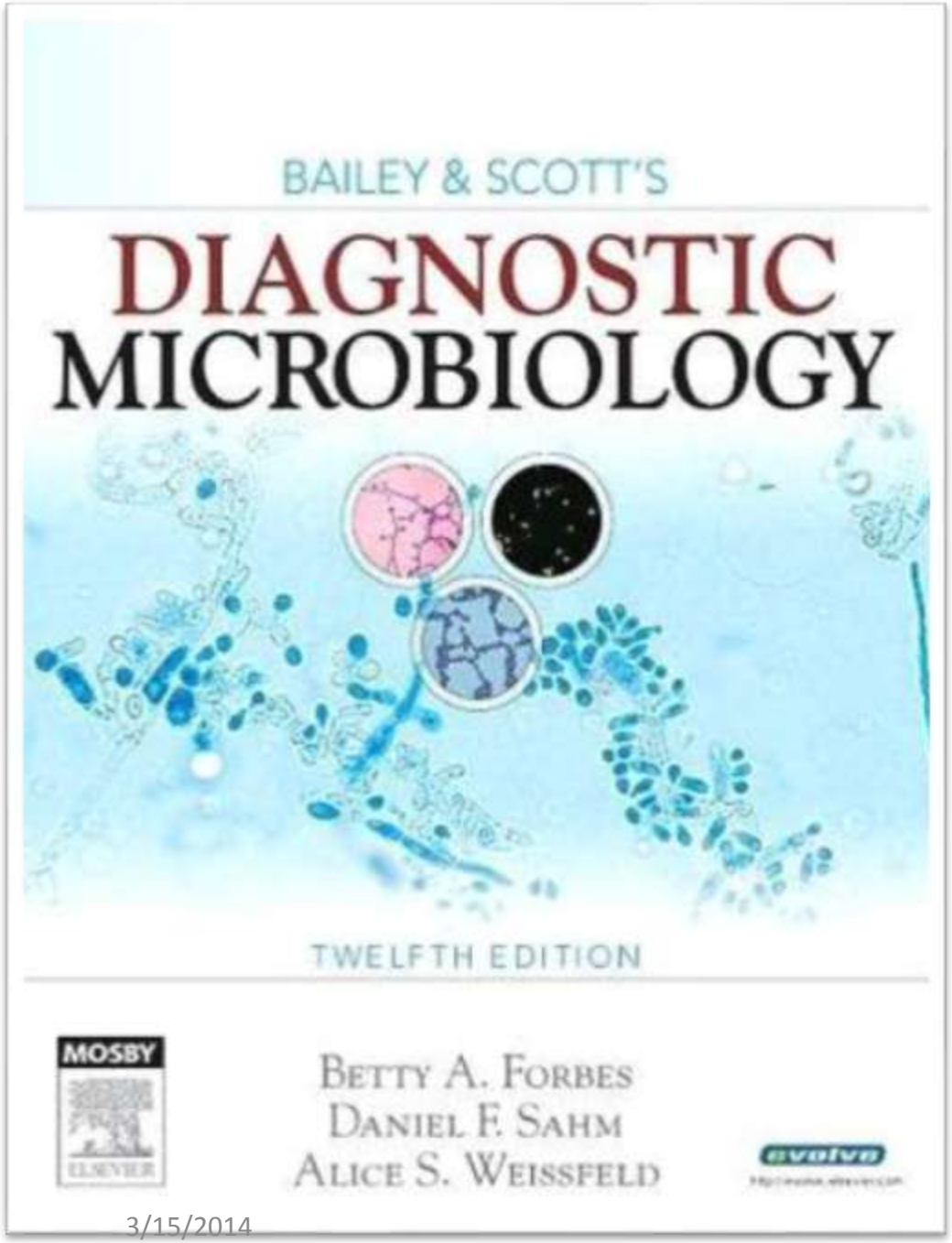


Diagnostic Microbiology

320 MIC

Lecture: 5

Identification of Microbes

Labeled Antibody Tests

- Use antibody molecules that are linked to some molecular “label” that enables them to be easily detected
- Used to detect either antigens or antibodies
- Extremely sensitive to detect trace antigens and antibodies

- **3 examples**

- **Enzyme-linked immunosorbent assay** (ELISA) –enzyme-antibody complex produces a colored product when an enzyme-substrate reaction occurs

– **Fluorescent antibody tests.**

– **Western blot test.**

Fluorescent – antibody test:

Fluorescent-antibody techniques use antibodies labeled with fluorescent dyes & detected by an U.V. microscope either directly or indirectly when antibody unites with antigen

- **If Ag is present fluorescence**
- **If Not No fluorescence**

Fluorescent – antibody test:

- **Direct fluorescent-antibody tests are used to identify specific microorganisms.**

–Tissue sample flooded with labeled antibody

–Antibody and antigen are allowed to bind for a short period

–Unbound antibody washed from the preparation

–Results observed under a fluorescent microscope

- Used to identify small numbers of bacteria in patient tissues

- **Indirect fluorescent-antibody tests are used to demonstrate the presence of antibody in serum.**
- Can be used to detect antigens in cells or patient tissues
- Also used to detect specific antibodies in serum via a two-step process

- **Disadvantage: expensive method (for each Ag we need specific labelled Ab).**

Group A streptococci from patient's throat

Fluorescent dye–labeled antibodies to group A streptococci

Fluorescent streptococci

(a) Reactions in a positive direct fluorescent-antibody test

T. pallidum from laboratory stock

Specific antibodies in serum of patient

Antibodies bind to *T. pallidum*

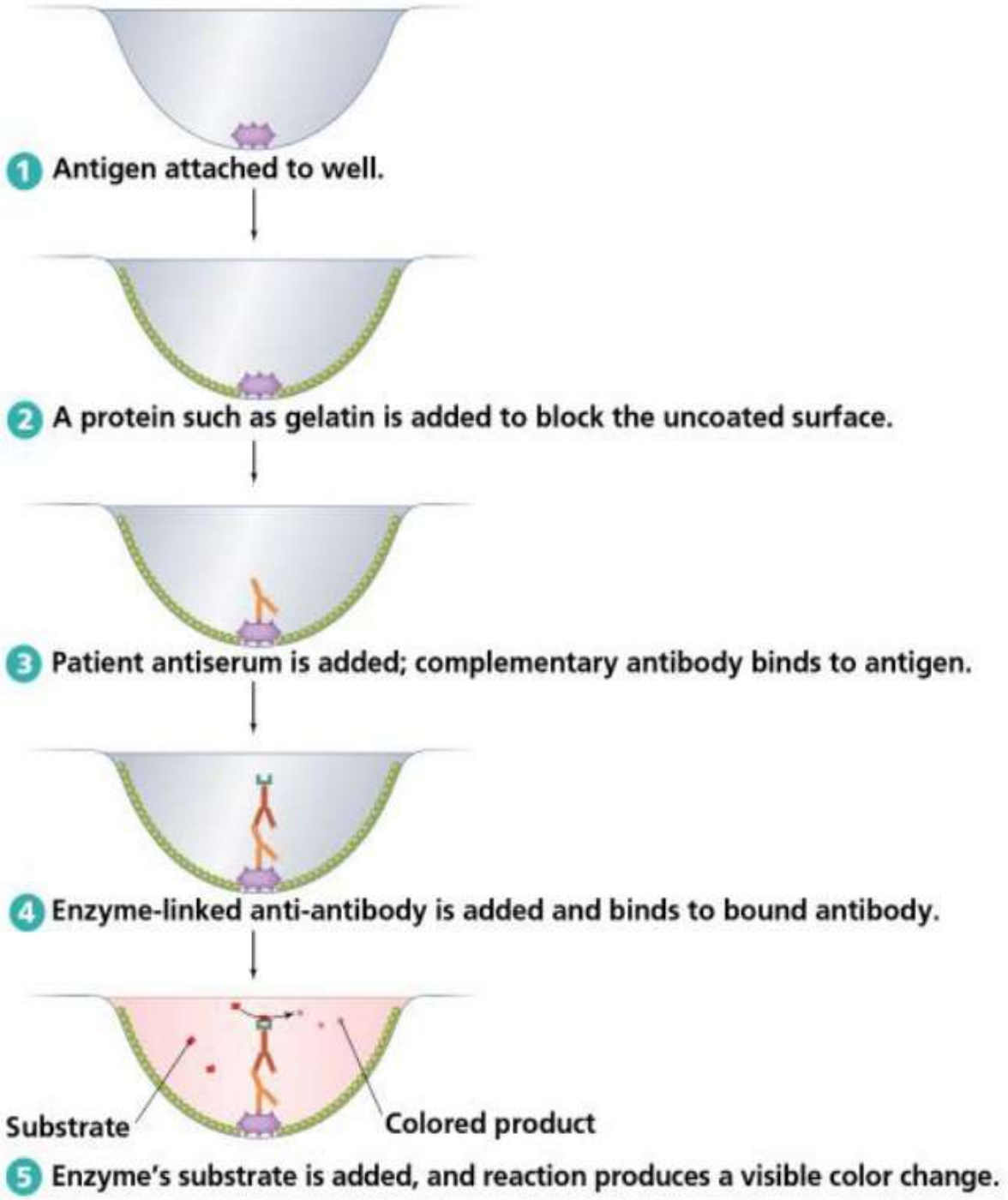
Fluorescent dye–labeled anti human immune serum globulin (This will react with any immunoglobulin)

Fluorescent spirochetes

(b) Reactions in a positive indirect fluorescent-antibody test

Enzyme-Linked Immunosorbent Assay (ELISA) Technique

- **ELISA:** an enzyme is linked to the known antibody and used to detect the homologous antigen.
- The principle of ELISA: is based on the high specificity of Antigen – antibody reactions and are detected by enzyme activity. An antibody linked to the indicator enzyme is added to the test well and is bound in the well if the antigen it is specific for is present. To determine whether or not the enzyme-linked antibody is bound in the well substrate for the enzyme is added. If the enzyme linked antibody is present the substrate is converted to a product that causes a color change.
- ELISA may be quantified by determining with a spectrophotometer the rate at which the enzyme converts a clear substrate to a colored product. The assay is called enzyme-linked immunosorbent assay. ELISA is now widely used in many areas in the medicines
- Most common form of ELISA is used to detect the presence of antibodies in serum



- **Advantages of The ELISA:**

-Can detect either antibody or antigen

-Can quantify amounts of antigen or antibody

-Easy to perform, inexpensive, and can test many samples quickly

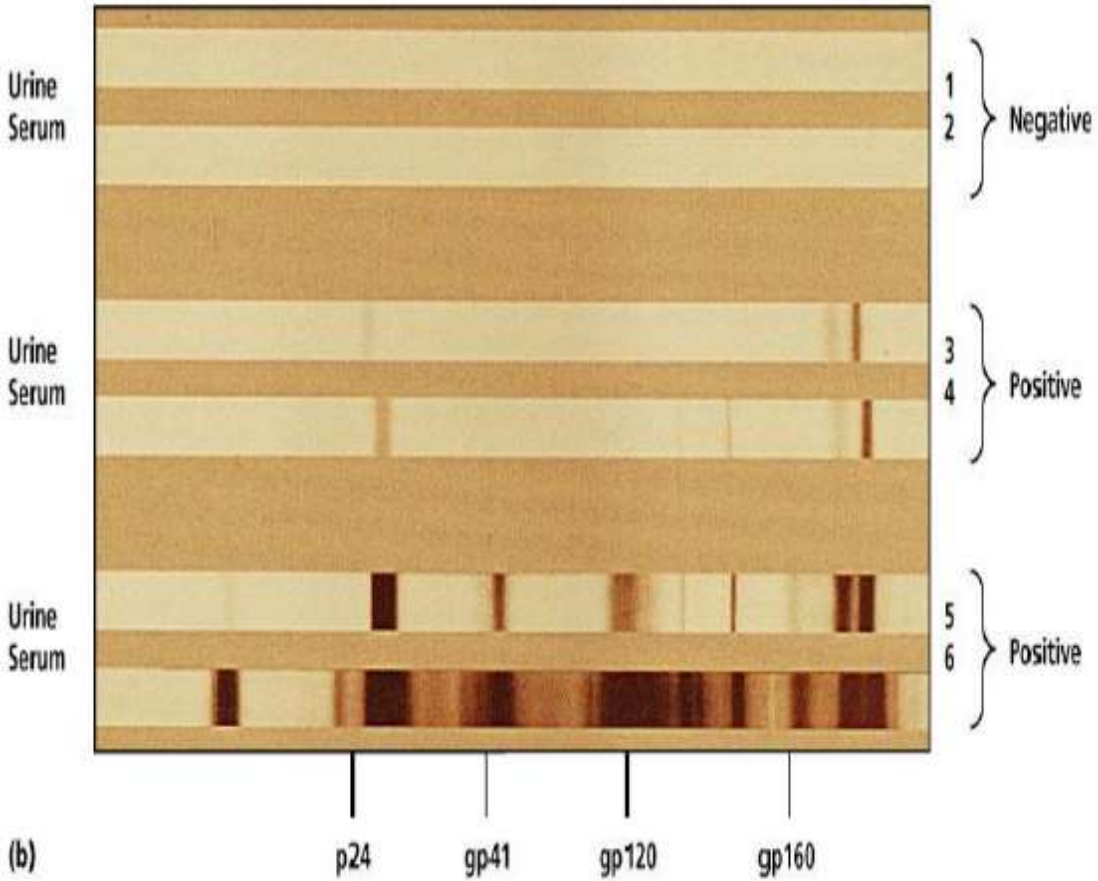
-Plates coated with antigen and gelatin -can be stored for later testing

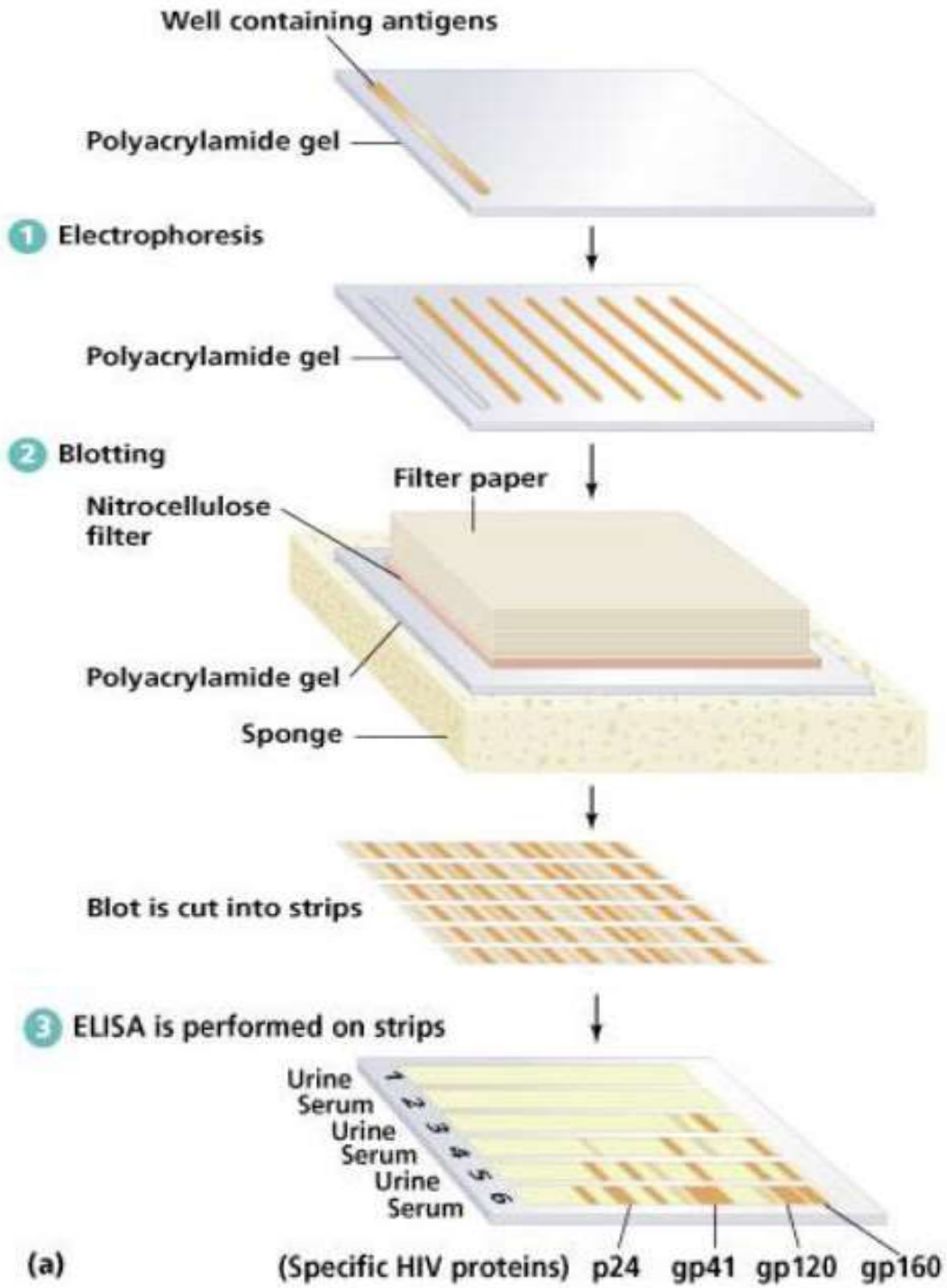
-Avoid the hazards of radioactivity.

ELISA kits are available for both clinical diagnostics and home use

Western Blot Test

- Technique for detecting antibodies against multiple antigens in a complex mixture.
- Can detect more types of antibodies and are less subject to misinterpretation than other tests.

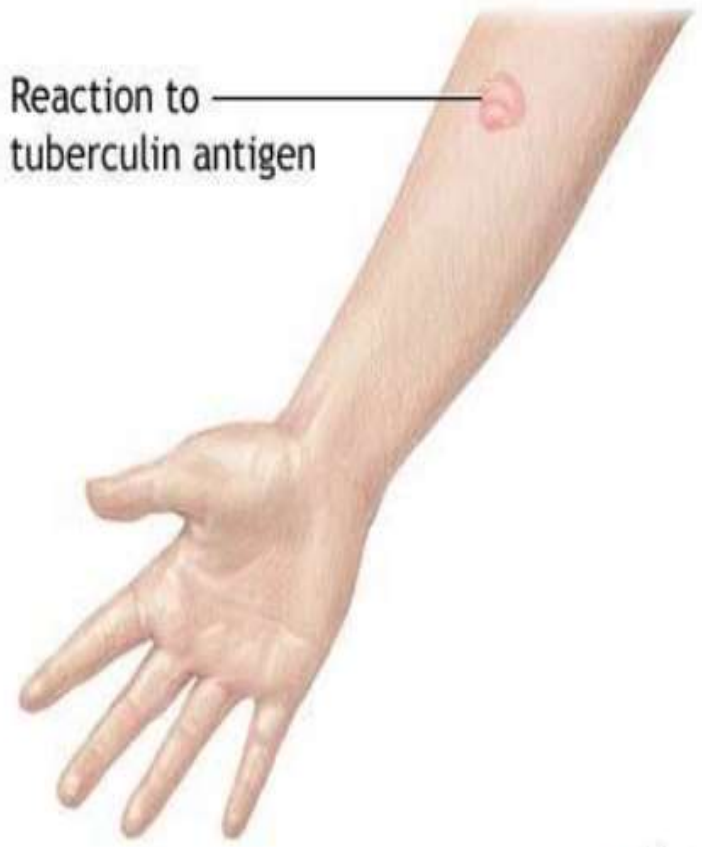




In Vivo Testing

- Antigens are introduced directly into the body to determine the presence or absence of antibodies

- –Tuberculin skin test, allergy testing



Recent Developments in Immune Testing

- Development of simple immunoassays that give results in minutes.
- Generally are useful in determining a preliminary diagnosis.
- Most common are:

– **Immunofiltration**

- Rapid ELISA that uses antibodies bound to membrane filters rather than polystyrene plates.
- Membrane filters have a large surface area making the assay quicker to complete.

– **Immunochromatography**

- Very rapid and easy to read ELISAs.
- Antigen solution flows through a porous strip where it encounters antibody labeled with either pink colloidal gold or blue colloidal selenium.
- Antigen-Antibody immune complexes flow through a region and encounter antibody against them, resulting in a visible pink or blue line.
- Used in pregnancy testing to detect human growth hormone.

Test	Use
Immunodiffusion (precipitation)	Diagnosis of syphilis, pneumococcal pneumonia
Immunoelectrophoresis (precipitation)	Assay production of particular classes of antibodies
Agglutination	Blood typing; pregnancy testing; diagnosis of salmonellosis, brucellosis, gonorrhea, rickettsial infection, mycoplasma infection, yeast infection, typhoid fever, meningitis caused by *Haemophilus*
Viral neutralization	Diagnosis of infections by specific strains of viruses
Viral hemagglutination inhibition	Diagnosis of viral infections including influenza, measles, mumps, rubella, mononucleosis
Complement fixation	Diagnosis of measles, influenza A, syphilis, rubella, rickettsial infections, scarlet fever, rheumatic fever, infections of respiratory syncytial virus and *Coxiella*
Direct fluorescent antibody	Diagnosis of rabies, infections of group A *Streptococcus*
Indirect fluorescent antibody	Diagnosis of syphilis, mononucleosis
ELISA	Pregnancy testing; presence of drugs in urine; diagnosis of hepatitis A, hepatitis B, rubella; initial diagnosis of HIV infection
Western blot	Verification of infection with HIV, diagnosis of Lyme disease