Exercise No. 1

Preparation of antigen

Killed bacterial suspensions are used for the production of antibodies in man and animals are known as bacterins. Bacterins may be used prophylactically to prevent bacterial infection or for the production of antisera which may be used for various serological reactions. An effective bacterial vaccine should contain requisite number of bacterial cells(1,000,000,000 cells per ml),which may be either avirulent or inactivated by means of agents like heat, formalin, phenol, alcohol, etc., to make it safe for the animal. The vaccine should not lose its antigenecity during the process of inactivation and should give a good response of antibody production in suitable animals. Antibody may be tested *in vivo* (protection test) or *in vitro* (serological test).

In this exercise *Escherichia coli*, a Gram negative, motile organism will be used to prepare a vaccine. These organisms contain flageller (H) as well as somatic (O) antigens. H antigen are protein in nature and are resistant to formalin but they are destroyed by alcohol and prolonged heating. While O antigens are sugars of lipopolysacharrides and are resistant to alcohol and prolonged heating.

Materials:

1. *Escherichia coli* culture,
2. Nutrient agar slants - Two
3. 0.5% formalin saline 25 ml, blood agar plate one, Ma Conkey agar plate one.
4. McFarland nephelometer

Procedure:

1. Inoculate two nutrient agar slants with *Escherichia coli* culture. Cover the entire surface of the slants with the inoculums to get maximum amount of growth. Incubate the slants at 37°c for 24 hours.
2. Using a sterile pipette, add 2 ml of 0.5% formalin saline in each of the culture slants.
3. Remove the growth from the surface of medium and dissolve in the formalin saline to make a uniform suspension. Use a sterile platinum loop for this purpose. Shake the agar with the loop. Shake the bacterial suspension to disperse the cell evenly.
4. Remove the bacterial suspension from both of the slants to a large screw cap tube. The slants may be washed by adding small quantity of fresh formalin saline if there are bacterial clumps they may be broken with the help of sterile glass beads. The suspension may be filtered with the sterile cotton filter.
5. Adjust the opacity of suspension to tube no.4 of McFarland nephelometer by adding required amount of formalin saline & store at room temperature for 72 hours for inactivation.
6. Test for sterility in Maconkey and blood agar.
7. The bacterins should be tested for purity, sterility. Safety test is conducted in susceptible laboratory animals.
8. Tube no.4 contains $1.2X\ 10^9$ organisms /ml.

Questions:

1. Write characteristics of an antigen.
   Ans.

2. Why are somatic and flagellar antigens referred to as ‘O’ and ‘H’ antigens respectively?
   Ans.

3. Why is the bacterial suspension heated for the preparation of ‘O’ antigen, while formalin treatment is done to prepare ‘H’ antigen?
   Ans.

4. What is the difference between an antigen and a hapten?
   Ans.

5. What is the difference between Freund’s complete and incomplete adjuvant?
   Ans.

6. Why should repeated freezing and thawing be avoided?
   Ans.

Draw Flow chart for preparation of antigen:

Date: Signature of Instructor