Diagnostic tests for antibody or antigen

- ELISA
- Immunofluorescence
- Flow cytometry
- Western blotting
**ELISA**

- We use an antibody that’s bound covalently to an enzyme to bind:
  - antigen (direct ELISA)
  - antibody that is bound to antigen (indirect ELISA)

- After washing, we add a colorless substrate for the enzyme in a reaction that produces color (qualitative detection)
  ...quantitative measurement can also be done by certain techniques

- The enzyme is usually ALK (alkaline phosphatase) or HRP (horseradish peroxidase)

- Example: Detection of antibodies to HIV
Immunofluorescence

• We use antibody that is bound to a fluorochrome (fluorescent compound)...usually fluorescein isothiocyanate (FITC)

• Fluorescent compound emits a greenish light when exposed to UV light...we use fluorescence microscope which provides UV light

• Usually done on tissues and cells to detect antigens (direct) or antibodies (indirect)

Fluorescent treponemal antibody absorption (FTA-ABS) test

Treponema pallidum spirochetes
Flow cytometry

• To enumerate live cells that express certain antigen

• Fluorochromes of different colors are used

• Counting cell populations with certain fluorochrome label

• Separating populations of cells according to physiochemical and fluorescence characteristics
Western blotting

- Protein antigens (e.g., of known HIV) are separated by SDS-PAGE (SDS-polyacrylamide gel electrophoresis) according to molecular weight and charge

- The separated proteins are transferred to a nitrocellulose membrane (blot)

- The blot is incubated with the antiserum, washed and an enzyme-coupled anti-Ig is then added

- The antigen bands that reacted with the antibody are colored