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

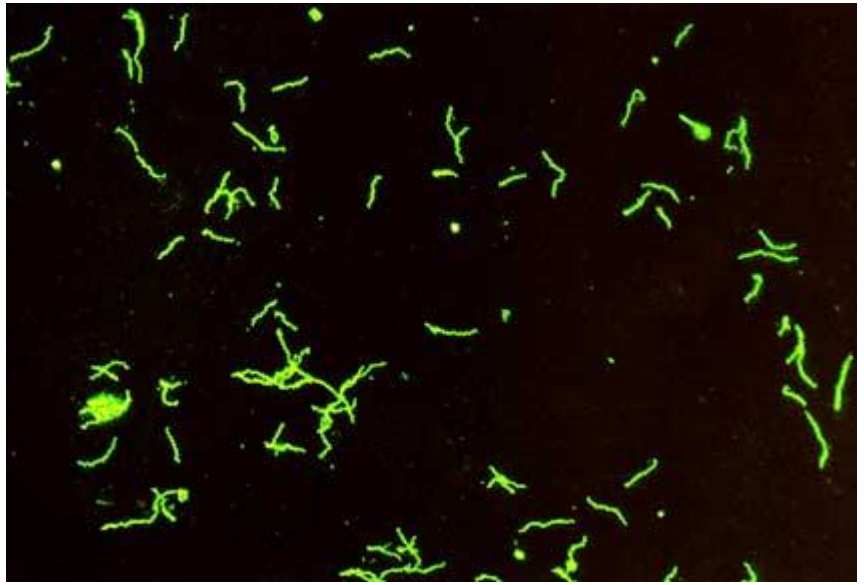
- The enzyme is usually ALK (alkaline phosphatase) or HRP (horseradish peroxidase)

- 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