Antigens Preparation

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Antigen:-

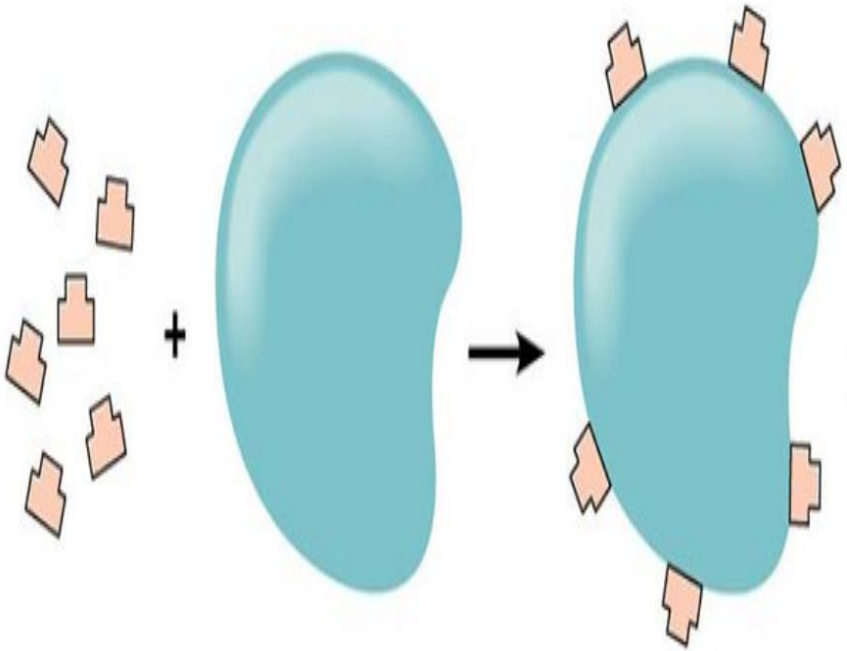
A molecule or part of molecule that recognized by immune system and bind specifically to antibodies or lymphocytes receptors. Examples of antigens are pollen, bacteria, parasitic worms, and viruses.

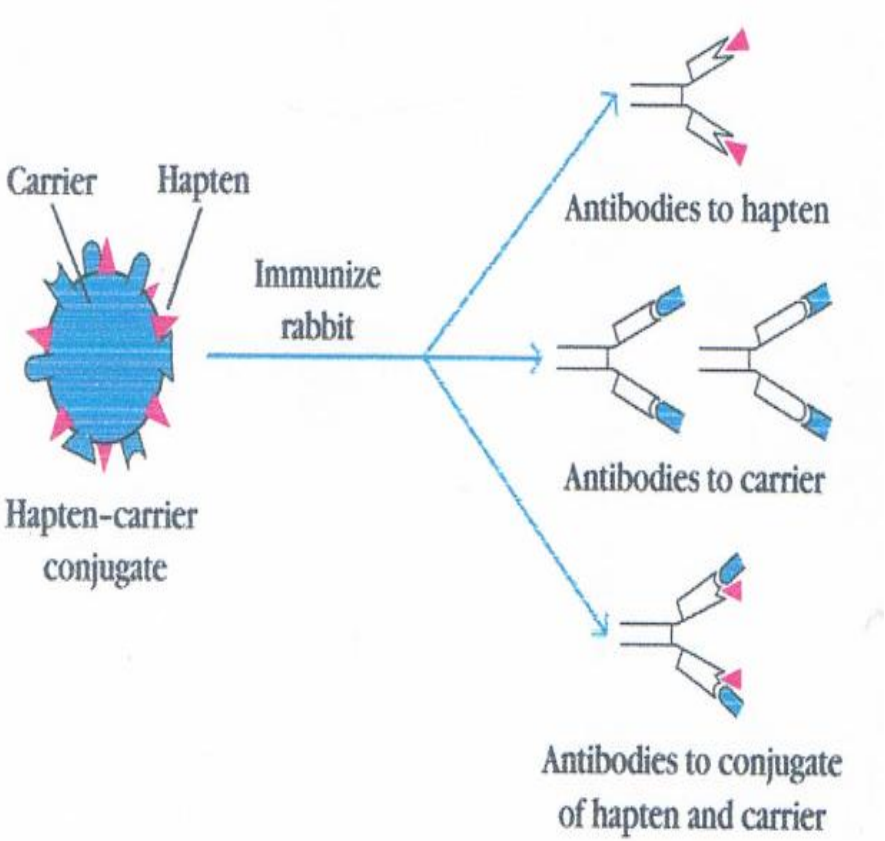
Hapten:-

A low molecular weight molecule that can not initiate an immune response unless first bound to an immunogenic carrier molecule . Example of hapten is penicillin Which are not antigenic by themselves unless binding with serum proteins of sensetive individuals.

Immunogen:-

A high Molecular weight molecule > 10000 D that is capable of inducing an immune response {humoral or cell mediated immunity }.The most potent immunogen such as proteins and polysaccharides .





Some types of antigens :-

Physically:

1- Particulate Ags :-

Bacteria , viruses , parasites and erythrocytes .

2- Soluble Ags :-

Serum , serum protein ……etc.

Bacterial antigens:

Somatic antigen (O-Ag):

- It is heat – stable Ag .

-Carbohydrate – Protein - lipids , complex in nature .

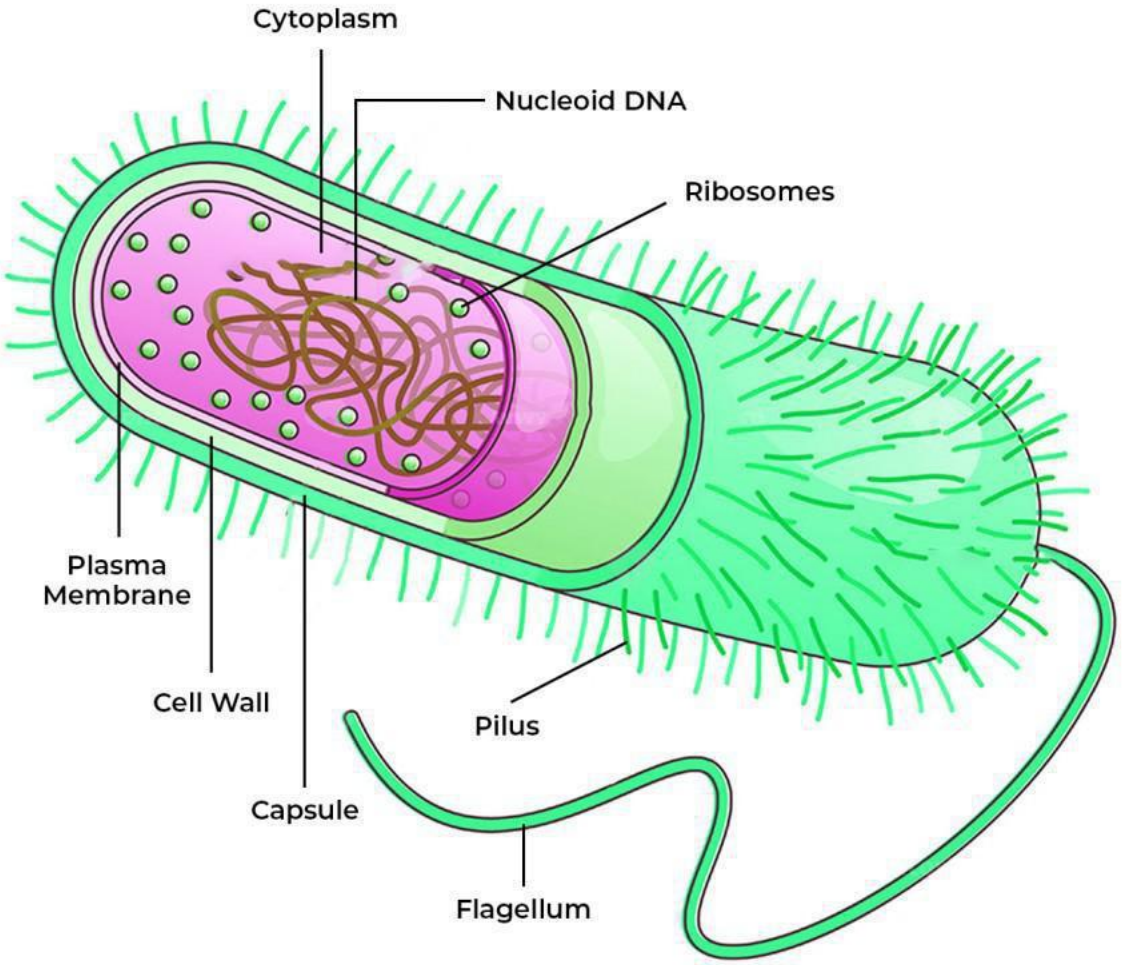
- Resistant to weak acids and alkaline .
- Example :Cell wall (LPS) of Gram negative bacteria.

Flagella Ag (H-Ag):

- It is heat labile Ag .
- Protein in nature .
- Example : Flagella of *Salmonella* spp except avian *Salmonella* (*S. gallinarum and S. pullorum*) which have no flagella.

Capsular Ag (K –Ag):

-like *Klebsiella pneumonia*


Cytoplasm
Nucleoid DNA
Ribosomes
Plasma Membrane
Cell Wall
Capsule
Pilus
Flagellum

Preparation of antigens (Methods):-

1- Purification of bacteria by selective techniques cultured the bacteria on the suitable media .

2- To obtain massive growth or culturing of bacteria we must culture the

bacteria on agar media for somatic antigen (LPS) , to prepare flagellar Ag we must cultured on broth media & examine the motility test .

3- Harvesting by preparation of bacteria suspension from culture with normal saline or PBS do not use distilled water to prevent lysis of

bacteria .

4- Wash three times with Phosphate Buffered Saline (PBS) by centrifugation 3000 rpm for 20-50 minutes.

5- Heating or boiling in water bath at 100 ^{o} C for 2.5 hours used for preparation of O-Ag and get rid of H-Ag

, while in case of preparing H-Ag we used PBS with formaline (0.6 % Formal saline) over night at 37^{o}C incubation to prevent denaturation of flagellar proteins.

(0.3% formale saline) for preservation of both types of Ags.

6- Wash three times by centrifugation .

7- Resuspend in PBS (pH 7.2) .

8 – Sterility test to check purity & contamination on suitable media for 24-48

hours .

9 – Standardization by using McFarland 's tubes (10 capacity tubes contains

different concentrations of 1% BaCl2 & 1% H2SO4 .

Tube ……..3----------9× 10^8 cell/ ml .

Tube …….4 ----------1.2 × 10^9 cell/ ml .

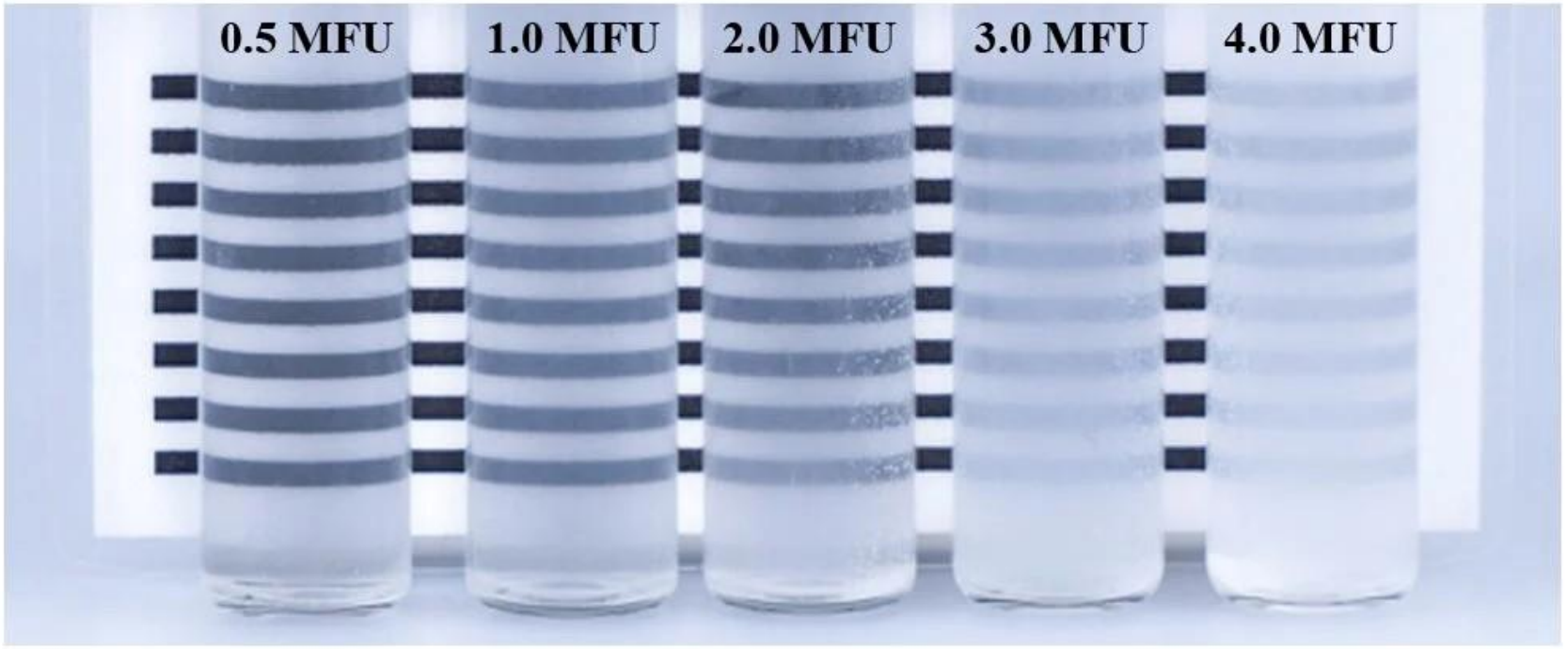
Or by using haemocytometer chamber , also , in case of H-Ag we used spectrophotometer .

McFarland Method :-

The method consists of comparing the opacity of the vaccine with a series of ten standard tubes containing varying amounts of barium sulfate in suspension:-

McFarland tube	BaCL2 1% ML	H2SO4 1% ML	No. of Bacteria
1	0.1	9.9	300.000.000
2	0.2	9.8	600.000.000
3	0.3	9.7	900.000.000
4	0.4	9.6	1.200.000.000
5	0.5	9.5	1.500.000.000
6	0.6	9.4	1.800.000.000
7	0.7	9.3	2.100.000.000
8	**0.8**	**9.2**	**2.400.000.000**
9	0.9	9.1	2.700.000.000
10	1.0	9	3.000.000.000

McFarland Standards



These turbidities correspond to varying concentration of bacteria in the size range of *Staphylococcus* , *Streptococcus* ,and the enteric bacteria .

Preparation of RBCs:-

Collect blood in sterile test tube containing suitable anticoagulants , Centrifugation and wash RBCs three times with isotonic saline solution , injection of RBCs in laboratory animals recording anti RBCs serum .

Preparation of immunoglobulin:-

Collect hyper immune serum , precipitation of immunoglobulin by using highly concentration of salts like ammonium sulfate , ligation of Ig with adjuvant , injected in laboratory animals , recording of anti immunoglobulin (Antibodies) .


THANKS
FOR YOUR
ATTENTION