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

Precipitation reactions

- Immunodiffusion
- Immunelectrophoresis

Figure 17.2 - Quantitation of immunologic tests
Figure 17.3
Precipitation reaction

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

Figure 17.4
Immunodiffusion

Figure 17.6
Immunoelectrophoresis

Can be used to analyze
patient Ab types

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)
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

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

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
- Cytotoxic T - cell function
- Cell – mediated immunity to infectious agents
  *Ag used instead of mitogen to stimulate lymphocytes*