Antigen Preparation

Antigen is a molecule that binds to Ag-specific receptors, but cannot necessarily induce an immune response in the body by itself. Antigens are usually proteins, peptides (amino acid chains) and polysaccharides (chains of monosaccharides/simple sugars) but lipids and nucleic acids become antigens only when combined with proteins and polysaccharides. Saccharides and lipids it must be a large enough size, since peptides too small will also not elicit an immune response

The Antigens classification on basis of Source/Origin

1-**Exogenous antigens**: These are antigens which are foreign to host body hence are also called foreign antigens. These are antigens that enters the body of the organism from the outside, e.g. through inhalation, ingestion, or injection. . E.g. Bacteria, Fungi, Viruses et

2-**Endogenous antigens**: These are antigens which originate from own body of host organisms.. The endogenous antigens are processed by the macrophages which are later accepted by the cytotoxic T – cells. E.g. Blood group antigens, HLA (Histocompatibility Leukocyte antigens) etc

3-Auto antigens: These are usually a normal protein or protein complex (and sometimes DNA or RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease. These are not immunogenic under normal condition however due to genetic and environmental changes or factors immunological tolerance is lost and immune response is generated. E.g. Nucleoproteins, Nucleic acids, etc.

The Antigens classification on the basis of immune response

1. **Immunogens/ Complete antigens:** A substance that induces specific immune response can be called as immunogen. Antigens which are able to generate immune response by themselves are known as complete antigens. These are generally molecules with high molecular weight (more than 10,000 Daltons).

2. **Haptens/ Incomplete antigens:** Antigens which are unable to generate the immune response themselves are termed as incomplete antigens however on coupling with carrier proteins they can be immunogenic. They are also called haptens. When a molecule of haptens are coupled to carrier proteins they become accessible to immune system and function as an immunogen. They generally have low molecular weight (Less than 10,000 Daltons) and are usually non-protein substances. E.g. Capsular polysaccharide of pneumococcus, polysaccharide "C" of β-haemolytic streptococci, etc.

Property of antigens/ Factors Influencing Immunogenicity

1. Foreignness: An antigen must be a foreign substances to the animal to elicit an immune response.

2. Molecular Size: The most active immunogens tend to have a molecular mass of 14,000 to 6,00,000 Da.

3. Chemical Nature and Composition: In general, the more complex the substance is chemically the more immunogenic it will be.

4. Physical Form: In general particulate antigens are more immunogenic than soluble ones.

5. Antigen Specificity: Antigen Specificity depends on the specific actives sites on the antigenic molecules (Antigenic determinants).

6. Species Specificity: Tissues of all individuals in a particular species possess, species specific antigen.

7. Organ Specificity : Organ specific antigens are confined to particular organ or tissue.

PREPARATION OF ANTIGEN Heat killed / formalinized whole cell bacterin

1. Plate out the bacterial culture from appropriate stock culture onto a BHI agar plate,or other suitable medium. Incubate overnight at 37°C (±2°C).

2.Bacterial Growth is transferred to normal saline solution. Thoroughly mixed .

3.Expose to heat by heating the broth culture for 30 min at 100°C (Free Steam). OR Add Formalin (40% w/v) was added to the broth culture at a final concentration of 0.5% (V/V) and left 48 hrs at room temperature.

4.Centrifuge the killed bacterial suspension and resuspend the sediment in a small volume of normal saline. Repeat the step twice or thrice.

5.Adjust the opacity of suspension to the tube No.4 of Mac Farlands nephlelometer by adding required amount of normal saline.

6. The antigen thus prepared can be used for immunization or testing antibodies against specific antigen by agglutination tests.

Preparation of somatic O antigen

1- Streaking a few typical *Salmonella* colonies from salmonella shigella agar (S.S agar) plate onto brain heart infusion agar using a sterile cotton swab.

2- Plate were incubated overnight at 37 C.

3- Serial normal saline was used to harvest the lawns and a bottle containing 250 ml of bacterial suspension was immersed in a boiling water bath for 2.5 hr (**OR :** Using a Pasteur pipette, wash off the culture, preferably inside a safety cabinet, with approximately 2 ml of absolute alcohol, and transfer into a sterile universal container to enable the alcohol to kill the bacteria and detach flagellae instead of boiling)

4- Leave the antigen at room temperature overnight and again immersed in boiling water for additional 1.5hr on the next day

5- The killed bacteria were washed 3 times with normal saline

6- It was judged sterile by its frailer to cause turbidity in brain heart infusion broth tubes after prolonged incubation at 37C and its failure to produce growth on S.S agar plates.

7- Storage was in 0.3 % Normal saline at 4 C.

Preparation of Flagellar H antigen

1- Inoculated a few typical Salmonella colonies from S.S agar plate onto a 250 ml brain heart infusion broth

2- Broth was incubated overnight at 37C

3- Then ,250 ml of 0.6 %formal saline was added ad the mixture was allowed to stand at room tempreture for 5 hr and sterility test were performed

4- Washing killed bacteria and discard the supernatant

5- Re suspend the precipitate with 0.3 formal saline and storage at 4C

Difference between O Antigen and H Antigen

S.N.	Characteristics	O Antigen	H Antigen
1.	Types	Somatic Antigen	Flagellar antigen
2.	Composition	Polysaccharide	Proteinaceous
3.	Antibody formation	Rapid and Early	Rapid and Sustained
4.	Level	Falls off quickly	Persists for longer periods
5.	Production	Produces granular clumps.	Produces cottony, fluffy precipitates.
6.	Observation	Round bottom Felix tube are used to see agglutination.	Conical bottom Dreyer's tube are used to see agglutination.
7.	Heat sensitivity	Somatic antigens are heat stable.	Flagellar antigens are heat-labile.
8.	Alcohol sensitivity	Resistance to alcohol	Sensitive to alcohol
9.	Extraction	Trichloro-acetic acid is used for extraction of O antigens.	Formaldehyde is used for extraction of H antigens.
10.	Immunogenicity	Less immunogenic	Highly immunogenic
11.	Antibody formation	Produces antibody formation with low titres.	Induces antibody formation with high titres.