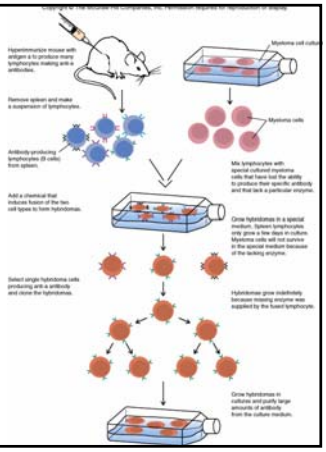
Immunological testing

- Monoclonal antibodies
- Serology
- Quantifying antigen – antibody reactions

17-1

Perspective 17.1
Monoclonal Antibodies



17-2

Serology

- Antibodies
- Antibodies detect and identify antigens

17-3

Quantifying antigen – antibody reactions

- Seroconversion or rise in titer
- Serial dilutions

17-4

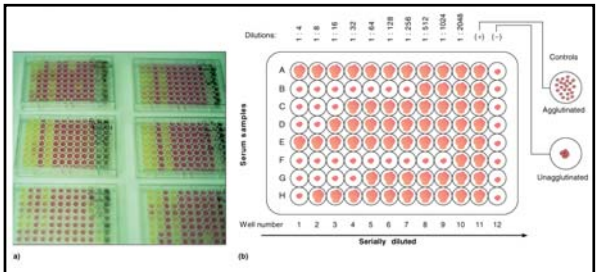


Figure 17.2 - Quantitation of immunologic tests

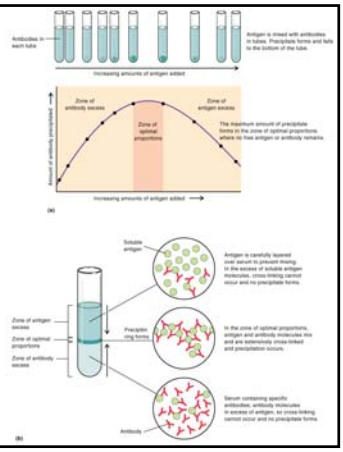
17-5

Precipitation reactions

- Immunodiffusion
- Immunoelectrophoresis

17-6

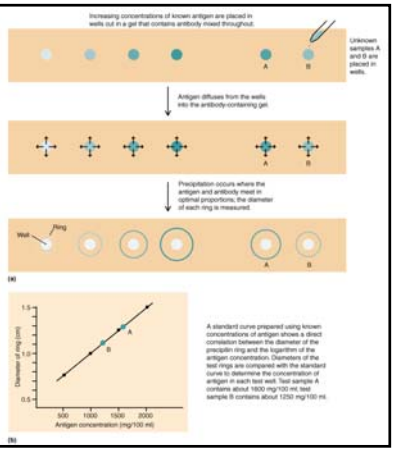
Figure 17.3
Precipitation reaction



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

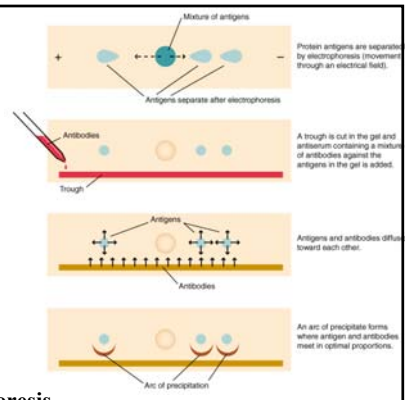
17-7

Figure 17.4
Immunodiffusion



17-8

Figure 17.6
Immunoelectrophoresis



Can be used to analyze
patient Ab types

17-9

Agglutination reactions

- Direct agglutination
- Indirect agglutination
- Hemagglutination

17-10

Direct agglutination

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

17-11

Indirect agglutination

- Soluble antigen is coated onto particles (red bood cells, latex beads)
- Allow for visible clumps (agglutination)

17-12

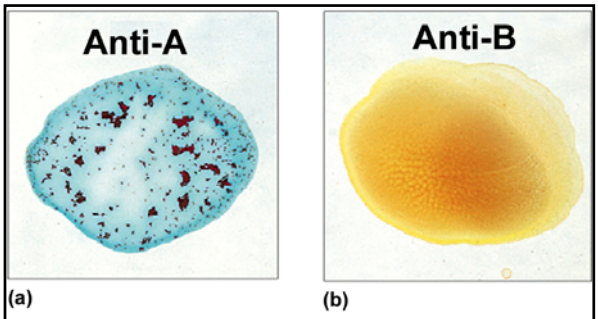


Figure 17.7 - Agglutination reaction

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)

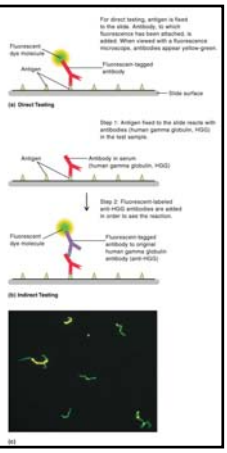
17-13

Immunofluorescence tests

- **Direct fluorescent antibody test**
- **Indirect fluorescent antibody test**

17-14

Figure 17.8 - Direct and indirect fluorescent antibody test



17-15

Antigen – antibody assays

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

17-16

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

17-17

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

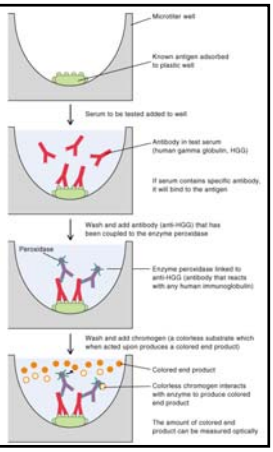
17-18

Enzyme – linked immunosorbant assay (ELISA)

- **Color reaction assay**
- **Indirect ELISA**
- **Direct ELISA**

17-19

Figure 17.9 - Indirect ELISA



17-20

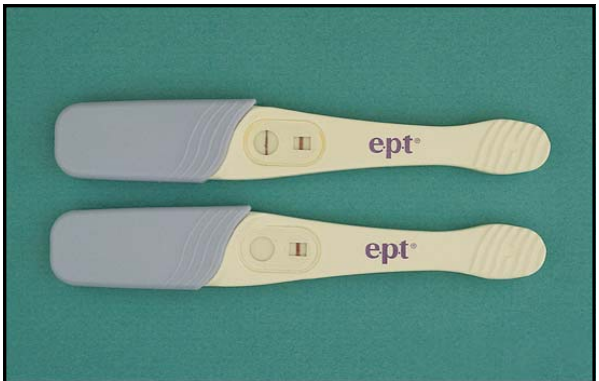


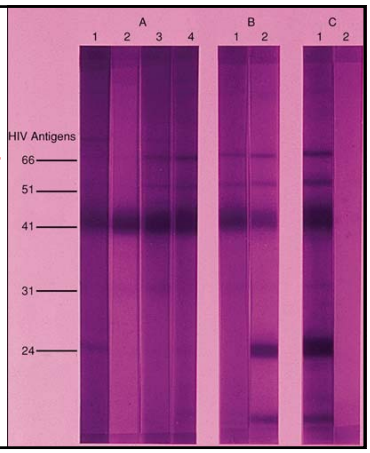
Figure 17.1 - Direct ELISA

Detects human chorionic gonadotropin
Present only in pregnant women

17-21

Figure 17.1
Western blot

Ags separated by electorphoresis
Transferred to membrane
Probed with specific Abs
Abs detected indirectly using anti-HGG



17-22

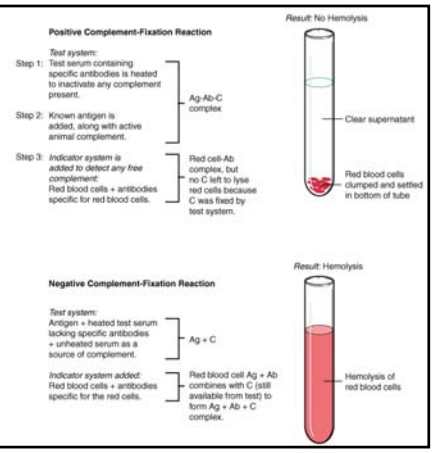
Complement fixation test

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

17-23

Figure 17.1
Complement fixation test

Used to detect specific Abs in serum



17-24

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***

17-25

Cellular immunology test

- **Identification of subsets of lymphocytes *(using FACS)***

17-26

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

17-27