Test Procedures: Processing Antisera from Whole Blood.

During an immunization series, periodic (e.g., weekly) serum samples should be tested, since then one can determine when the titer is appropriately high. In practice this is not always feasible. Immunization duration is then based on past successes. Most generally, three to four weeks of injections are appropriate for immunizations against red cells.

At the end of the immunization series of injections, a waiting period of about one week (usual range 4-10 days after the last injection) is allowed, hopefully for the antibody titer to rise to its maximum. The recipients then are bled in quantity.

Usually the blood is collected in clean dry glass bottles and allowed to clot. In mammals the clot shrinks expressing serum in a few hours' time. Placing the fresh clots at 37°C for the first two hours is helpful in getting the best yield. The clots should be broken away from the side of the tube at about half an hour if they have not already done so naturally. For large clots (300 ml or more), longer times are necessary for full clotting and shrinking. The serum is poured off at about 3-4 hours, centrifuged clear, often recentrifuged clear, then labeled and stored frozen at -20°C or colder. If the clots are allowed to stand overnight at room or refrigerator temperatures, additional yields may be obtained. The later yields are not demonstrably different in antibody content but are more contaminated with bacterial growth and should be heated at 56°C for 30 minutes. They are then conveniently complement deactivated and, therefore, ready for testing and absorbing.

Blood clots from birds are quite variable in shrinking and expressing serum. When saline agglutinating tests only are involved, it is more rewarding, generally, to obtain plasma from birds. The dilution effect is small and the yield considerably increased. Fibrin in the plasma will precipitate out after each freezing and thawing, sometimes obscuring reactions. The fibrin may be removed by heating at 56°C for 30 minutes and <u>then</u> standing 48 hours at refrigerator temperatures and finally centrifuging the fibrin precipitate out.

Always <u>label</u> completely antisera to be stored more than two or three days.