

Lecture 24

Immune Diseases - Chapter 16

- Allergies
- Autoimmunity
- Immunodeficiency

Disease Diagnostics - Chapter 17

- Sample Collections
- Phenotypic Method
- Genotypic Method
- Immunological Method

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Autoimmunity

- Antibodies, T cells or both, mount an **immune response against self antigens**
 - Systemic or organ-specific
 - Type II (fixed Ag) or III (soluble Ag) reactions

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An example some major autoimmune diseases.

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Disease	Target	Type of Hypersensitivity	Characteristics
Systemic lupus erythematosus (SLE)	Systemic	III	Inflammation of many organs; antibodies against red and white blood cells, platelets, clotting factors, nucleus DNA
Rheumatoid arthritis and ankylosing spondylitis	Systemic	III and IV	Vasculitis; frequent target is joint lining; antibodies against other antibodies (rheumatoid factor)
Scleroderma	Systemic	II	Excess collagen deposition in organs; antibodies formed against many intracellular organelles
Hashimoto's thyroiditis	Thyroid	II	Destruction of the thyroid follicles
Graves disease	Thyroid	II	Antibodies against thyroid-stimulating hormone receptors
Pernicious anemia	Stomach lining	II	Antibodies against receptors prevent transport of vitamin B ₁₂
Myasthenia gravis	Muscle	II	Antibodies against the acetylcholine receptors on the nerve-muscle junction alter function
Type I diabetes	Pancreas	II	Antibodies stimulate destruction of insulin-secreting cells
Multiple sclerosis	Myelin	II and IV	T cells and antibodies sensitize to myelin sheath destroy neurons
Goodpasture syndrome (glomerulonephritis)	Kidney	II	Antibodies to basement membrane of the glomerulus damage kidneys
Rheumatic fever	Heart	II	Antibodies to group A <i>Streptococcus</i> cross-react with heart tissue

Table 16.4 Selected autoimmune diseases.

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Origins of autoimmunity

- Sequestered antigens
- Clonal selection against self
- Immune deficiency
- Inappropriate expression of MHC II
- Molecular mimicry
- Viral infections

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Diseases

- Systemic autoimmunities
 - Systemic lupus erythematosus
 - Rheumatoid arthritis
- Endocrine
 - Graves disease
 - Hashimoto thyroiditis
 - Diabetes mellitus
- Neuromuscular
 - Myasthenia gravis
 - Multiple sclerosis

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An example of systemic lupus and rheumatoid arthritis.



Fig. 16.17 Common autoimmune diseases.

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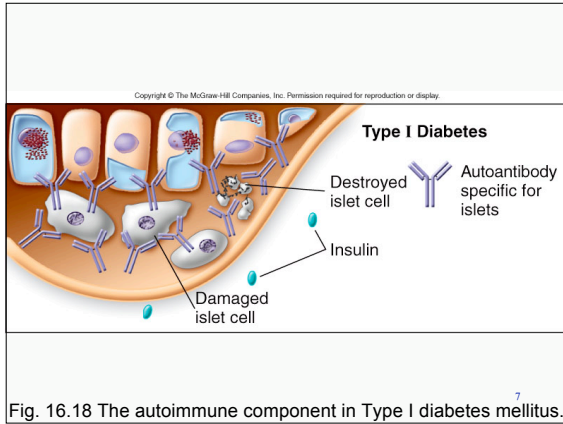


Fig. 16.18 The autoimmune component in Type I diabetes mellitus.

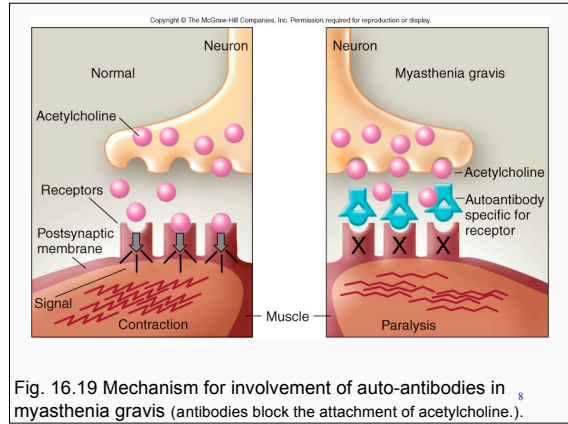


Fig. 16.19 Mechanism for involvement of auto-antibodies in myasthenia gravis (antibodies block the attachment of acetylcholine.).

Immunodeficiency

- A person can be born with or develop a weakened immune system
 - Primary
 - Secondary

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Primary

- Antibody production and phagocytosis
- Inherited abnormality
 - Deficiencies in B-cell or T-cell and development and expression
 - Combined B- and T-cell deficiency

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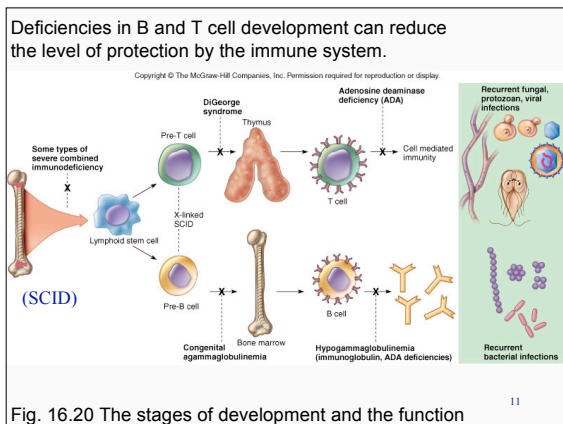


Fig. 16.20 The stages of development and the function

Secondary

- Caused by
 - Infection
 - Chemotherapy
 - Radiation

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Summary of the primary and secondary immunodeficiency diseases.

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TABLE 16.5 General Categories of Immunodeficiency Diseases with Selected Examples	
Primary Immune Deficiencies (Genetic)	Secondary Immune Deficiencies (Acquired)
B-Cell Defects (Low Levels of B Cells and Antibodies) Agammaglobulinemia (X-linked, non-sex-linked) Hypogammaglobulinemia Selective immunoglobulin deficiencies T-Cell Defects (Lack of All Classes of T Cells) Thymic aplasia (DiGeorge syndrome) Chronic mucocutaneous candidiasis Combined B-Cell and T-Cell Defects (Usually Caused by Lack or Abnormality of Lymphoid Stem Cell) Severe combined immunodeficiency disease (SCID) X-SCID due to an interleukin defect Adenosine deaminase (ADA) deficiency Wiskott-Aldrich syndrome Ataxia-telangiectasia Phagocyte Defects Chediak-Higashi syndrome Chronic granulomatous disease of children (see In The News, chapter 14) Lack of surface adhesion molecules Complement Defects Lacking one of C components Hereditary angioedema Associated with rheumatoid diseases	From Natural Causes Infection: AIDS, leprosy, tuberculosis, measles Other disease: cancer, diabetes Nutrition deficiencies Stress Pregnancy Aging From Immunosuppressive Agents Irradiation Severe burns Steroids (corticosteroids) Drugs to treat graft rejection and cancer Removal of spleen

Table 16.5 General categories of immunodeficiency diseases 13

Chapter 17

Topics

- Sample Collections
- Phenotypic Method
- Genotypic Method
- Immunological Method

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Sample Collection

- Typical samples
 - Saliva
 - Sputum
 - Blood
 - Urine
 - Fecal
 - Skin
 - Cutaneous membrane samples

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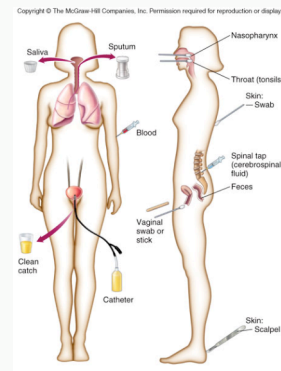


Fig. 17.1 Typical sampling sites and methods of collection 16

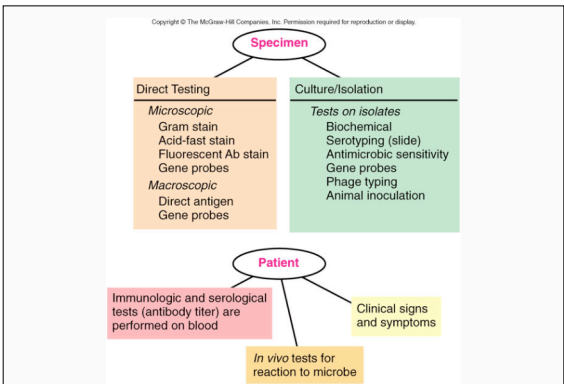


Fig. 17.2 A scheme of specimen isolation and identification 17

Fig. 17.3 Example of a clinical form used to report data 18

Phenotype

- Microscopic investigation
- Cultivation
- Biochemical analysis
- Clinical significance

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Microscopic investigation

- Common methods
 - Gram stain
 - Acid-fast stain
 - Direct fluorescence
 - Direct antigen testing

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Stains

- Determine cell shape
- Determine cell wall property
 - Gram-positive (thick peptidoglycan layer)
 - Gram-negative (thin peptidoglycan layer)
 - Mycolic acid OM in Gram-positive wall

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A direct fluorescent antibody test enables specific identification of microbes, and is visualized under the microscope.

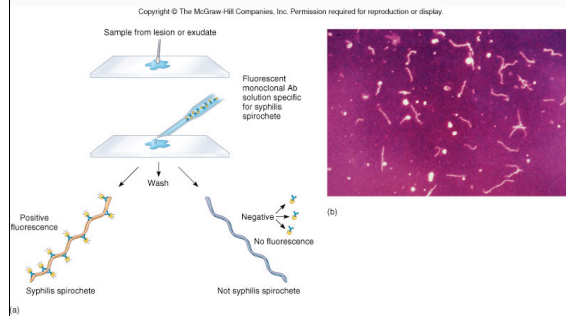


Fig. 17.4 Direct fluorescence antigen test

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Cultivation

- Enable growth of specific microbes
 - Specialized media
 - Selective media
 - Defined media
 - Differential media
 - Biochemical media
- Enable pure culture isolation

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Biochemical

- Indirect method
- Common tests
- Rapid test
- Schemes

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Indirect method of identification

- Identify enzyme and product
 - Express genes related to nutrient use and stress defense
- Common tests
 - Carbohydrate fermentation
 - Hydrolysis of gelatin, starch
 - Catalase
 - Oxidase
 - coagulase

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An example of a rapid test, which includes 23 biochemical tests.

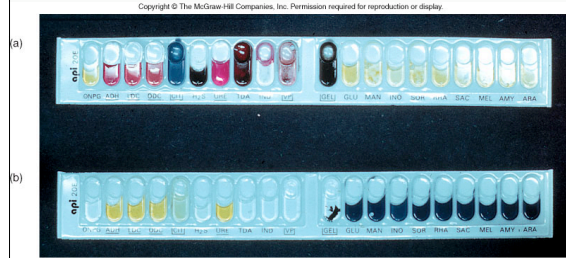


Fig. 17.5 Rapid tests

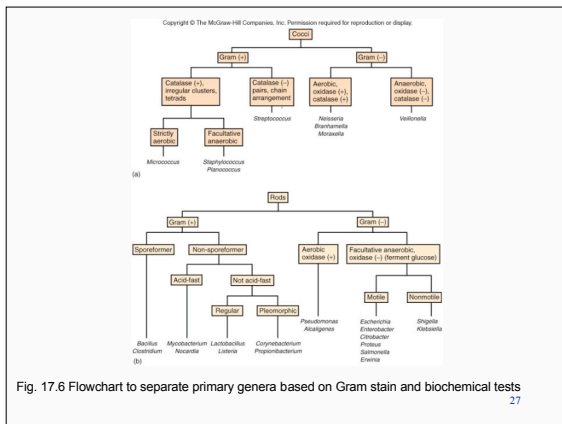


Fig. 17.6 Flowchart to separate primary genera based on Gram stain and biochemical tests

Clinical significance

- Number of microbes present
- Presence of microbes in a sterile site (eg. Cerebrospinal fluid, inner eye)
- Repeated isolation
- Isolation of a true pathogen (eg. *Mycobacterium tuberculosis*)

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Genotypic Method

- Genetic probes
- rRNA sequencing
- Polymerase Chain Reaction (PCR)
- G + C base composition

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Genetic probes

- Specific DNA probes for genes that identify a particular microbe
- Probes bind to complimentary sample DNA (hybridization), enabling positive identification

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Restriction digests, followed by hybridization with different probes generates specific patterns that enables identification of a particular microbe.

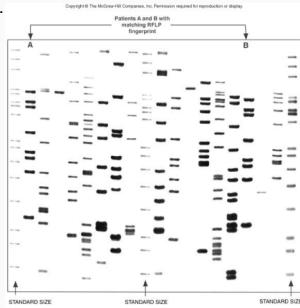


Fig. 17.7 DNA typing of restriction fragment length polymorphisms.

rRNA sequencing

- rRNA and protein specific to a ribosome
- rRNA sequence is stable
- Identification or signature for a particular microbe

Also:

- Establishes evolutionary relationships and distinguishes major taxonomic units

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PCR

- Amplify specific fragment DNA or gene
- Sensitive – amplify small amounts of sample DNA
- Quick identification – 1 day

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G + C base composition

- General indicator of relatedness
- Very small rate of change
- Allows taxonomic determination; however, cannot be used to identify specific microbe or virus

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Immunological Methods

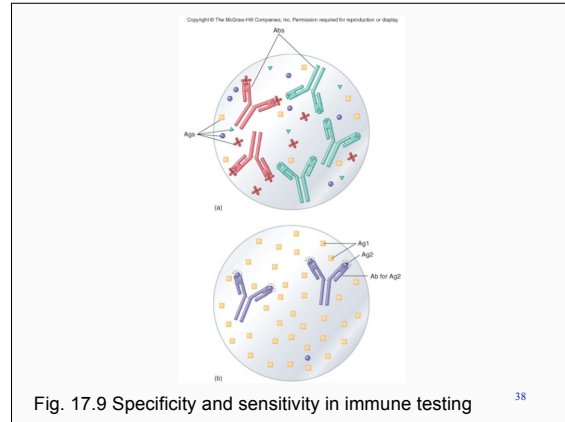
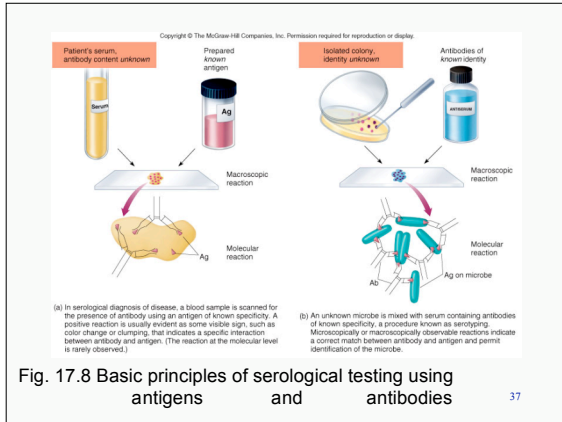
- Basic principle
- Agglutination
- Precipitation
- Western blot
- Complement fixation
- Immunofluorescence
- Common assays

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Basic principle

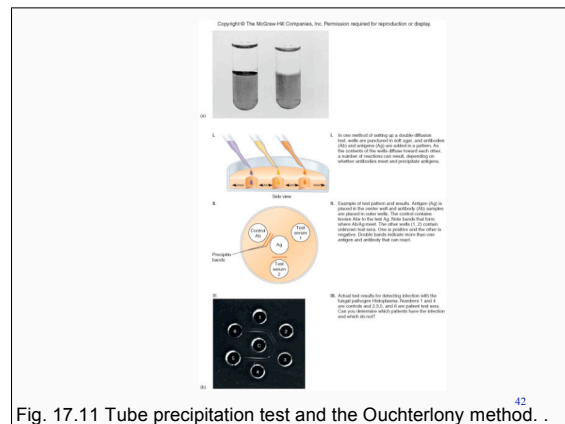
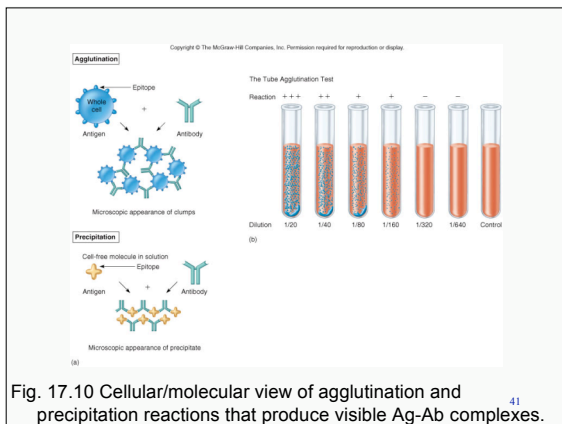
- Serology (*In vitro* method)
 - Antibodies have specificity for antigens
 - Detect
 - Identify
 - Quantify
 - Sensitive

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- ## Agglutination
- Antigens are whole cells (eg. RBC or bacteria)
 - Antibodies cross-link antigens => Ag-Ab complex
 - Allows determination of serum titer
 - Types of tests
 - Rapid plasma test (syphilis)
 - Cold agglutinin test (*mycoplasma*)
 - Weil-Felix test (ricketsia)
 - Latex agglutination test (pregnancy)
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- ## Precipitation
- Soluble antigens
 - Binding of antibody to antigen, makes the complex insoluble or visible
 - Types of test
 - Tube precipitation
 - Ouchterlony (double diffusion)
 - Immunoelectrophoresis
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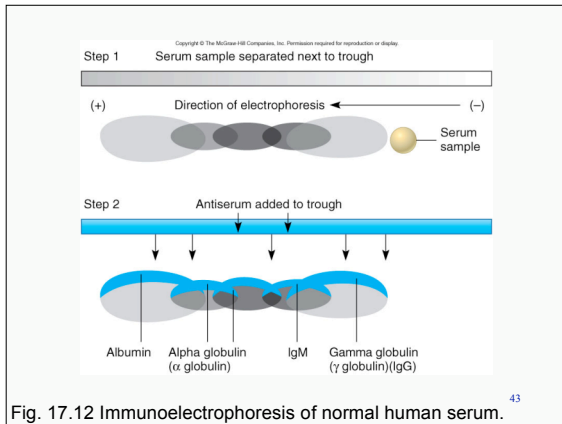


Fig. 17.12 Immunoelectrophoresis of normal human serum.

Western blot

- Electrophoretic separation of protein (antigen) sample
- Specific antibodies bind to protein sample
- Specific and sensitive
- Counterpart of the Southern blot (DNA test)

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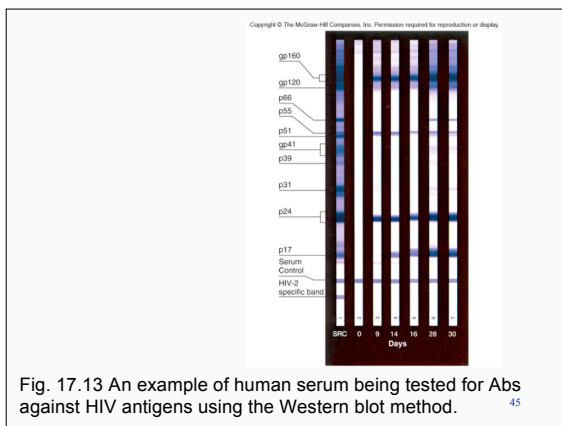


Fig. 17.13 An example of human serum being tested for Abs against HIV antigens using the Western blot method.

Complement fixation

- Antibody (lysin), antigen, complement, and sensitized sheep RBCs are required
- Complement is fixed to a Ab-Ag-complex
- Fixed complement cannot participate in rbc lysis = positive reaction or identification

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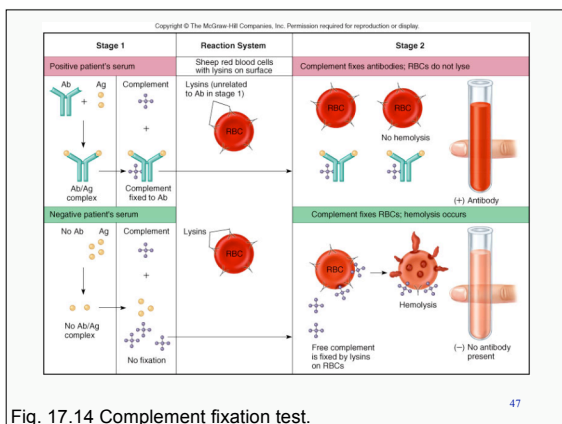


Fig. 17.14 Complement fixation test.

Immunofluorescence

- A monoclonal antibody labeled with a fluorescent dye
 - Direct testing
 - Indirect testing

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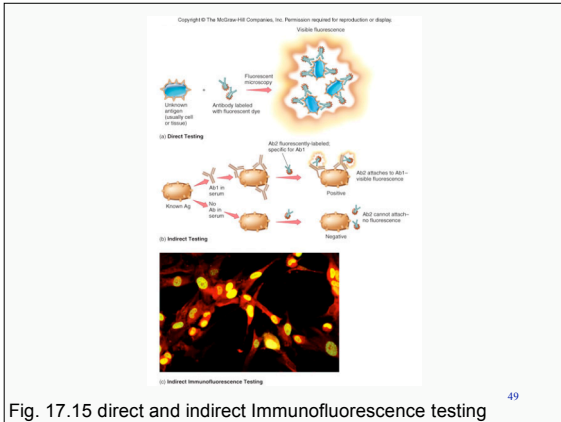


Fig. 17.15 direct and indirect Immunofluorescence testing

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Common assays

- Radioimmunoassay (RIA)
- Enzyme-Linked Immunosorbent Assay (ELISA)
- T and B cell differentiation
- Virus testing

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RIA

- Antibody or antigen is labeled
- Detect corresponding antibody or antigen
- Competition between unlabeled and labeled molecule

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ELISA

- Horseradish peroxidase or alkaline phosphatase used as indicators
- Enzyme releases dye (chromogen)
- Types of test
 - Indirect
 - Capture or Sandwich

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An example of the indirect and capture ELISA methods.

Fig. 17.16 Methods of ELISA testing.

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The rosette formation method and the fluorescent technique are used to identify lymphocytes (T or B cells), in order to evaluate immune dysfunctions.

Fig. 17.17 Tests for characterizing T cells and B cells.

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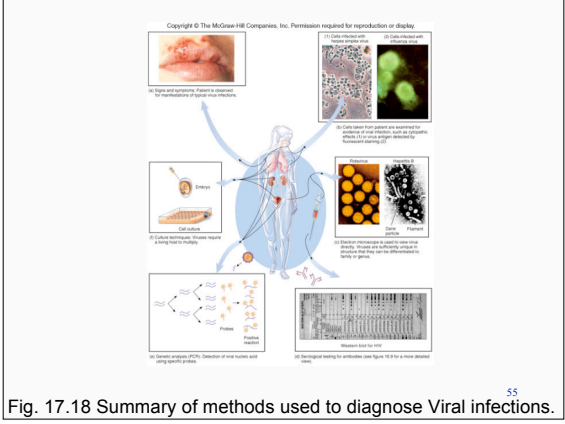


Fig. 17.18 Summary of methods used to diagnose Viral infections.