Enzyme-linked Immunosorbent Assay (ELISA)

Subject: Vertebrate Immunology
Subject Code: MZOO-401

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Enzyme-linked Immunosorbent Assays (ELISAs)

The ELISA technique was first conceptualized and developed by **Peter Perlmann** and **Eva Engvall** at Stockholm University, Sweden.

ELISA is a type of immunoassay.

An **immunoassay** is a highly selective bioanalytical method which is based on the *interactions between antigen and antibodies*.

Antibody (=antiserum): proteins produced by host immune system to defend against foreign molecules

Antigen, e.g., bacteria, virus, pollen, etc.

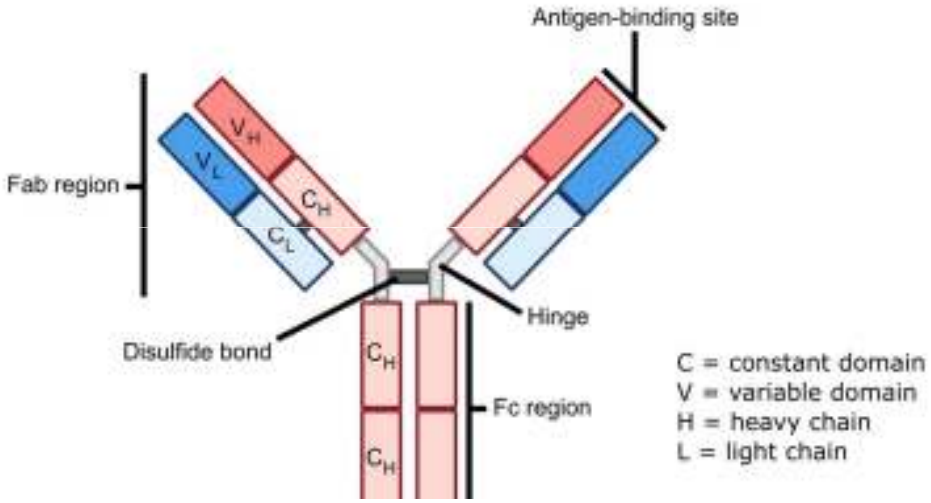
(Purpose in host body: Immune complex ---------> Transported to cellular systems --------> Destruction or Deactivation.)

ELISA is used to visualize and quantify antigens.

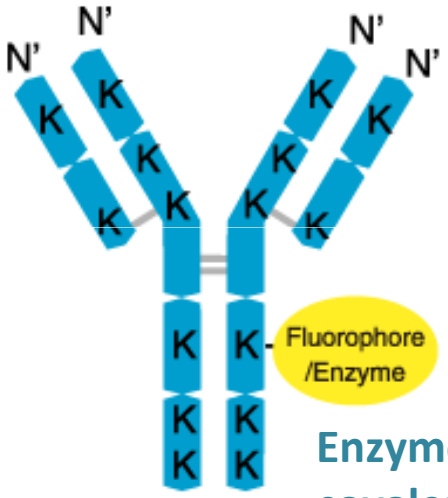
Antigen or antibody in ELISA: adsorbed onto the plastic surface (***sorbent***).

The tests use an **antibody** *conjugated to an* **enzyme** (commonly **Alkaline phosphatase, Horseradish peroxidase,** or **β-galactosidase** enzyme**).**

Antibody



Enzyme linked Antibody

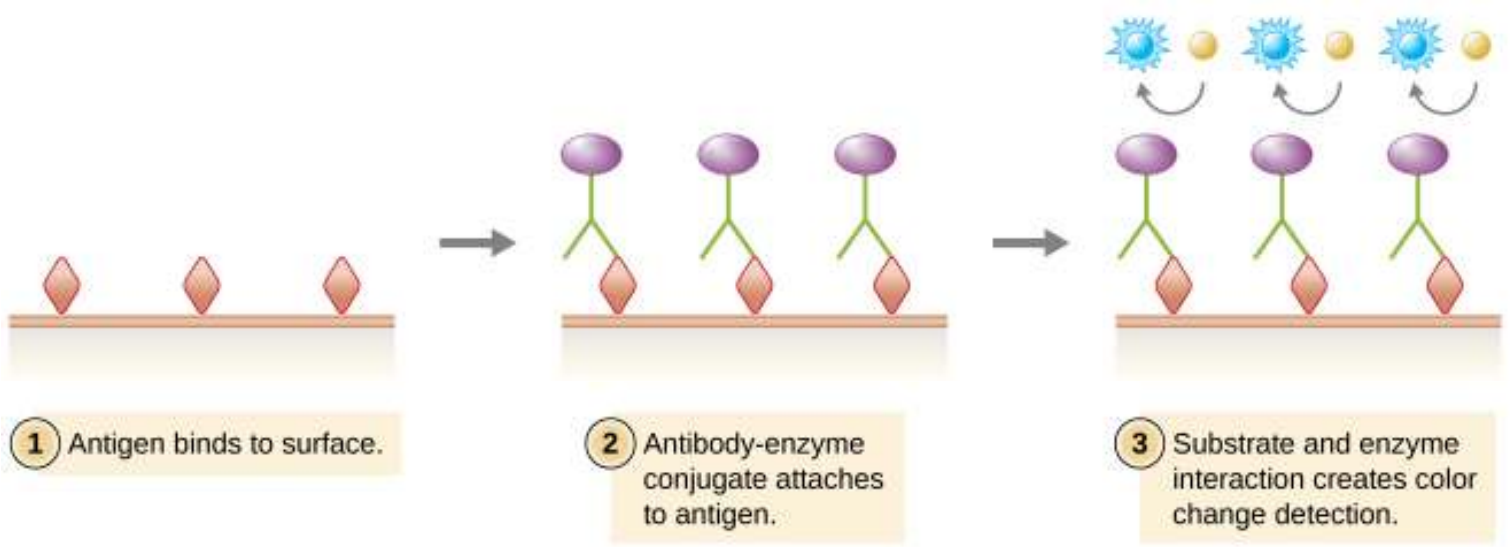


Enzyme is bound **covalently** to the **Constant** region of antibody.

The **enzyme** converts a substrate into an observable end product.

Substrate may be either: a **chromogen** (colored end product) or a **fluorogen** (non-fluorescent molecule is converted to the fluorescent product).

Reaction between E and S: Product (P) is **visualized** and **quantified**.



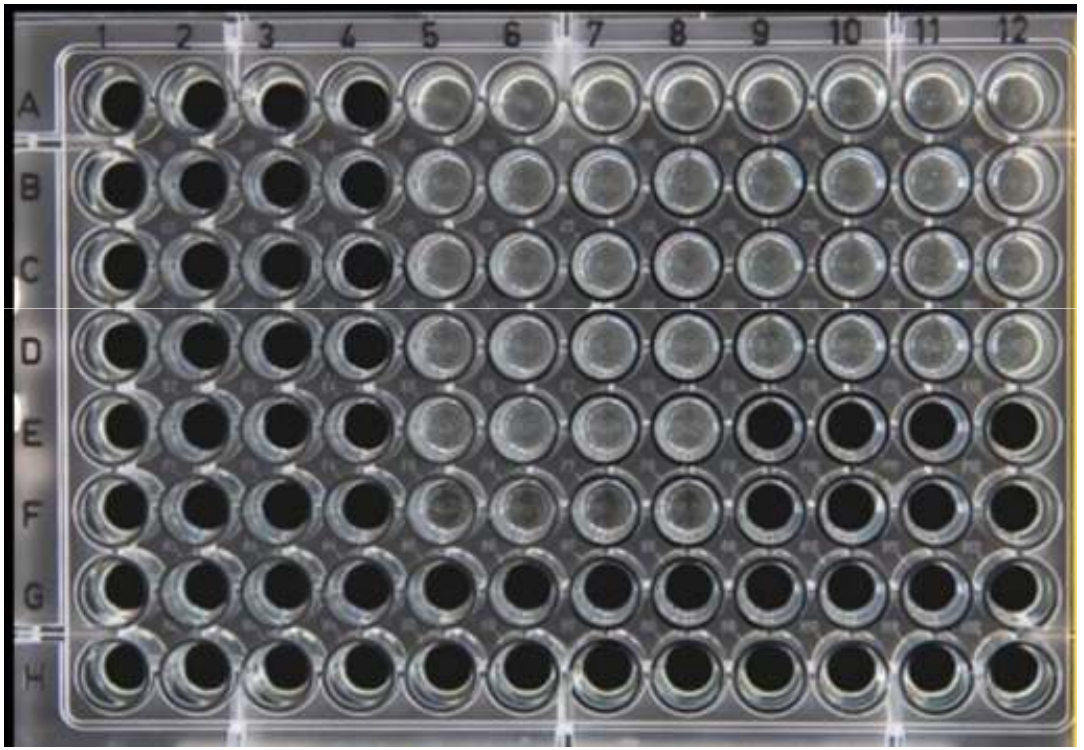
Amount of the color/ fluorescence produced: **proportional to** Ag-Ab interaction.

Amount of color produced: determined by **spectrophotometer**.

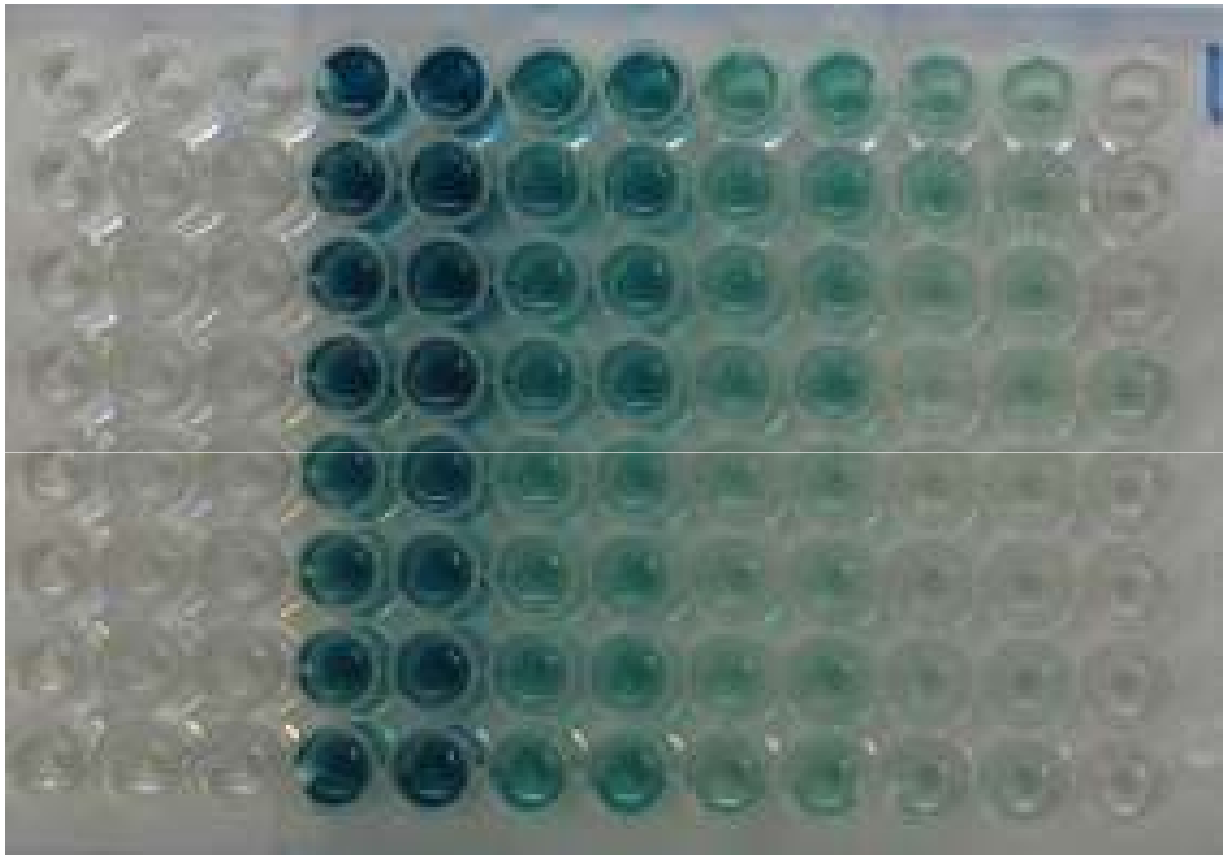
Amount of fluorescence produced: determined by **fluorescence microscope or spectrophotometer .**

Samples routinely used in ELISAs: serum, plasma, cell culture supernates, cell lysates, saliva, tissue lysates, and urine.

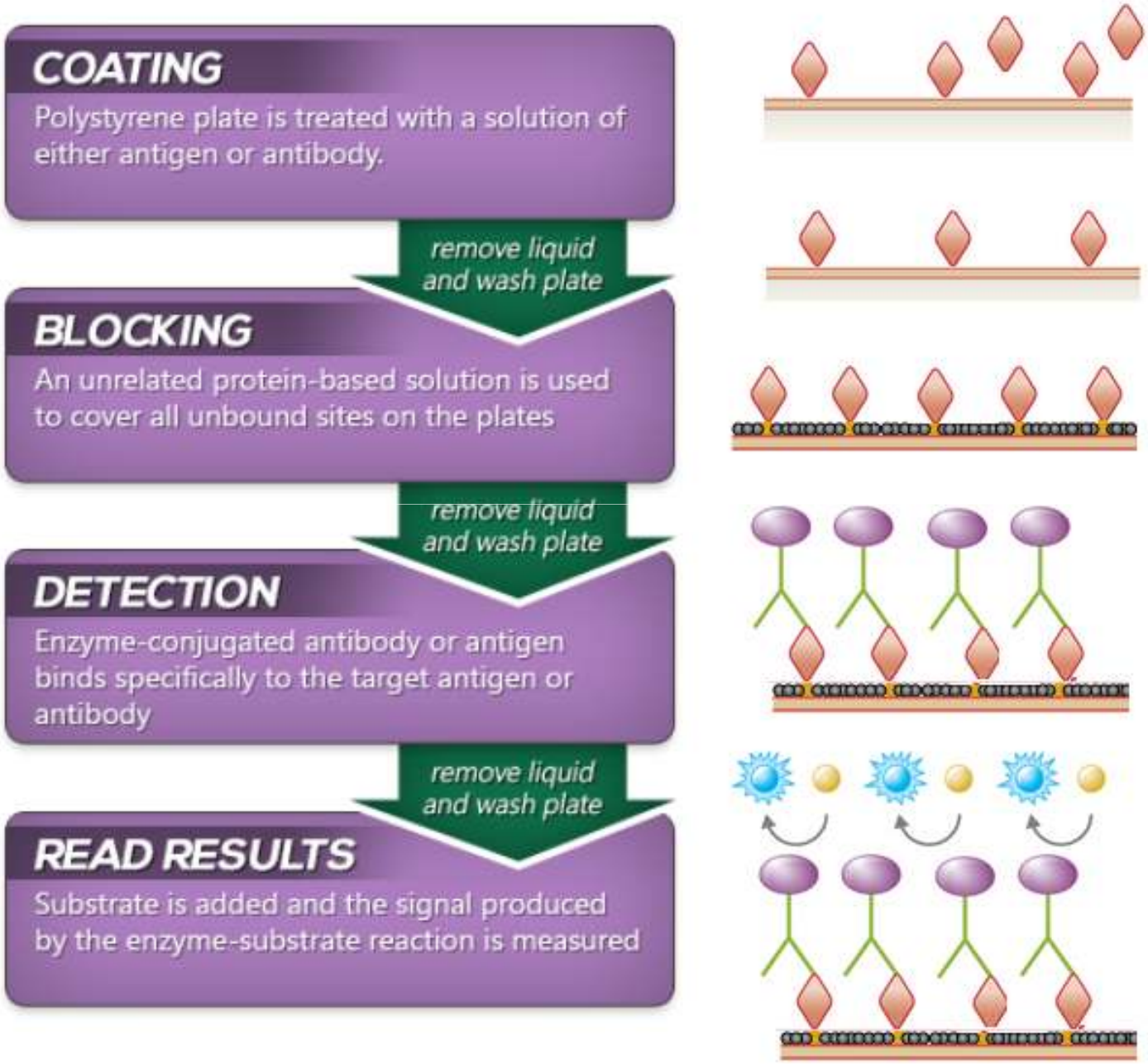
ELISAs are usually run in **96-well (12 columns x 8 rows) microplates.**



Colored end product visible in microtiter plate.



Procedure/ Steps:



Wash steps:

- *Critical* (only specific high-affinity binding events are maintained)
- Fill well with PBS (phosphate buffered saline) containing small amount of a non-ionic detergent such as Tween-20.
- Repeated 3-5 times between each step (to thoroughly remove unbound material).
- Reduce background signal (due to presence of unbound, conjugated antibody) and thereby increase the assay's signal-to-noise ratio.

Blocking:

- Prevents nonspecific binding of the antibodies.
- Uses specific blocking buffers (commonly bovine serum albumin or **BSA**, **non-fat dry milk**, and **whole normal serum**), etc.

Four types of ELISA tests

Direct ELISA

Antigen is attached to the microtiter plate.

↓

Enzyme-labeled antibody (primary) is added.

↓

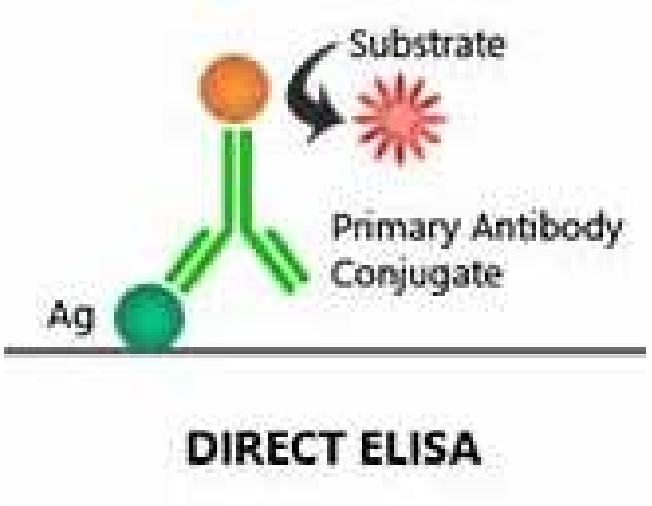
Ag-Ab interaction occurs

↓

Substrate is added

↓

Product is measured



Indirect ELISA

Attachment of Antigen to the microtiter plate.

↓

Addition of unlabeled primary antibody is added.

↓

Ag-Ab interaction occurs.

↓

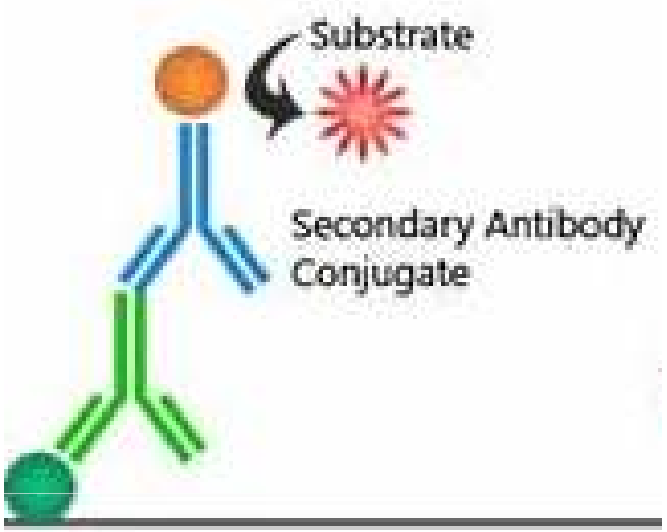
Addition of an enzyme-labeled (secondary) antibody

↓

Addition of Substrate.

↓

Addition of Product.



INDIRECT ELISA

Sandwich ELISA

Attachment of **capture antibody** to the microtiter plate.

↓

Addition of Antigen (Ag-Ab interaction occurs).

↓

Addition of second antibody (unlabeled). This antibody is also specific for the antigen & binds to it at epitope different from the one which attaches to capture antibody.

↓

Antigen is sandwiched between capture and second antibody (detection antibody).

↓

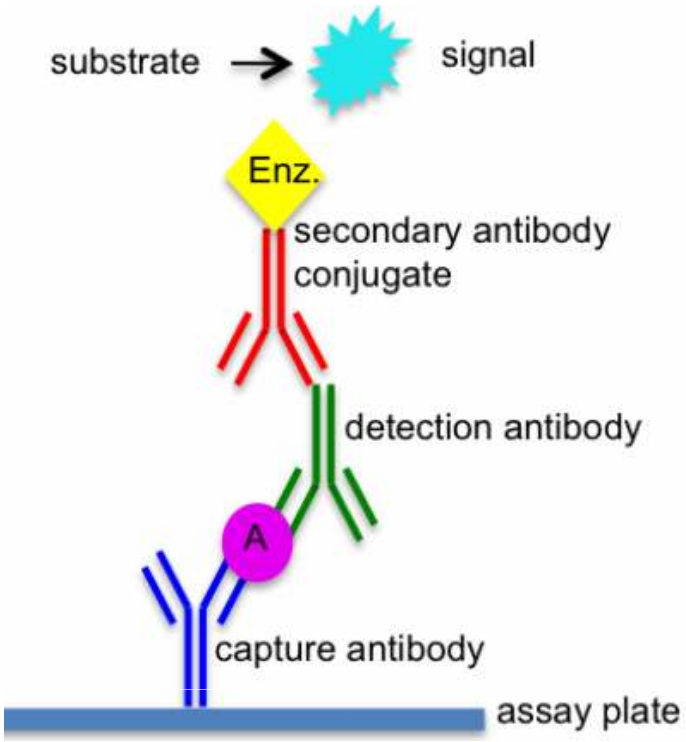
Addition of an enzyme-labeled antibody specific for the second antibody (indirect sandwich)

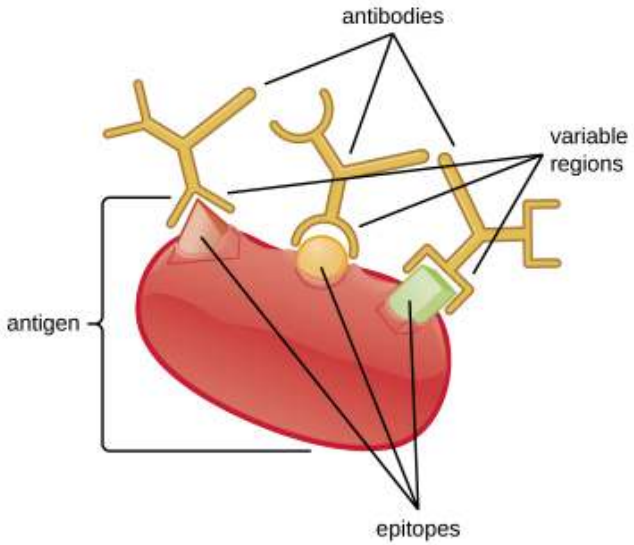
↓

Addition of Substrate

↓

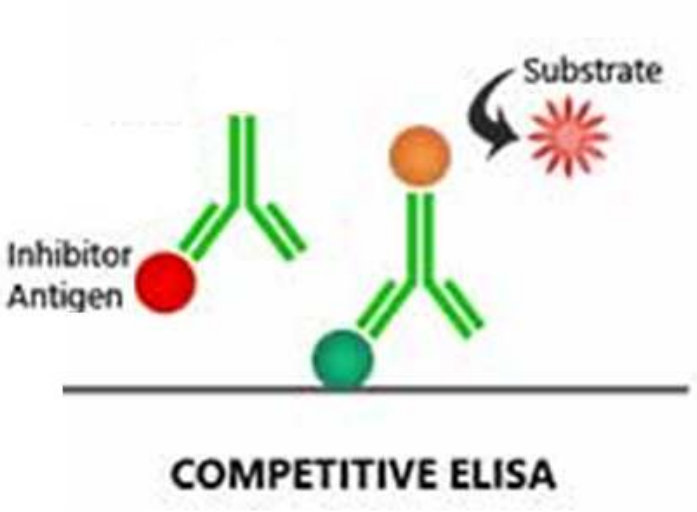
Measurement of Product.





Competitive ELISA

Principle of the test is that two specific antibodies, one conjugated with enzyme and the other unlabeled, compete for the same antigen.



Unlike other tests:-

Appearance of color: indicates a **negative test** (absence of antibodies)

Absence of color: indicates a **positive test** (presence of antibodies)

Incubation of **Test sample** (containing Ag to be tested) + unlabeled antibodies in a test tube. (Ab-Ag complex formation occurs only if specific Ag is present in test sample).

↓

Coating of microtiter plate with the same antigen which is to be detected in sample.

↓

Addition of incubated sample (containing Ag-Ab complexes, if formed) to the well plates.

↓

Removal of unbound antibody by washing.

↓

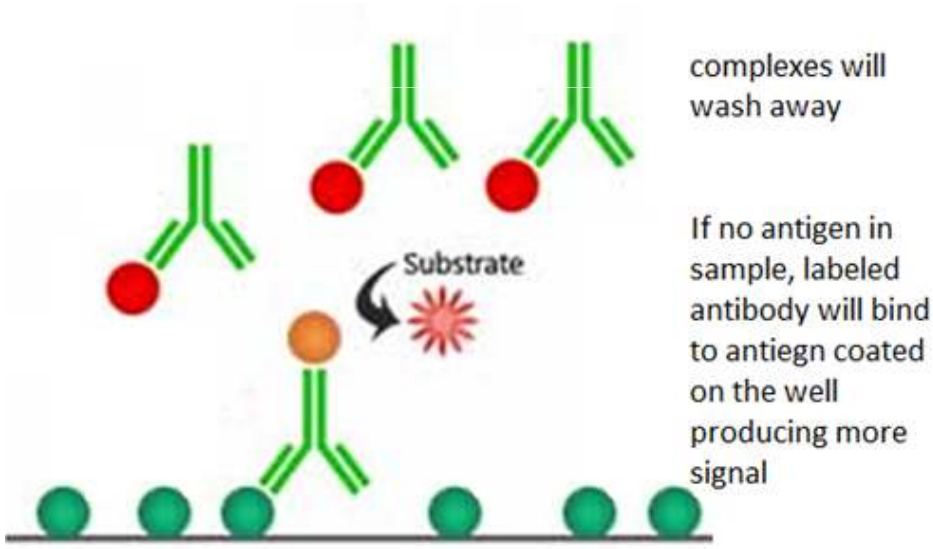
Addition of enzyme labeled antibody.

↓

Addition of substrate & product measurement.

↓

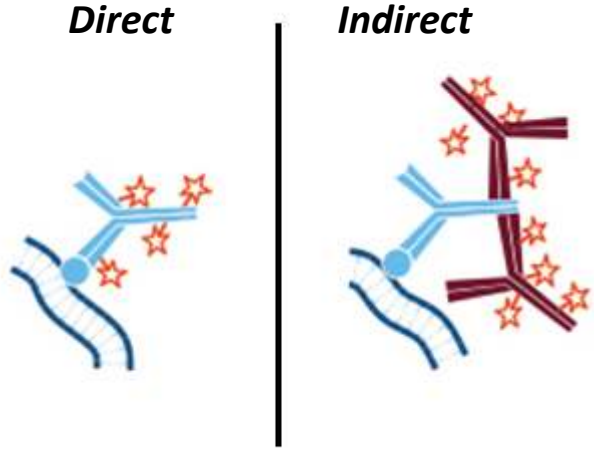
The less antigen in the sample, the more unlabeled antibody in the mixture will tend to bind to the antigen in the well, hence "competition" will occur between labeled and unlabeled Ab for the Ag coated over the plate).



	Direct ELISA	Competitive ELISA	Indirect ELISA	Indirect competitive ELISA	Sandwich ELISA
Advantage	Simple because only one antibody is used		Higher sensitivity and versatility than direct methods owing to usage of PAb that recognizes different epitopes of primary antibody		High specificity as two antibodies possessing different epitopes are used
Disadvantage	Labeling antibody is necessary for each ELISA, which may result in inactivation of antibody		Nonspecific signal is induced through cross-reactivity of secondary antibody		To prepare two different antibodies is labor-intensive and expensive
Target	Macromolecules	Macromolecules (Hapten)	Macromolecules	Macromolecules (Hapten)	Generally macromolecules
Signal (as target antigen increase)	Increase	Decrease	Increase	Decrease	Increase

How indirect methods enhance signal?

*Each primary antibody (unlabeled) is bound with **multiple** polyclonal secondary antibodies (enzyme labeled). Thus, signal amplification occurs compared to when directly labeled primary antibody is used for visualization.*



Polyclonal antibodies are a mixture of Abs that attach to different epitopes on the same antigen.

Thank You