

Test Procedures

Not all, but most Immunogenetic work, has been done with particulate antigens, specifically erythrocytes (red blood cells = RBC). Two main tests are involved: (1) the agglutination test, (2) the hemolytic test.

1. Agglutination tests:

The antigen is held constant and the antibody titered -- the reverse of precipitin tests. Another difference: multiple injections are usual in the recipient.

General standard procedure (does vary):

1. Fill out protocol or test sheet headings.
2. Wash RBC in isotonic (0.75 - 0.92%) saline 3 times in 10 volumes (or more).
3. Resuspend to 3% (later by eye.)
4. Titer antiserum in doubling, quadrupling or tens dilutions.
5. Add two drops (pipette drops 20-40 drops - I prefer 30 per ml) to the tubes or reaction wells, horizontally: 1st row undiluted, 2nd row 1/2, 3rd row 1/4, etc. for doubling dilutions. The last row should be a saline control.
6. Wash pipette by aspiration between dilutions (and later between cell samples).
7. Add one drop of the 3% washed cell samples to each dilution vertically straight back (i.e., perpendicular to the horizontal rows of antisera.)
8. Shake well.
9. Read at 5 minutes, 30 minutes, and 2 hours after the setup. Agitate or shake well after each reading. Check for cold agglutinins by leaving overnight in the refrigerator, 4°C. By precedent the 5 minute reading is recorded in pencil at the top of the square, the 30 minute reading is taken in permanent blue or black (India ink preferred) in the lower left, the 2 hour reading is taken in red (lower right) and the overnight reading (if any) in green.

Cold agglutinins are rare. They are characterized by reacting only at low temperatures, 4°C, and not at room temp. 25+2°C or at 37°C, body temperature. Further, the reaction disappears if the test is warmed to room temperature. Further, they are absorbed out only at the low temp.

Agglutination is read by gentle shaking or swirling the mixture, and distinguishing grades of clumping of the red cells. The degree of agglutination is relative and slightly subjective.