Exercise No. 2

(A) Raising of immune serum against particulate antigen

Serum is the amber coloured fluid that is obtained after clotting of the peripheral blood. Serum can be electrphorectically separated into different proteins as albumin, α, β and γ globulins. Antibodies are in the β and γ globulins. When the serum is having very large amount of specific antibodies it is known as hyperimmune sera. Hyperimmune sera are obtained by repeated immunization of the animal and use of adjuvants. When adjuvants are mixed with an antigen they enhance the activity of that antigen.

Immune serum against particulate antigen is raised to conduct agglutination test.

Materials:

E. coli bacterin approximately 6ml, 1 ml & 5 ml graduated syringes with 24 gauge 1 inch needles, scissor 1, 70% alcohol, 3 to 4 month old rabbits 2

Procedure:

1. Secure each rabbit in inoculation box & clip the hairs of the area over marginal ear vein & sterilize with 70 % alcohol.
2. Follow the following schedule of vaccination:

First day	0.1 ml intravenously
Third day	0.25 ml intravenously
Fifth day	0.5 ml intravenously
Ninth day	2.0 ml intravenously
Twelfth day	3.0 ml intravenously

Bleed the animal one week after the last injection, separate serum from clotted blood, inactivate at 56°c for 30 min, add merthiolate as preservative and store in refrigerator in sealed ampoules.

Exercise No. 2

(B) Raising of immune serum against soluble antigen

Immune serum against soluble antigen is raised to conduct precipitation test.

Materials:

Horse serum approximately 6ml , sterilized normal saline approximately 6ml, 1 ml and 10 ml graduated syringes with 24 gauge 1 inch needles, 70% alcohol and Two 3 to 4 month old rabbits.

Procedure:

1. Collect approximately 30ml blood from horse, separate serum and inactivate it at 56°C for 30 min in water bath and filter it through Seitz filter.
2. Dilute the serum with 50 % normal saline solution and add merthiolate as preservative and inoculate in the rabbit as below
   a. Secure each rabbit in inoculation box & clip the hairs of the area over marginal ear vein & sterilize with 70 % alcohol.
3. Follow the following schedule of vaccination:

First day	0.2 ml intravenously
Fourth day	0.5 ml intravenously
Seventh day	1.0 ml intravenously
Twelth day	10.0 ml intravenously

4. Bleed the animal one week after the last injection, separate serum from clotted blood, inactivate at 56°c for 30 min, add merthiolate as preservative and store in refrigerator in sealed ampoules.

Questions:

1. Define serum?
   Ans.

2. What do you understand by hyper immune serum.
   Ans.

3. How is serum sterilized?
   Ans.

4. How can non-sterile serum be preserved for transport without refrigeration?
   Ans.

5. Does serum contain secretory IgA?
   Ans.

Vaccination schedule:

Date: Signature of Instructor