by use of microorganism.

Applications:

Selective Introduction of Functional groups at certain portion which are not attacked by chemical agents. E.g. 11 α- hydroxylation of progesterone by Rhizopus nigricans or R. arrhizus.

Regioselective conversions of functional groups: e.g. Selective dehydrogenation of Hydroxy at C-2 positon of D-sorbitol to produce L-Sorbose by Acetobacter Suboxydans.

Racemic modification: Production of L-aminoacid by acyl-DL-amino acids by enzyme L-amino acylase (from Asperligus oryzae) which hydrolyze only the L-enantiomer of the substrate.

Chiral Biotransformation:

- Bioconversion of Fumaric acid to L-aspartic acid by asymmetric addition of NH_3 by *E.Coli*.
- Bioconversion of Fumaric acid to L-Maleic acid by addition of water by *Braevibacterium flavum.*

Pertusis´ Killed bacterial vaccine

Disease or Pathogen	Type of Vaccine
Bacterial Cells	
Anthrax	Inactivated
Cholera	Inactivated
Plaque	Inactivated
Tuberculosis	Live attenuated
Typhoid	Live attenuated
Virus Particles	
Hepatitis A	Inactivated

Influenza	Inactivated
Measels	Live attenuated
Mumps	Live attenuated
Polio (Sabin)	Live attenuated
Polio (Salt)	Inactivated
Rabies	Inactivated
Retrovirus	Live attenuated
Rubecella	Inactivated
Yaricella zoster	Live attenuated
Yellow fever	Live attenuated
Small pox	Live attenuated

Toxoids

Diptheria, Tetanus — Inactivated Exotoxin

Capsular Polysacchrides

Haemophilus Influenza	Polysacchride + Protein Carrier
Neisseria Meningitsidis	Polysacchrides
Streptococus Pneumonac	23 distinct capsular polysacchrides

Surface antigen

Hepatitis B Recombinant Surface antigen (HBsAg)

Passive immunizing agents

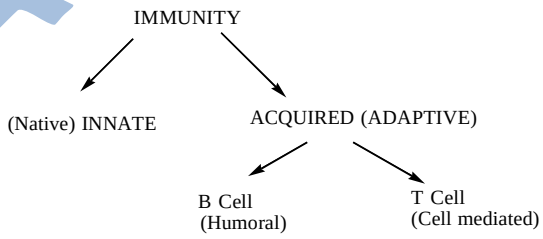
Gas- Gangrene antitoxin, Hummoral Immunoglobulin, Plaque vaccine, Rabies antisera

SEROLOGICAL TESTS

Ducrey Test	Haemophilia
Vaqden-berg test	Hepatitis
Widal's test	Typhoid
VDRL Test	Syphilis
Kahn's (tube flocculation) test	Syphilis
Wassermann (Complement fixation) test	Syphilis
Mantaux's test & Tuberculin test	Tuberculosis
Western Test	AIDS (Diagnosis)
ELISA	AIDS
FUJI	AIDS
Frie's Test	Lymphogranuloma venerum
Ouch tertony test	Small pox
Schick's test	Scarlet fever
Schultz Charlatan test	Scarlet fever
Radial immunodiffusion	Influenza virus
Radial Immunoassay	HCG in pregnant woman for pregnancy

	detection
Cold Haemegglunation test	Pneumonia
Dick's test	Diphtheria
Coomb's test	Brucellosis
Rosewater test	Rheumatoid arthritis
Lepromine test	Leprosy
Shick's test	Susceptibility of Diptheria
Van-den Berghn Test	Serum Bilitubin
O-toludine test	Glucose
Gerhardia test	Acetoacetate in Urine
Mureoxide test	Uric acid
Jeff's Test	Creatinine
Rothra's test	Ketone bodies
Saliwonoffi's test	Ketone bodies
Corr-price test	Vitamin A
Heller Nitric acid ring test	Proteins
Sulfo salicylic acid	Proteins
Heat coagulation test	Proteins
Biuret test and urease test	Urea
Salkowski test	Steroids
Libermann's Buchard test	Cholesterol
Mac-clean test & Hapkinis test	Lactic acid
CNBr	Nicotinic acid
Benzidine test	Bile pigments
Sakaguchi test	Urobie (Jaundice)
Raybin's test	Sucrose
Molish test	1^0 test for carbohydrates
Hay's test	Bile salts

IMMUNITY



- B-cells proliferate and mature in bone marrow.
- T-cell proliferate in Bone marrow but mature in thymus gland.

INNATE IMMUNITY/NATURAL IMMUNITY/NATIVE IMMUNITY

- It consists of cellular and biochemical defense that are present even before infection.
- It provides first line of dense against infection.

- It is non-specific but recognizes certain pattern like lipopolysacchrides in Gram –ve and telchoic acid in Gram +ve bacteria, double stranded replicating virus, N-formylmethionine in bacterial proteins.
- It recognizes microbial products that are often essential for microbial survival. E.g. double stranded RNA, complex microbial lipids.

COMPONENTS OF THE INNATE IMMUNE SYSTEM

- **Physical and chemical barriers** (epithelial barrier and antimicrobial substance produced at epithelial surface).
- **Circulating effector cells:** Phagocytes (Neutrophils, macrophages), NK cells. Neutrophils kills microbes by forming radicals like H_2O_2, O_2^-, OH^- due to absence of superoxide mutase, catalase, peroxidase enzymes.
- **Circulating effector proteins** (complement system, mediators of inflammation).
- **Cytokines** (Cell to cell communication proteins).

Natural Killer Cells: These are subsets of lymphocytes but are neither B or T-lymphocytes. These are first line of dense against viruses and some intracellular microbes. They mostly kill the tumour cells as they have very low expression of MHC – I which isgenerally high in case of normal cells.

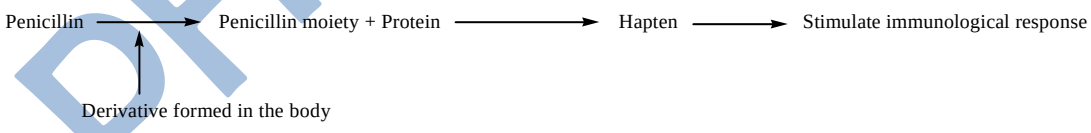
Neutrophils: They uses both oxygen dependent and oxygen independent pathways to generate antimicrobial substances. Neutrophils exhibit a larger respiratory burst than macrophages and consequently are able to generate more reactive oxygen intermediates and reactive nitrogen intermediates. Neutrophils have high level of defense than macrophages.

ADAPTIVE IMMUNITY/SPECIFIC IMMUNITY/ACQUIRED IMMUNITY

Immunity develops as a response to infection and adapts to the infection. It increases in magnitude and capabilities with repeated exposure to microbes.

Antigen: Protein or peptide substance (mostly made up of G-10 Amino acids). Antigen may or may not have immune response but immunogen must have immune response.

HAPTEN:



Complement System: C_7-C_9 protein formed in liver

- Forms membrane attacking complex
- Causes Obsonization (tagging)
- Causes Anaphylactic
- Exhibits Chemotaxis mechanism (cell calling)

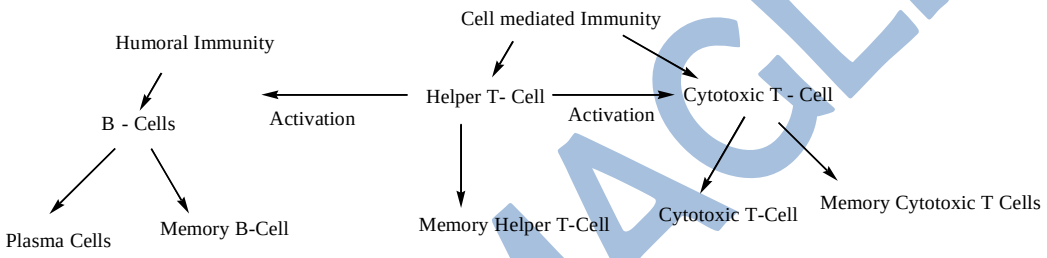
TYPE S OF ADAPTIVE IMMUNITY

(A) Humoral Immunity:

- It mediates by antibodies in the blood and mucosa secretion. B-lymphocytes produces antibodies.
- Antibodies are produced by Gamma globins which can be separated from albumins by centrifugation.
- Antibodies recognizes antigens and target for elimination by various effector mechanism.
- Principal defense against extracellular microbes and their toxins.

(B) Cell Mediated Immunity:

- It is mediated by T-lymphocytes (T_H and T_C) principle defense against intracellular microbes (virus and some bacteria).
- Destroys microbial infected cell by T_H and Tumar Cells by T_C.
- **T Cells** only recognizes the **antigen presented by MHC molecules**.



Primary (central) Lymphoid Organs:

They provides microenvironment for development of Lymphocytes e.g. Bone marrow, Thymus.

Secondary (Peripheral) Lymphoid organs:

Site of interaction of antigen with mature lymphocytes e.g. Lymph nodes, spleen, mucosa associated lymphoid tissue and cutaneous associated Lymphoid tissue.

Active Immunity: Generated in host by exposure to foreign antigen.

Passive Immunity: Generated by transferring serum, antitoxin, monoclonal antibodies and lymphocytes from a specified immunization to recipient.

E.g. Maternal antibodies to fetus, anti-tetanus.

It contains preformed antibodies rather than active production of antibodies after exposure to antigen.

PASSIVE IMMUNITY ACQUIRED THROUGH

- Natural material antibody
- Immune globulin
- Humanized monoclonal antibody
- Antitoxin

Increased Neutrophils	Bacterial infection
Increased basophils	Leukemia
Increased Eosinophils	Allergic reaction
Increased monocytes	Leukemia and TB

Increased lymphocytes	In Viral infection
Decreased Lymphocytes	In AIDS

Active Immunity Acquired Through:

- Natural Infection
- Vaccines
- Attenuated Organisms
- Inactivated Organisms
- Purified microbial macromolecules
- Cloned microbial antigen (as cloned DNA alone or in virus vectors) expressed as Recombinant protein.
- Multivalent complex
- Toxoids
- **Granulocytes:** Neutrophils, Eosinophils, Basophils
- **Agranulocytes:** Monocytes, Lymphocytes

Common agents of Passive Immunization

Disease	Agent
Black widow spider Bite	Horse antivenum
Botulism	Horse antitoxin
Diphtheria	Horse antitoxin
Hepatitis	Pooled Human immune γ-globulin
Measles	Pooled Human immune γ-globulin
Rabies	Pooled Human immune γ-globulin
Respiratory Disease	Monoclonal anti Respiratory Syncytical Virus
Snake Bite	Horse antivenum
Tetanus	Pooled Human immune γ-globulin or Horse antitoxin

Antitoxin: It is an antibody with the ability to neutralize a specific toxin. These are produced by plants, animals, bacteria. They also kills bacteria and other microorganisms. **Antitoxins are made within organisms,** but can be injected into other organisms (Human). Then, the animals body makes the antibodies needed to neutralize the toxin. Later, the blood is withdrawn from the animal. When the antitoxin is produced from the blood, it is purified and injected into a human or other animal for inducing passive immunity. To prevent serum sickness, it is often best to use antitoxin generated from the same speices (i.e. use human antitoxin to treat humans).

Antiserum: It is a **blood serum containing polyclonal antibodies**. **Antibodies in the antiserum** bind the **infectious agent or Antigen**. The immune system then recognizes foreign substances bound to antibodies and then triggers the human response.

The use of antiserum is particularly effective against pathogens which are capable of evading the Immune response in un-stimulated state but which are not strong enough to evade (escape out) the stimulated Immune system.

Toxoid: It is a **Bacterial toxin** (usually an antitoxin) whose toxicity has been weakened or suppressed either by chemical (formalin, β-propionlactone) or heat treatment, while immunogenic property are maintained.

Toxoids are used as vaccines as they induce an immune response to the original toxin or increase the response to another toxin. E.g. DPT toxin.

Immunogenicity: Ability to induce immune response.

It is determined by four properties of Immunogen:

- Its foreigness.
- Molecular size
- Chemical composition
- Complexity and ability to be processed and presented with an MHC molecule on the surface of an antigen presenting cell.

Antigenicity: Ability to bind with secreted antibodies or surface receptors.

- All immunogen are antigen but not vice versa.
- Many small molecules can binds to antibodies but can not active β-cells on their own. E.g. Haptens

Adjuvants: These are the substances, when mixed with an antigen and injected with it, enhances immunogenicity of that antigen. These are often used when an antigen has low immunogenicity or when only small amount of antigens are available.

For example: Antibody response of mice to immunization with BSA can be increased 5 times if BSA is adjusted with alum.

Aluminium Potassium Sulfate (alum) prolongs the presistance of antigen. Injection of this alum precipitate results in slower release of antigen from the injection site, so that the effective time of exposure to the antigen increases.

W/O emulsion adjuvants also prolongs the persistence of antigen. Freund's incomplete adjuvants contains antigens in aqueous solution, mineral oil, an emulsifying agent such as mannide monoleate which depresses the oil into small droplet surrounding the antigen.

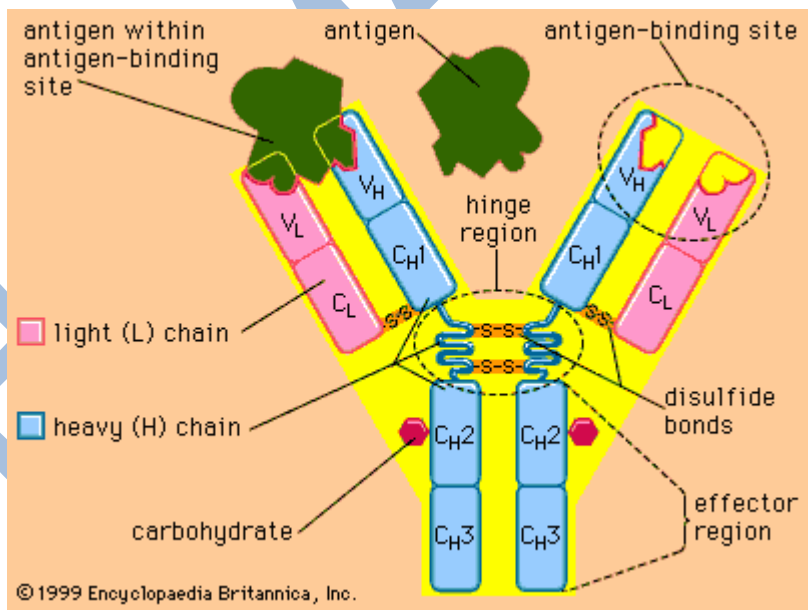
The antigen is then released very slowly, from the site of Injection. This preparation is based on **Freund's complete adjuvant.** The first containing heat killed mycobacterial as an additional ingredient. Muramyl dipeptide, a component of bacterial cell wall activates macrophages, making Freund's complete adjuvant for more potent than incomplete form.

Haptens: These are antigenic but not immunogenic i.e. these are small molecules that can binds to the antibodies but can not themselves induces immune response. However, the conjugate formed by coupling a hapten to large carrier protein is immunogenic and starts production of anti-hapten antibodies when injected into an animal.

- In the body, the formation of Hapten-carrier conjugates is the basis of allergic response to drugs such as penicillin.
  Pencilloyl protein behaves as an hapten-carrier conjugate, with the pencilloyl group acting as Haptenic epitope.
- Streptomycin, Aspirin, Some sulfonamides, some anesthetics (Succinyl choline), opiates are some another examples.
- All of these molecules first react with protein to form drug protein derivatives. After this, immune system will produce an anti-hapten response to the drug.
- Drugs that are incapable of forming drug-protein conjugates rarely elicits allergic response.

AVIDITY: Strength of multiple interactions called avidity. IgM has maximum avidity because it is pentameric so each arum can bind with two antigen simultaneously.

ANTIBODIES: These are part of humoral immunity. They have molecular weight 100000-900000. These are protein made up of 2 heavy and 2 light chains. B-lymphocytes produces antibodies. They are produced and matured in Bone marrow. Both H and L chains consists of N-terminal **variable (V) regions** and **Constant (C) regions**. V region participate in antigen recognization and provides diversity. **H chain C Chain mediates** effector functions also **anchor** antibody molecules on B-cells. On recognization, antigen divides and differentiate into the memory B-cell and antibody secreting plasma cells.



The four-chain structure of an antibody, or immunoglobulin, molecule

The basic unit is composed of two identical light (L) chains and two identical heavy (H) chains, which are held together by disulfide bonds to form a flexible Y shape. Each chain is composed of a variable (V) region and a constant (C) region.

- Fab fragments had antigen binding activity [fragment, antigen binding].
- Fc fragment [Fragment, crystalizable] No antigen binding activity because it was found to crystallize during storage. When antibody is digested with different enzymes then following fragments are obtained:

  IgG + Papain → Fab fragment + Fc fragment

  IgG + Pepsin → $F(ab)_2$ fragment + Degraded peptides.

  IgG + Mercaptoethanol → 2 H + 2 L chains

  IgG → Most abundant in serum. It crosses the placenta and plays and important role in developing foetus. Having life span (22 days).

  IgM → Largest molecular weight antibody. IgM is a monomer but secrete IgM in serum is a pentamer.

  - It contains J (Joining) chain attached to µ-chain needed for polymerization.
  - Firstly Ig produces in primary response to an antigen.
  - Because of its high valency, pentameric IgM is more efficient.
  - When RBCs are incubated with specific antibody, they clump together into large aggregates in a process called agglutination.
  - It is the first isotype produced by neonates and during 1^0 response.
  - It activates complement, system maximally.
  - It has maximum avidity.

IgA: Predominant Ig in external secretion like external secretion such as breast milk, saliva, tears and mucus of bronchial, genitourinary, digestive tracts. It also has a secretory component. IgA exists as a monomer, dimer, trimer, tetramer.

IgG, IgE and IgD always exist as monomers.

Membrane bound IgM is a monomer, but secreted IgM in serum is a pentamer.

IgE → Involved in the inflammation and have shortest half-life (2.5 days).

IgE antibodies medicates hypersensitivity reactions that are responsible for the symptoms of Hay fever, asthma, anaphylactic shock (vasodilation due to release of Histamine).

Different antibodies are separated by Size exclusion chromatography.

Cell Mediated Immunity

- T-lymphocytes are produced in bone marrow but mature in thymus while B cells are produced and mature in bone marrow.
- Antigen binding receptor on the membrane is called T-cell receptor. They recognizes only peptide antigen presented by MHC protein of other cells.
- MHC first proesses the larger protein into smaller peptides and prevents the process peptides to T-cell receptor.
- MHC molecules-p are polymeric membrane Glycoproteins.
- MHC are highly polymeric i.e. vary individual to individual.

- MHC is the main cause for Graft rejection.
- Class I MHC (all nucleated cells)
- Class II MHC (Only by antigen presenting cells)
- Subpopulations: T_H and T_C.
- T_H cells bears CD_4 while T_C cells bears CD_8 membrane, Glycoprotein receptor. T_H recognizes the peptides presented by MHC II, while T_C recognizes the peptides presented by MHC – I.

T_H ´ CD_4 ´ MHC II

T_C ´ CD_8 ´ MHC I

HYPERSENSITIVITY

An abnormal response to antigens

1. **Type I (Anaphylactic) Reactions**

- Occurs with in minutes of exposure to antigen
- Antigens combines with IgE antibodies.
- IgE Binds to most cells and Basophils causing them to undergo degranulation and release several mediators:

Histamine: Dilates and increases permeability of Blood vessels, increases mucus secretion, smooth muscle contriction.

Prostaglandins: Contraction of smooth muscle of Respiratory system and increases mucus secretion.

Leukotrienes: Bronchial spasms

- Massive drop in Blood pressure.

Type II (CYTOTOXIC) REACTIONS:

- Involvement activation of complement by IgG or IgM binding to an antigenic cell. Antigenic cell is lysed.
- E.g. Transfusion reactions. Incompatible donor are lysed as they enter blood stream.
- E.g. Rh system: 85% of population is Rh +ve. Those who are Rh –ve can sensitized to destroy Rh positive blood cells.
- E.g. Haemolytic disease of Newborn: Fetal Cells are destroyed by maternal anti-Rh antibodies that cross the placenta.

TYPE III (IMMUNE COMPLEX) REACTIONS:

- Involves reaction against soluble antigens circulating in serum usually involves IgA antibodies.
- Antibody-Antigen Immune complexes are deposited in organs, active complement and causes Inflammatory kidney damage.
- Glomerulonephritis: Inflammatory kidney damage.
- Rheumatoid arthritis, systemic lupus erythematosus

TYPE IV (Cell Mediated) Reactions:

Reactions are delayed by one or more days (delayed type hypersensitivity). Delay is due to migration of macrophages and T-cells to site of foreign antigens. Reactions are frequently displayed on skin. Graft rejection, tubercular lesions, dermatitis.