

Laboratory Diagnosis

Lecture 12

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Three approaches.

- ▶ **Bacteriologic** approach: the organism is identified by staining and culturing the organism,
- ▶ **Immunologic (serologic)** approach: the organism is identified by detection of antibodies against the organism in the patient's serum.
- ▶ **Molecular (nucleic acid-based) tests.**



How to Diagnose a Bacterial Infection When the Culture Is Negative.

- ▶ Detect antibody in the patient's serum. Detection of IgM antibody indicates a current infection.
 - ▶ A fourfold or greater rise in antibody titer between the acute serum sample and the convalescent serum sample also indicates a current infection
 - ▶ Detect antigen in the patient's specimen. Use known antibody to detect presence of antigens of the organisms, e.g., fluorescent antibody to detect antigens in tissue, latex agglutination to detect capsular polysaccharide antigens in spinal fluid.
 - ▶ Detect nucleic acids in the patient's specimen. Use polymerase chain reaction (PCR) and DNA probes to detect the DNA or RNA of the organism.
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Immunological methods

- ▶ Two basic approaches:
- ▶ (1) using known antibody to identify the microorganism and
- ▶ (2) using known antigens to detect antibodies in the patient's serum.



Antigen-Antibody tests

Used for:

▶ **Diagnosis of infectious diseases:**

- When organism cannot be cultured. E.g. Hepatitis B,C.
- When organism is too dangerous to be cultured. E.g. Rickettsial diseases.
- When culture techniques not readily available. E.g. HIV.
- When organism takes too long to be cultured. E.g. Mycoplasma.

▶ **Diagnosis of autoimmune diseases.**

▶ **Typing of blood.**

▶ **Tissue typing prior to transplantation.**



Identification of an Organism with Known Antiserum

- ▶ **Capsular Swelling (Quellung) Reaction**
- ▶ Microscopic observation that the capsule swells in the presence of homologous antiserum.
- ▶ Antisera against the following organisms are available: all serotypes of
 - ▶ *S. pneumoniae* (Omniserum),
 - ▶ *H. influenzae* type b, and
 - ▶ *N. meningitidis* groups A and C.



Slide Agglutination Test

- ▶ Antisera can be used to identify *Salmonella* and *Shigella* by causing agglutination (clumping) of the unknown organism.
- ▶ Antisera directed against the cell wall O antigens of *Salmonella* and *Shigella* are commonly used in hospital laboratories.



Slide Agglutination Test



Latex Agglutination Test

- ▶ Latex beads coated with specific antibody are agglutinated in the presence of the homologous bacteria or antigen.
- ▶ To determine the presence of the capsular antigen of *H. influenzae*, *N. meningitidis*, several species of streptococci, and the yeast *C. neoformans*.



Identification of Serum Antibodies with Known Antigens

- ▶ **Slide or Tube Agglutination Test**
 - ▶ Serial twofold dilutions of a sample of the patient's serum are mixed with standard bacterial suspensions. The highest dilution of serum capable of agglutinating the bacteria is the titer of the antibody.
 - ▶ As with most tests of a patient's antibody, at least a fourfold rise in titer between the early and late samples must be demonstrated for a diagnosis to be made.
 - ▶ Diagnosis of typhoid fever, brucellosis, tularemia, plague, leptospirosis, and rickettsial diseases.
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Immunochromatographic technique

- ▶ **Immunochromatographic technique (ICI) / Lateral flow system:** In this technique a capture antibody is immobilized on the surface of a porous membrane & sample passes along the membrane. Most tests are produced in strip & cassette form.
 - ▶ **Principle:** Antigen in the specimen first meets specific antibody, conjugated to colloid gold particles & antigen binds to antibody. The antigen antibody complex migrates up the strip where it becomes bound by line of specific antibody, producing a pink line. A further pink line is of control. The test results are available in 15-20 minutes. These are usually used for screening of different diseases.
 - ▶ **Clinical application of ICI:** Detection of:
 - ▶ HBsAg
 - ▶ Dengue IgM antibodies
 - ▶ Anti-HCV antibodies
 - ▶ Salmonella typhi IgM antibodies (Typhoid)
 - ▶ Anti-HIV antibodies
 - ▶ Malarial parasite
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Imunochromatographic strip

a

Positive

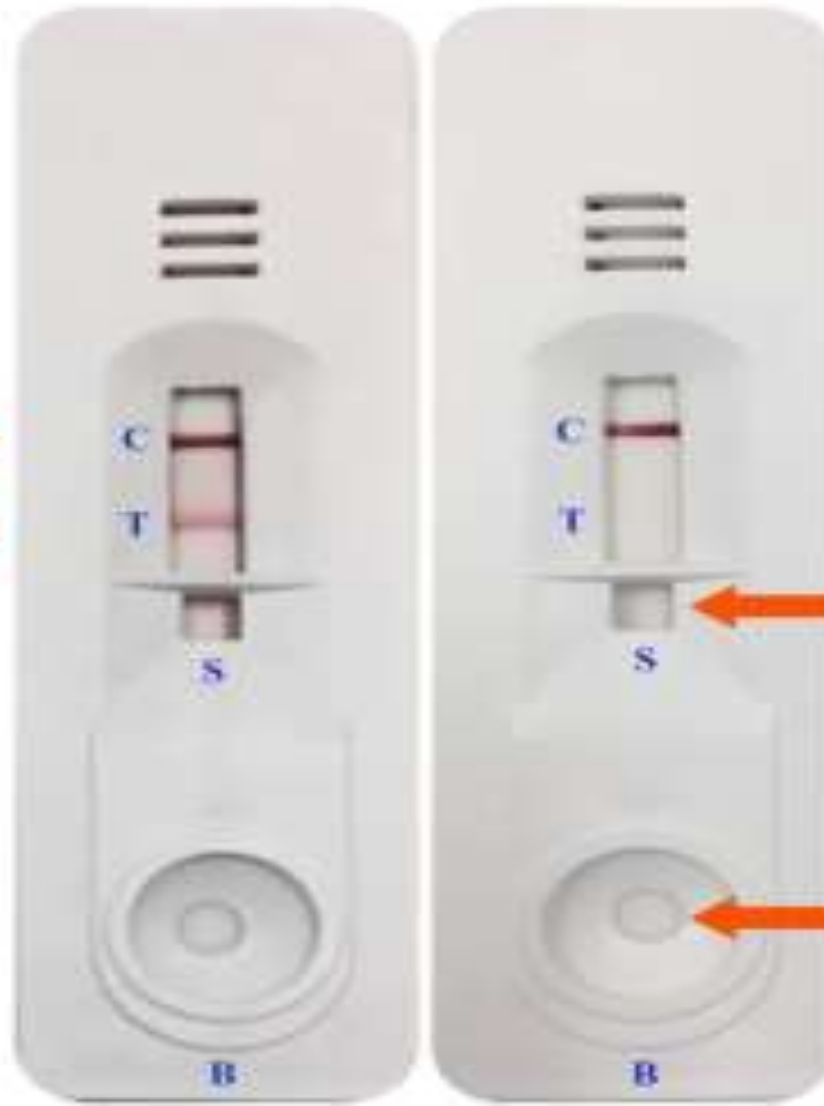
Negative

Control line

Test line

Sample

Buffer



Counter-Immunoelectrophoresis Test

- ▶ The unknown bacterial antigen and a known specific antibody move toward each other in an electrical field.
 - ▶ If they are homologous, a precipitate forms within the agar matrix.
 - ▶ Because antibodies are positively charged at the pH of the test, only negatively charged antigens, usually capsular polysaccharides, can be assayed.
 - ▶ Used to detect the presence in the spinal fluid of the capsular antigens of *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, and group B streptococci.
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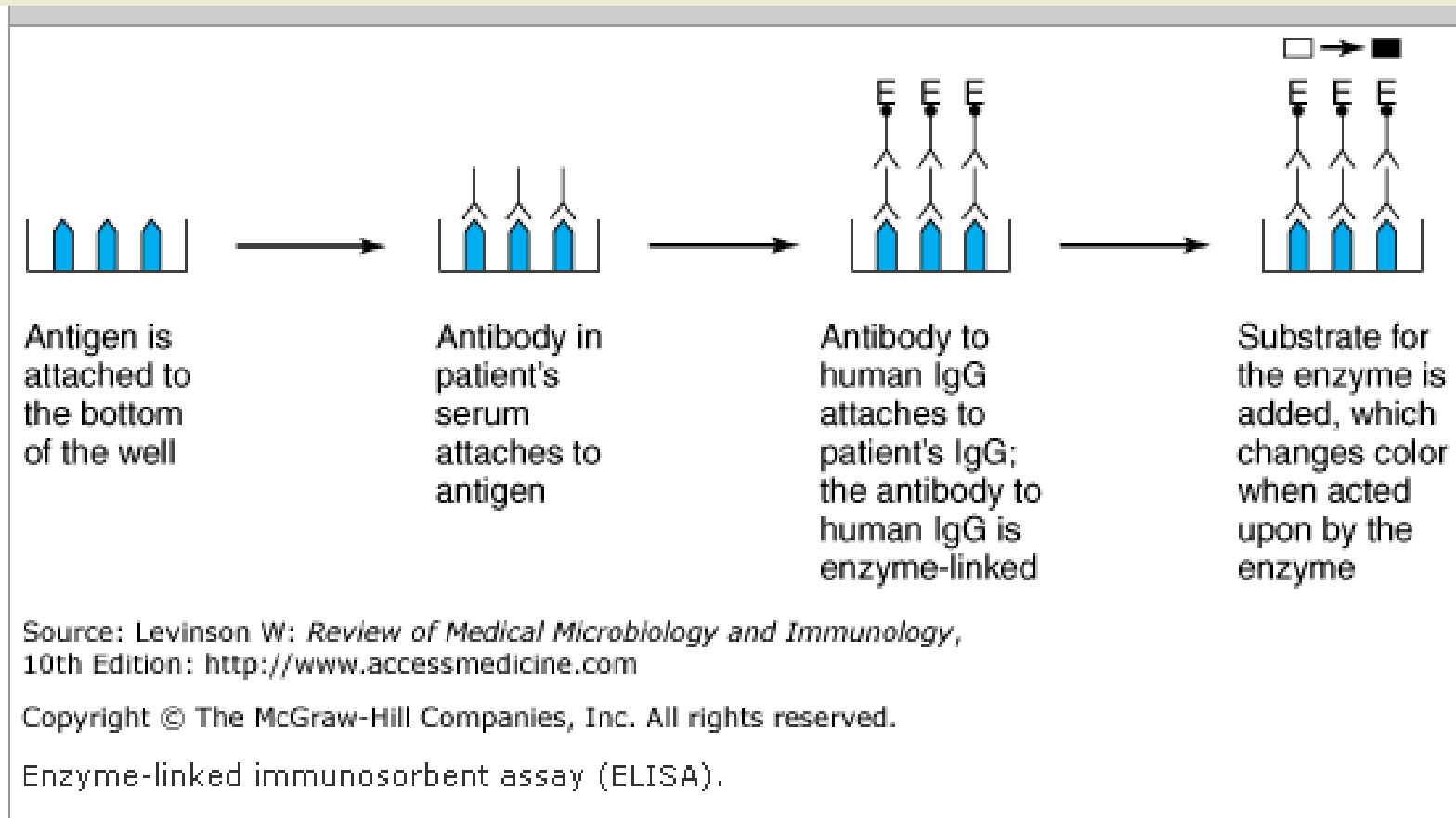


Enzyme-Linked Immunosorbent Assay

- ▶ A specific antibody to which an easily assayed enzyme has been linked is used to detect the presence of the homologous antigen.
- ▶ Useful in detecting a wide variety of bacterial, viral, and fungal infections.



Enzyme-Linked Immunosorbent Assay



Microtiter plate for ELISA

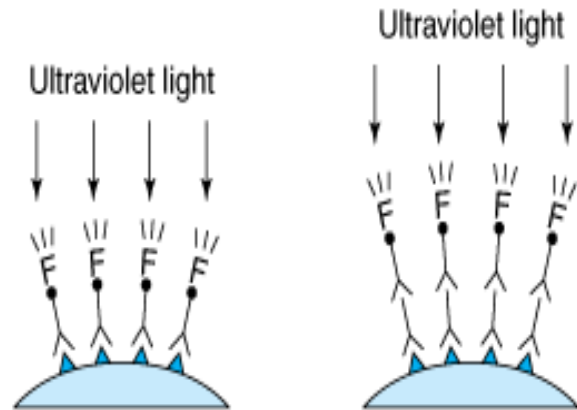


Fluorescent-Antibody Tests

- ▶ Exposure to known antibody labeled with fluorescent dye, which is detected visually in the ultraviolet microscope.
- ▶ Various methods can be used, such as the direct and indirect techniques



Fluorescent-Antibody Tests



A. Direct fluorescent-antibody test

B. Indirect fluorescent-antibody test

Source: Levinson W: *Review of Medical Microbiology and Immunology*, 10th Edition: <http://www.accessmedicine.com>

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Fluorescent-antibody test. **A:** In the direct fluorescent-antibody test, the fluorescent dye is attached directly to the antibody that is interacting with the antigen (dark triangles) on the surface of the cell. **B:** In the indirect fluorescent antibody test, the fluorescent dye is attached to antibody made against human IgG.



Serologic Tests for Syphilis

- ▶ The detection of antibody in the patient's serum is frequently used to diagnose syphilis, because *T. pallidum* does not grow on laboratory media.
- ▶ Two kinds of tests.
- ▶ The nontreponemal tests use a cardiolipin-lecithin-cholesterol mixture as the antigen, not an antigen of the organism. Flocculation (clumping) of the cardiolipin occurs in the presence of antibody to *T. pallidum*.
- ▶ The VDRL and RPR tests .
- ▶ The treponemal tests use *T. pallidum* as the antigen.
- ▶ The two most widely used treponemal tests are the
- ▶ FTA-ABS
- ▶ MHA-TP tests.



Cold Agglutinin Test

- ▶ Patients with *Mycoplasma pneumoniae* infections develop autoimmune antibodies that agglutinate human red blood cells in the cold (4°C) but not at 37°C.
- ▶ These antibodies occur in certain diseases other than *Mycoplasma* infections; thus, false-positive results can occur.



Nucleic Acid-Based Methods

- ▶ There are three types of nucleic acid-based tests used in the diagnosis of bacterial diseases:
 - ▶ nucleic acid amplification tests,
 - ▶ nucleic acid probes, and
 - ▶ nucleic acid sequence analysis.
- ▶ Much faster than culturing the organism.
- ▶ Especially useful for those bacteria that are difficult to culture such as *Chlamydia* and *Mycobacterium* species.
- ▶ Nucleic acid amplification tests utilize the PCR (polymerase chain reaction) or other amplifying process to increase the number of bacteria-specific DNA or RNA molecules so the sensitivity of the test is significantly higher than that of unamplified tests.



PCR Test

- ▶ Amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.



PCR Test

