Immunological testing

- Monoclonal antibodies
- Serology
- Quantifying antigen – antibody reactions

Perspective 17.1
Monoclonal Antibodies
Serology

• Antibodies
• Antibodies detect and identify antigens

Quantifying antigen – antibody reactions

• Seroconversion or rise in titer
• Serial dilutions
Figure 17.2 - Quantitation of immunologic tests

Precipitation reactions

- Immunodiffusion
- Immunelectrophoresis
Figure 17.3
Precipitation reaction

Immune complexes
large removed by phagocytes
small can remain in circulation
and cause disease

Figure 17.4
Immunodiffusion
Immunoelectrophoresis

Can be used to analyze patient Ab types

Figure 17.6
Immunelectrophoresis

Agglutination reactions

- Direct agglutination
- Indirect agglutination
- Hemagglutination
Direct agglutination

- Cross – linking and lattice formation
- Antibodies react with particulate antigens (red blood cells, bacteria, fungi)
- Visible clumps
- Estimate amount of antibody

Indirect agglutination

- Soluble antigen is coated onto particles (red blood cells, latex beads)
- Allow for visible clumps (agglutination)
Figure 17.7 - Agglutination reaction
Anti-A antibodies agglutinate; Anti-B antibodies do not. Therefore the patient has type A blood (A antigens on the surface of their red blood cells)

Immunofluorescence tests

- Direct fluorescent antibody test
- Indirect fluorescent antibody test
Antigen – antibody assays

- Radioimmunoassay (RIA)
- Enzyme – linked immunosorbant assay (ELISA)
- Western blot
Radioimmunoassay (RIA)

- Competitive inhibition assay
- Measure antigen or antibody
- Ex. Measure small amounts of hormones or drugs in a clinical sample
- Ex. Measure small amounts of IgE antibody (radioallergosorbent test)

Unlabeled Ab is used to coat well
Labeled specific Ag is added with sample
Ability of unlabeled Ag in sample to compete with labeled Ag binding to Ab is measured
Reduced binding indicates competition by unlabeled Ag in sample
Amount of competition a measure of unlabeled Ag levels

Enzyme – linked immunosorbant assay (ELISA)

Widely used; very sensitive; small volumes; little reagent; lots of samples
Used for HIV testing of blood before it is used for transfusion
Enzyme – linked immunosorbant assay (ELISA)

- Color reaction assay
- Indirect ELISA
- Direct ELISA

Figure 17.9 - Indirect ELISA
Figure 17.1 - Direct ELISA

D dete human chorionic gonadotropin
Present only in pregnant women

Figure 17.1 - Western blot

Ags separated by electrophoresis
Transferred to membrane
Probed with specific Abs
Abs detected indirectly using anti-HGG
Complement fixation test

- Measures the binding of complement by an antigen–antibody interaction
- Indicator system determine positive or negative reactions

![Diagram of Complement Fixation Test](image.png)

**Figure 17.1 Complement fixation test**

*Used to detect specific Abs in serum*
Neutralization test

- Antibody bind to specific antigen (virus, toxin)
- Antibody – antigen complex prevents antigen from binding (neutralization)
- *Viral or toxin activity is diminished in tests*

Cellular immunology test

- Identification of subsets of lymphocytes (*using FACS*)
Cellular immunology test

- Identification of subsets of lymphocytes
- Lymphocyte response to mitogens
- Cytoxic T-cell function
- Cell-mediated immunity to infectious agents

*Ag used instead of mitogen to stimulate lymphocytes*