TITRATION of ANTIBODY

The purpose of this experiment is to illustrate what is meant by the titration of a substance. A titration is the estimation of the activity or content of a reagent. Although sera are not used in this experiment, the same general method is often used in measuring antibody content of sera. Usually, doubling, or \( \log_2 \), dilutions are made when titering a serum.

*PROCEDURE I Preparation of 2% sheep red blood cell (SRBC) suspension

1. Centrifuge 5 ml of SRBC in a clinical centrifuge (10 min.).
2. After centrifugation, aspirate off the serum and buffy coat (the thin layer of white blood cells on top of the sedimented SRBC).
3. Resuspend up to 5 ml with PBS.
4. Using a wooden applicator stick, resuspend the SRBC.
5. Centrifuge again, aspirate supernatant, add up to 5 ml of PBS and resuspend.
6. Centrifuge again. After aspirating supernatant, note the packed volume and resuspend with PBS to a 2% suspension. (This is done by multiplying the packed volume by a factor of 50, i.e., if the packed volume is 1 ml add PBS until a total volume of 50 ml is achieved.) Store cells in 250 ml. Erlenmeyer flask.

USE OF MICROTITER EQUIPMENT

In practice, gross dilutions with pipettes have often been replaced by special equipment designed to speed up the process and to economize the amount of reagents needed for standard titrations. The principle is exactly the same, but the equipment is quite different. Plastic trays with indented wells are used. Titrations are commonly performed in 25 or 50 ul quantities.

PROCEDURE I: Using crystal violet practice making two fold serial dilutions with micropipettes.

1. Label the microtiter plate as designated on black board
2. Into the first well add 50 ul of crystal violet
3. Into the rest of the wells in the row add 25 ul of PBS
4. Transfer 25 ul from the first well into the second well, draw up and down in pipette several times, transfer to third well etc., until dilutions have been made in all the wells. Discard 25ul from last well.

**PROCEDURE II: Titration of antibody**

1. Dispense 25 ul of saline in wells 2 through 12 in one row of a microtiter tray, using a micropipette (usage will be demonstrated). In the row above or below design appropriate control wells.

2. Using a similar dispenser, add 50 ul of 1/10 anti-sheep RBC serum to well

3. Transfer 25ul from well 1 to well 2.

4. Carefully mix solution up and down in pipetter and then transfer to well 3.


6. Dispense 25 ul of 2% sheep RBC's into all wells.

Mixing of the microtiter plates and incubation:

1. Put a piece of sealing tape over the top of your microtiter plate.

2. Place the tray on the micromixer and allow it to mix for 1 minute.

3. Incubate your microtiter plate for 30 minutes at room temperature.

4. Observe your plate and record reactions in each well as 0-4+, 0 representing a button of SRBC, 4+ being a diffuse blanket of SRBC covering the bottom of the well. Record the titer as the last well that gives a positive reaction. The titer is the reciprocal of the highest dilution of reagent that gives any evidence of the desired reaction.