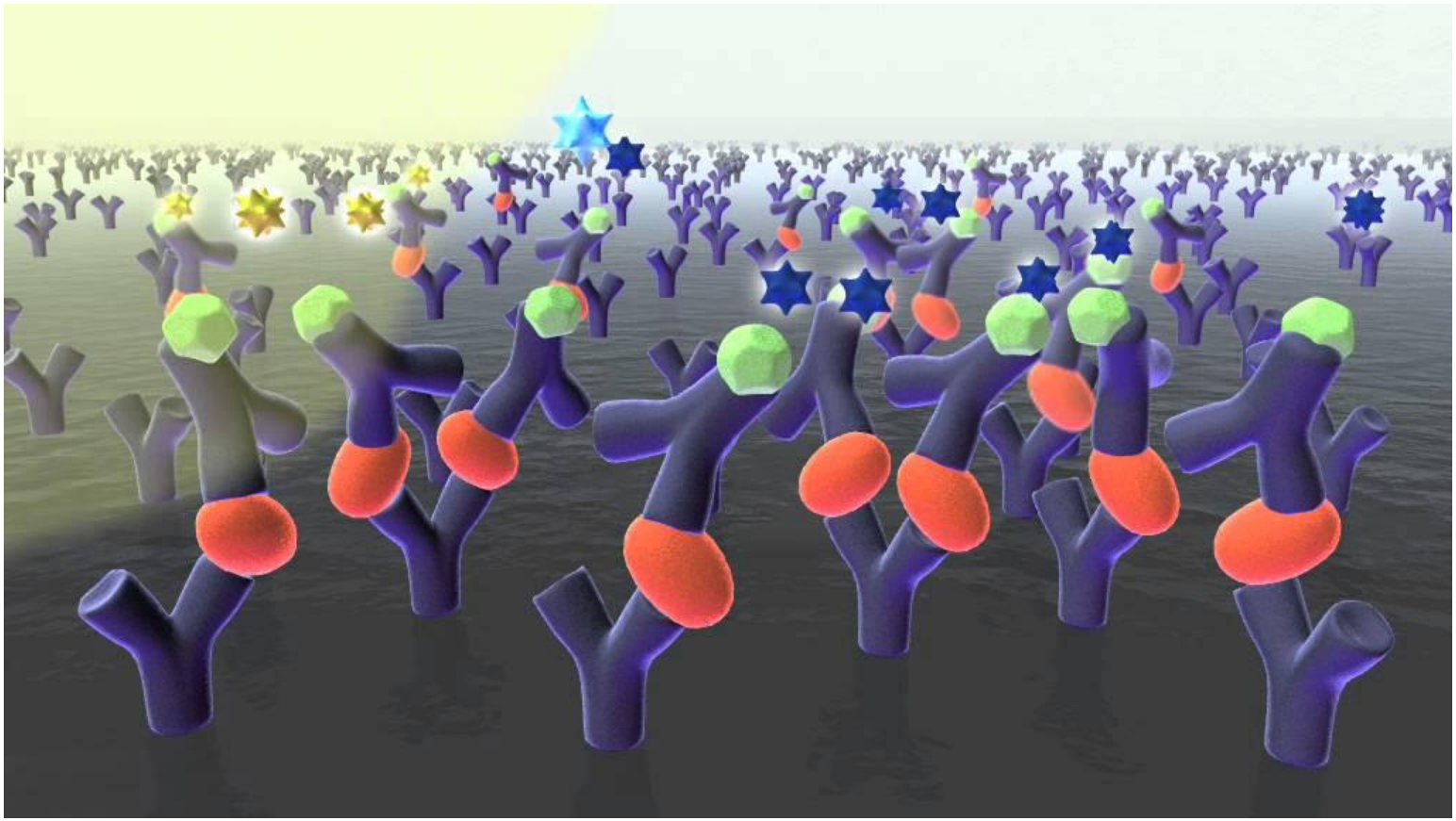
ELISA

Enzyme-linked immunosorbent assay



Overview

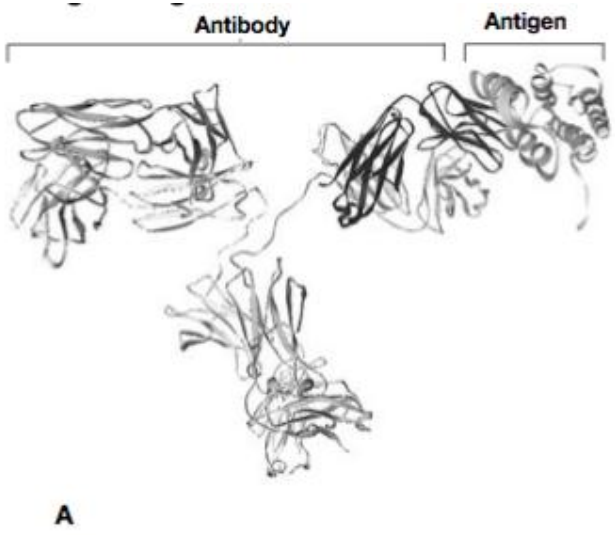
- Immunoassay

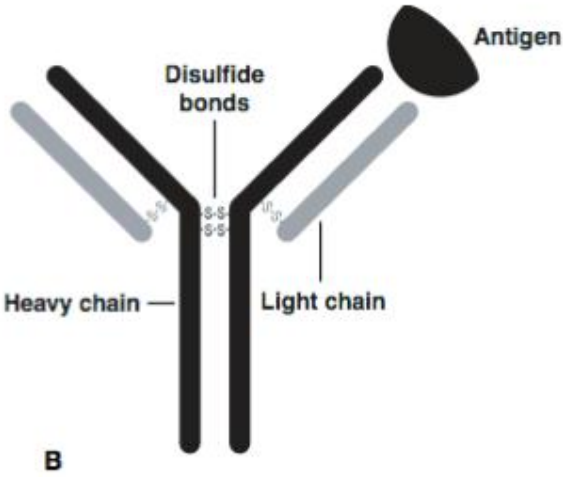
  a laboratory technique that makes use of the binding between an antigen and its homologous antibody in order to identify and quantify the specific antigen or antibody in unknown sample.

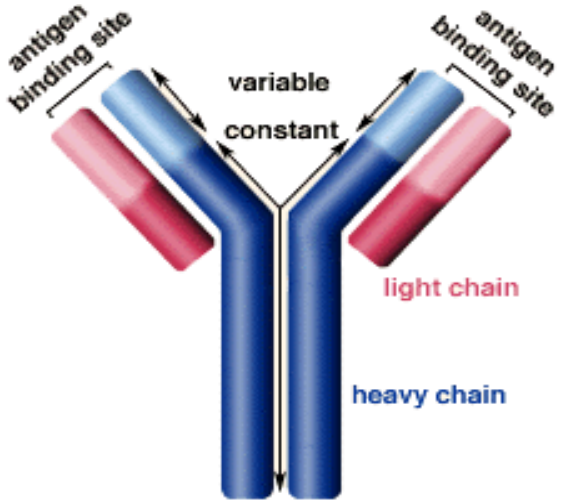
- Antigens

  a substance that when introduced into the body stimulates the production of an antibody

- Antibodies (also known as **immunoglobulins** abbreviated **Ab**)
- globulin proteins that make up to 15% of your total blood serum protein, and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.
- very specific; each Ab recognizes only a single antigen.







ELISA

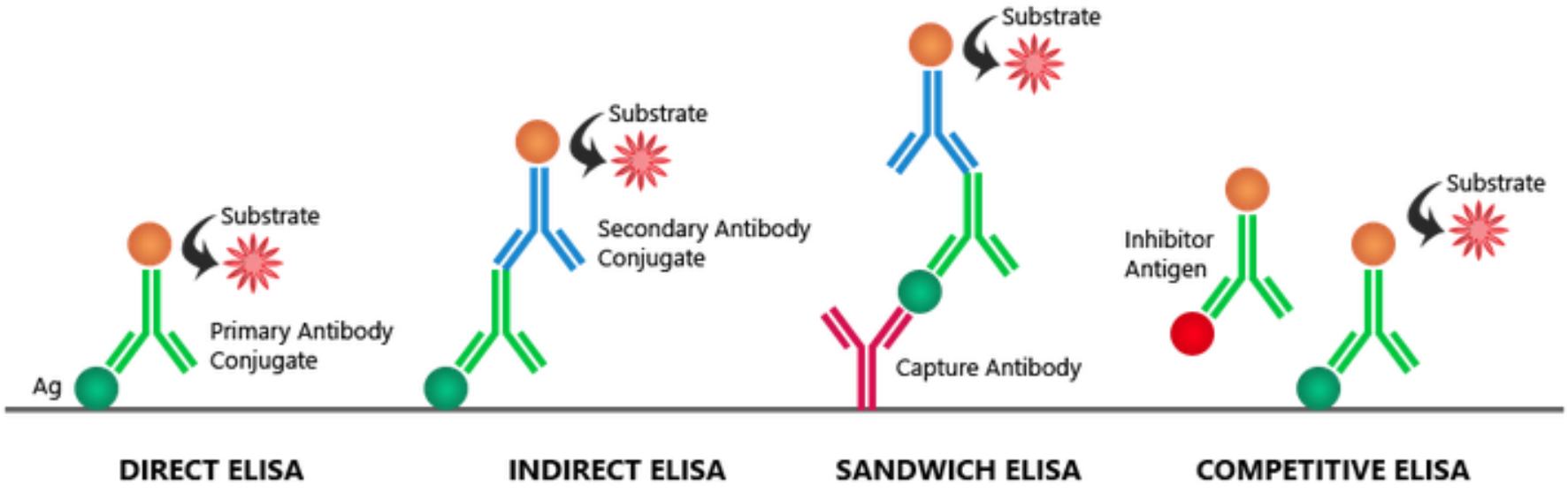
- Is the most common immunoassay type

- Applications:
  - diagnostics, in detecting cases of infection with viruses, e.g. HIV, SARS, tuberculosis
  - research

- Materials to be tested:
  - serum
  - purified antigen
  - hormones
  - cytokines
  - urine

Four most common ELISA types:

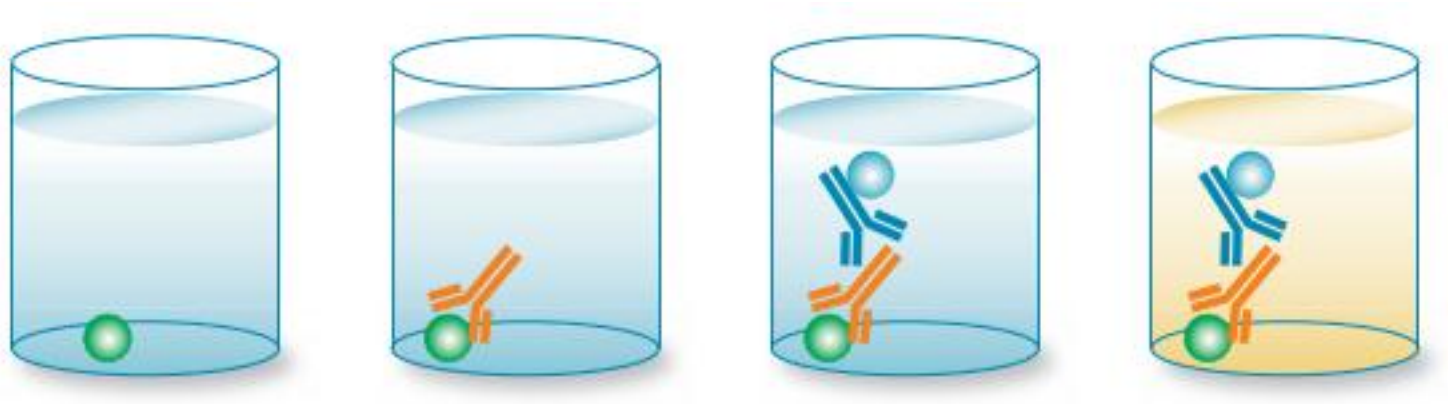
1) Direct
2) Indirect
3) Sandwich
4) Competitive



1) Direct ELISA

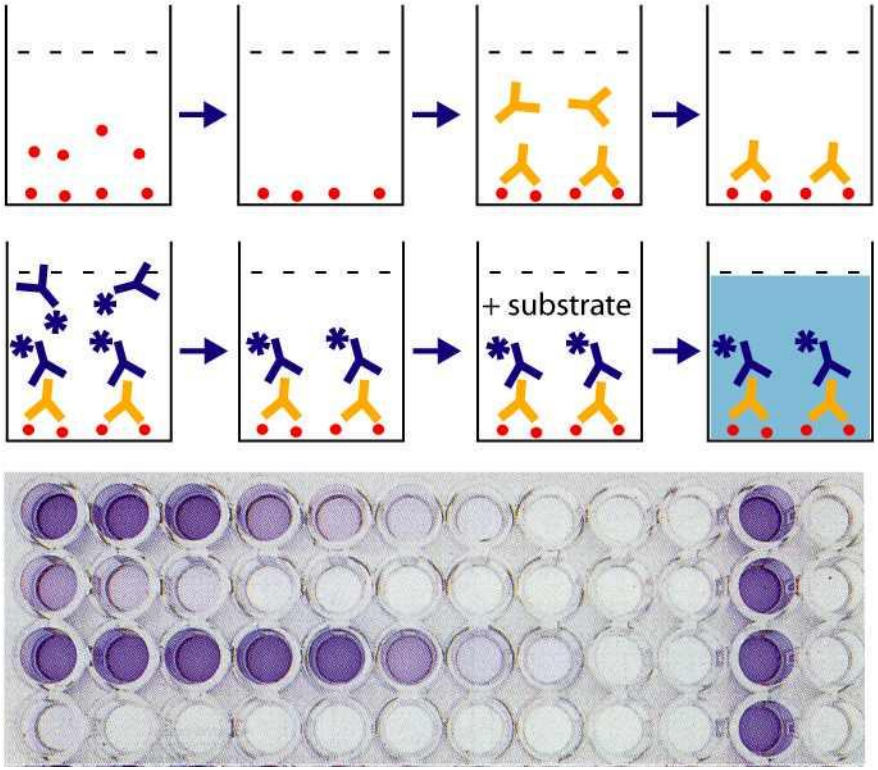
- is less prone to error, and direct i.e. no potentially cross-reacting secondary Ab needed.
- the antigen immobilization is not specific, higher background noise may be observed in comparison to indirect ELISA.
- as no secondary Ab is used there is no signal amplification, which reduces assay sensitivity.

2) Indirect ELISA



- detection is a two - step process
- high sensitivity - more than one labeled secondary ab can bind the primary
- economical - fewer labeled antibodies are needed
- greater flexibility - different primary antibodies can be used with a single labeled secondary ab.

Indirect ELISA

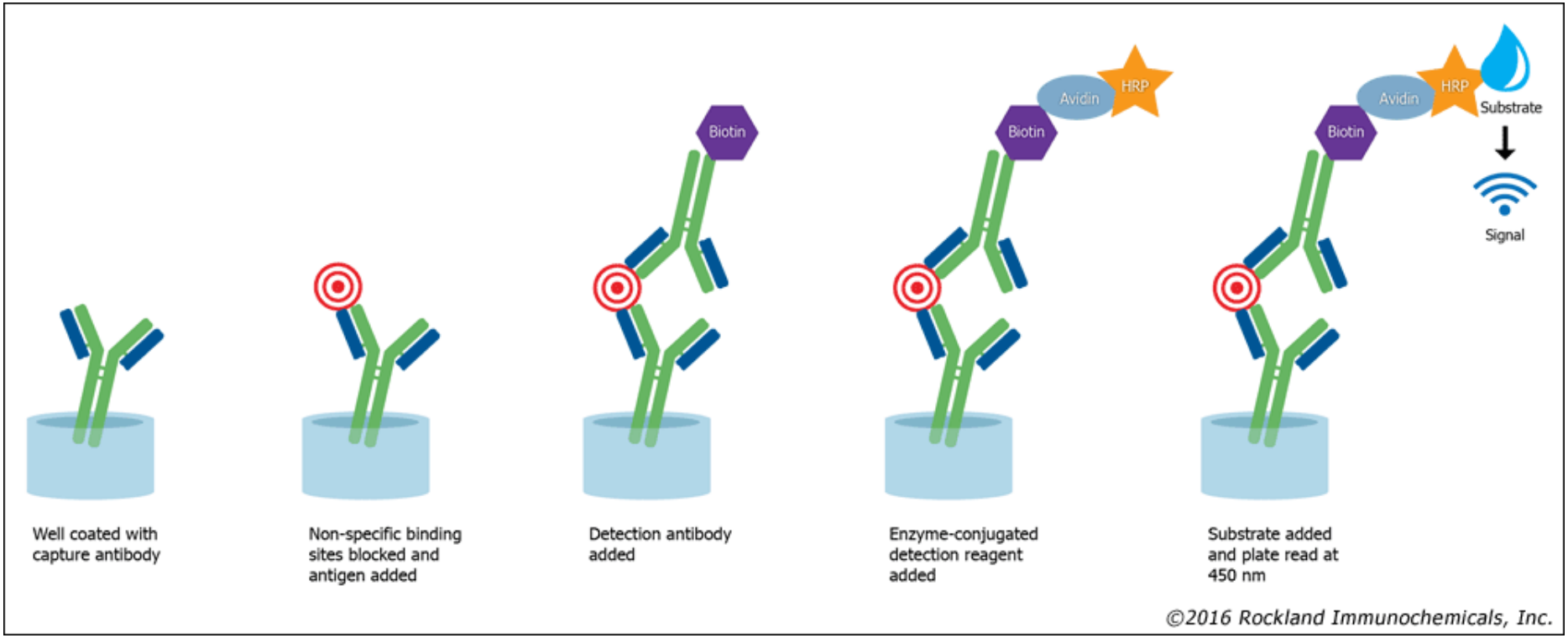


Video

Sandwich ELISA

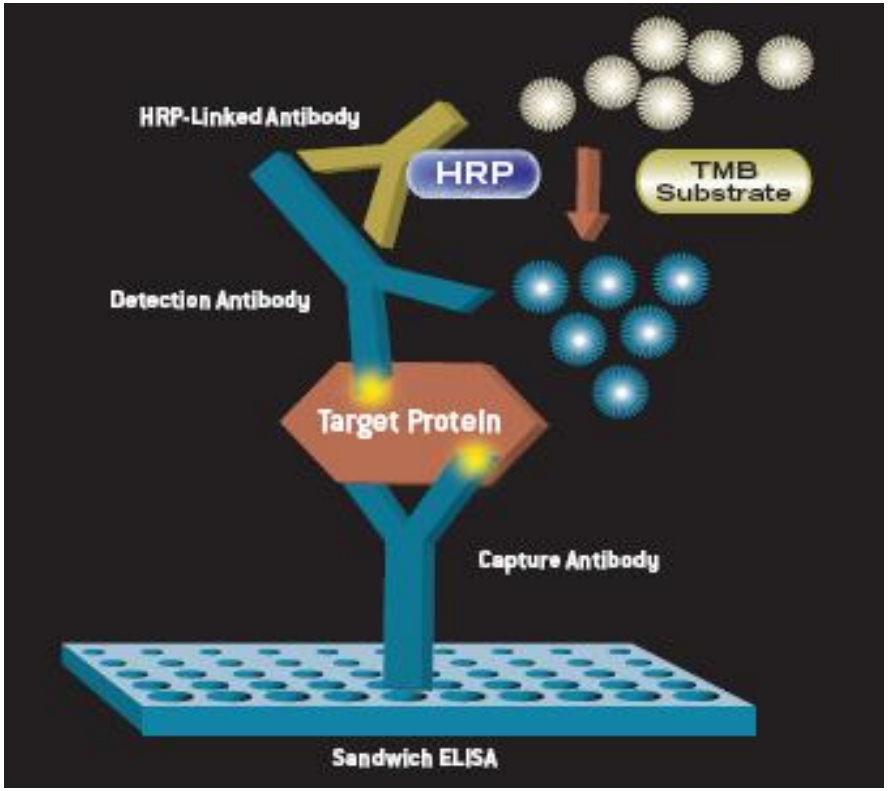
- it is 2-5 times more sensitive than direct or indirect ELISAs.
- delivers high specificity as two Abs are used to detect the Ag.
- is particularly suited to the analysis of complex samples, since the antigen does not need to be purified prior to measurement.

Sandwich ELISA



Sandwich ELISA

- The ELISA plate is coated with antibody to detect specific antigen



Competitive ELISA

Coat well with known antigen and block as usual

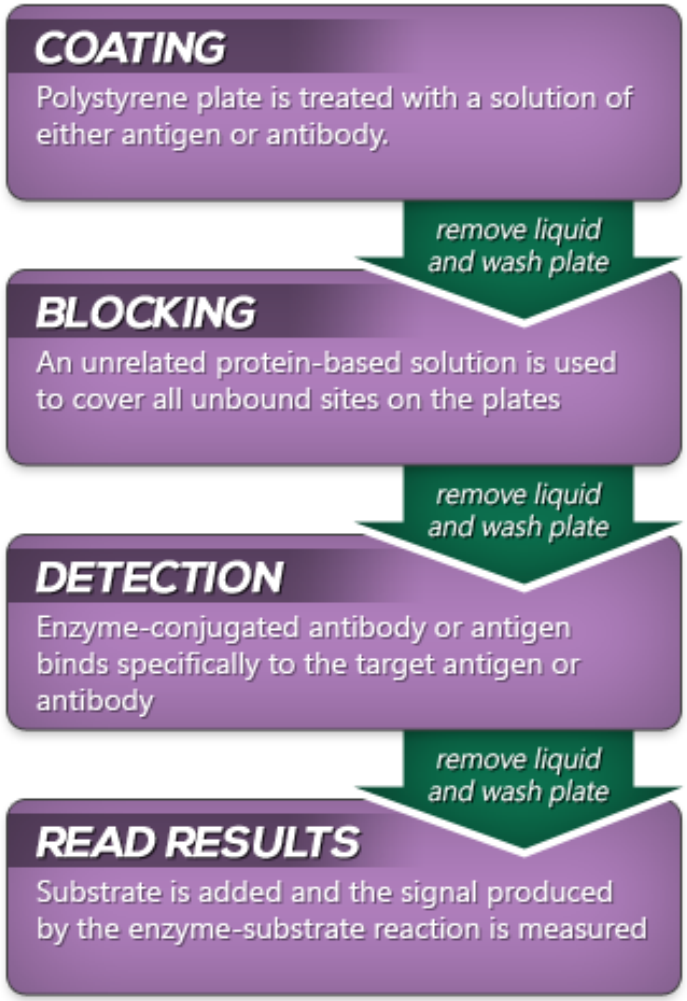
Add sample containing unknown antigen

Add labeled detection antibody

Detection antigen bound by antigen in a sample is washed away

No signal results because no labeled detection antibody has bound. his indicates a high level of antigen in the sample

Assay that is used to determine the amount of HIV p24 protein in a patient's serum



Antigen is added to the wells and Incubated to allow binding.

Unbound Ag is washed from the wells with detergent (blocking reagent)

Primary Ab solution is added to the wells and incubated.

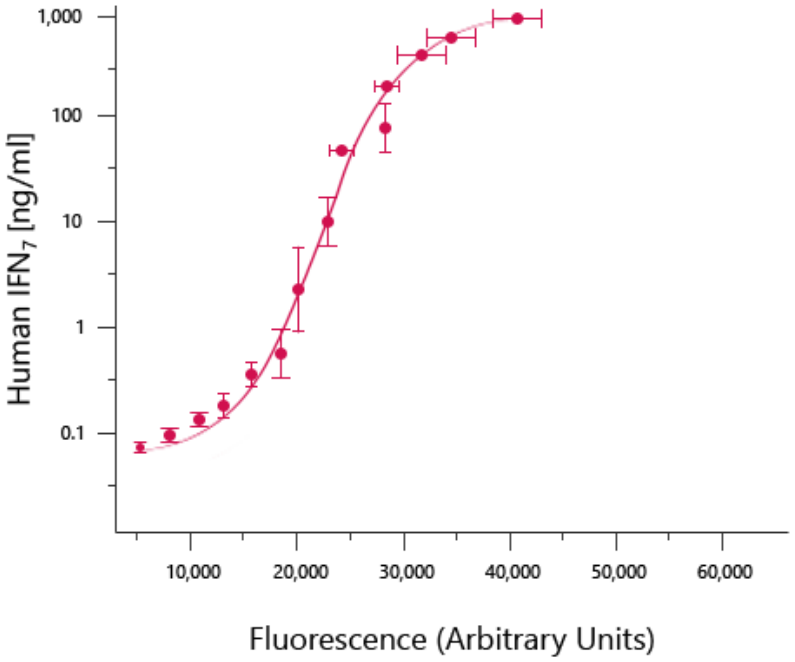
Unbound Ab is washed from the wells.

Enzyme-labeled (horseradish peroxidase) secondary Ab is added to the wells and incubated.

Unbound Ab is washed from the wells.

Chromogenic (color-producing) enzyme substrate (H_2O_2) is added to the wells and incubated to allow color to develop.

ELISA data interpretation



A. Quantitative: in comparison to a standard curve (a serial dilution of a known, purified antigen)

B. Qualitative: used to achieved a YES or NO answer indicating whether a particular Ag is present in a sample.

Post-Lab Questions?

1. Did your sample contain the antigen?

2. Why did you need to wash the wells after every step?

3. When you added primary Ab to the wells, what happened if your sample contained the antigen?

4. When you added secondary Ab to the wells, what happened if your sample contained the Ag?

5. If the sample gave a negative result for the antigen, does this mean that the Ag is not present? What reasons could there be for a negative result when the the Ag is actually present?

6. Why did you assay your samples in triplicate?

7. What antibody-based tests can you buy at your local pharmacy?

What problems can prevent the immune system from working properly?

1) Hypersensitivity (when immune system overreacts to an antigen)

2) Immunodeficiency (can be genetic or induced by a disease, or by immunosupressive drugs)

3) Autoimmune disease (inappropriate an immune response to itself)