Hormone Analysis

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BCH 560

Types of Assays

- **Antibody-based immunologic assays**
  - **Competitive immunoassays**
  - **Immunometric (sandwich) assays**
- **Chromatographic assays**
- **Mass spectroscopy**
- **Nucleic acid-based assays**

Immunoassays

- **An immunoassay is a test that uses antibody and antigen complexes as a means of generating a measurable result. An antibody:antigen complex is also known as an immuno-complex.**
- **"Immuno" refers to an immune response that causes the body to generate antibodies, and "assay" refers to a test.**

  Thus, an immunoassay is a test that utilizes immunocomplexing when antibodies and antigens are brought together.

Competitive immunoassays

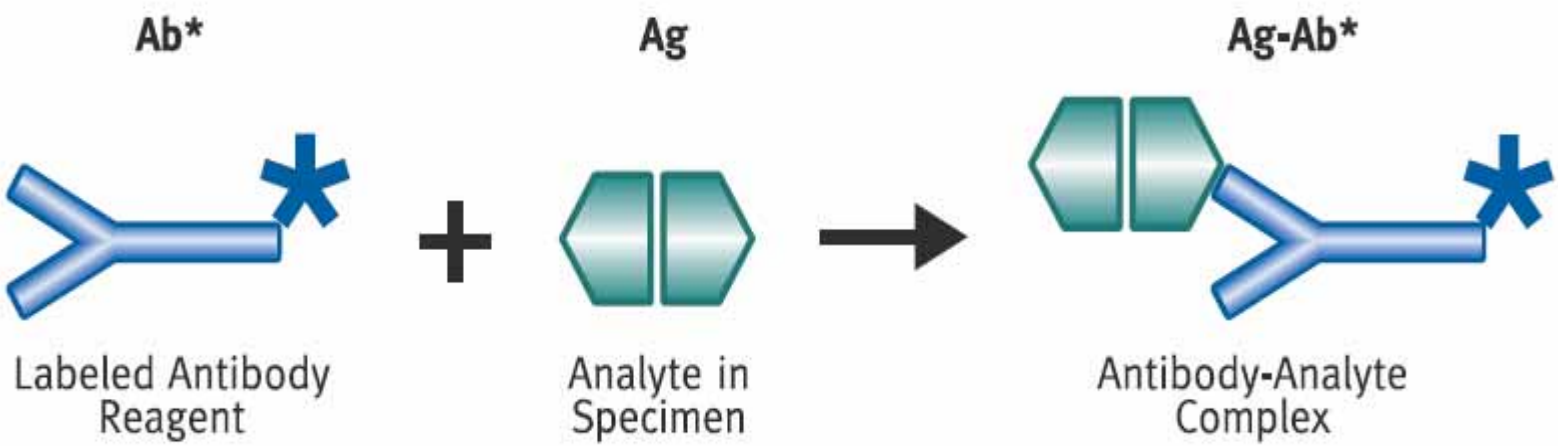
- **Refer to an assay method in which an antigen (e.g., a hormone) in a specimen competes with radiolabeled reagent antigen for a limited number of binding sites on a reagent antibody.**
- **Three basic components are:**
  - **Antiserum specific for a unique epitope on a hormone or antigen**
  - **Labeled antigen that binds to this antiserum**
  - **Unlabeled antigen in the specimen or standard that is to be measured**

- **All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody present.**
- **A label is a molecule that will react as part of the assay, so a change in signal can be measured in the blood:reagent solution.**
- **Examples of a label include:**
  - **a radioactive compound,**
  - **an enzyme that causes a change of color in a solution,**
  - **or a substance that produces light.**

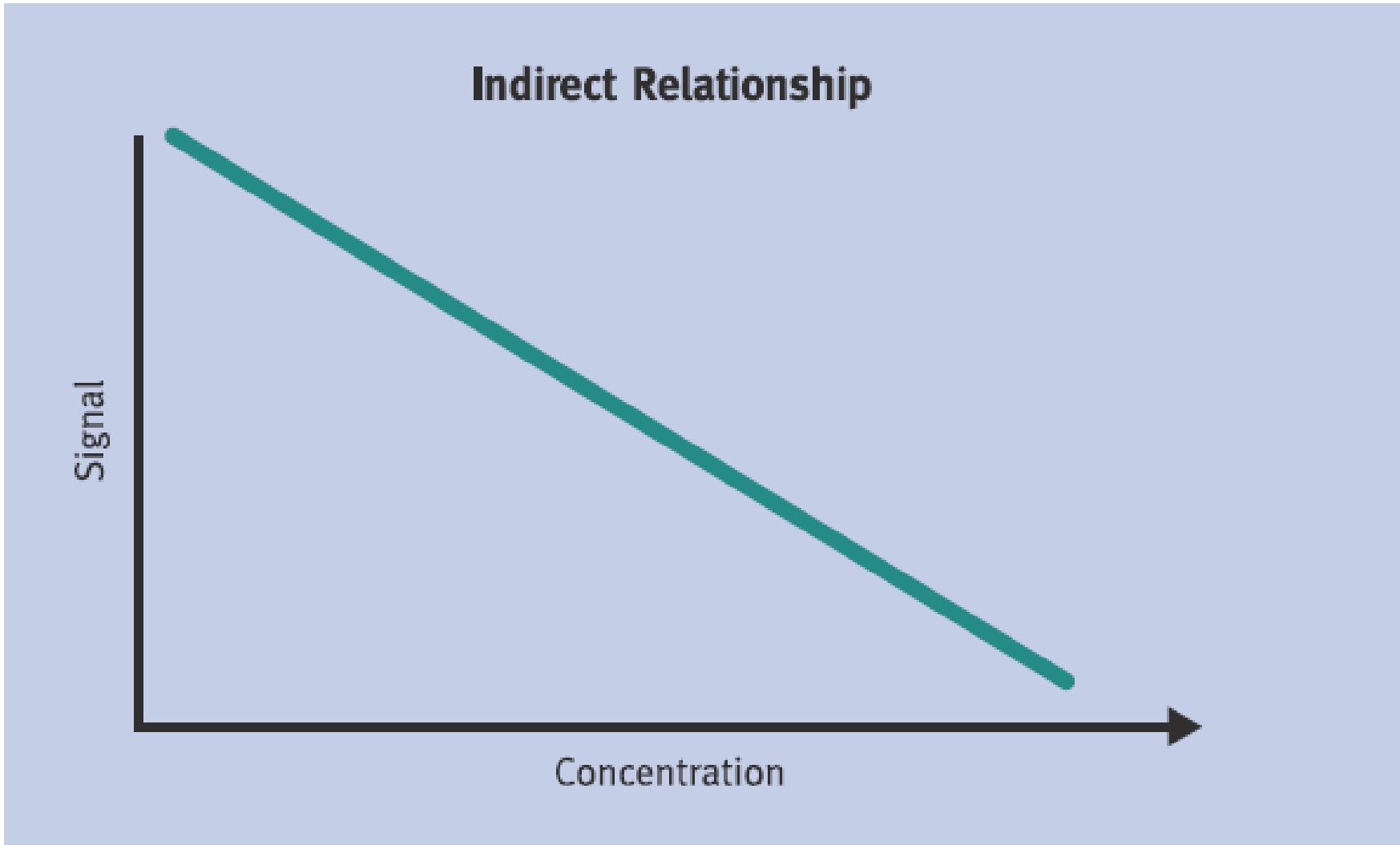
Competitive assays

- **In competitive assays, unlabelled analyte (usually antigen) in the test sample is measured by its ability to compete with labeled antigen in the immunoassay.**
- **The unlabeled antigen blocks the ability of the labeled antigen to bind because that binding site on the antibody is already occupied.**
- **Thus, in a competitive immunoassay, less label measured in the assay means more of the unlabeled (test sample) antigen is present.**
- **The amount of antigen in the test sample is inversely related to the amount of label measured in the competitive format.**

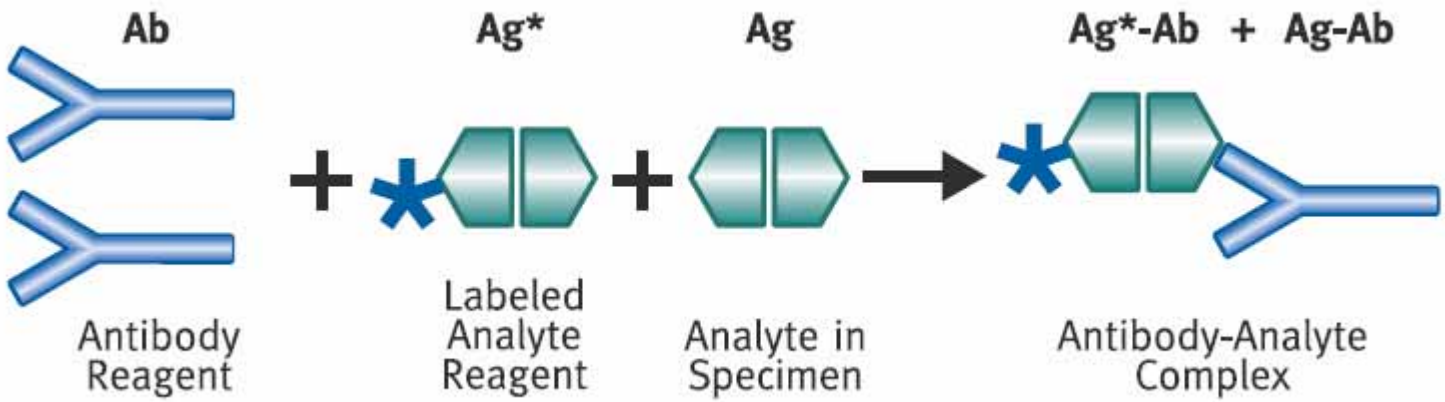
Labeled antibodies allow detection of antigen/antibody complexes in immunoassays



Amount of antigen is indirectly related to the amount of label (signal) in competitive formats



Labeled antigen also allows detection of antigen/antibody complexes in immunoassays



Radioimmunoassay

- **Highly sensitive laboratory technique used to measure minute amounts of substances including antigens, hormones, and drugs present in the body.**

Preparation of Ab against the antigen

- **The substance or antigen to be measured is injected into an animal, causing it to produce antibodies.**
- **Serum containing the antibodies is withdrawn and treated with a radioactive antigen and later with a nonradioactive antigen.**
- **Measurements of the amount of radioactivity are then used to determine the amount of antigen present.**
- **The technique was developed by Solomon Berson and Rosalyn Yalow. Yalow was awarded the 1977 Nobel Prize in Physiology or Medicine for her work.**

Radioimmunoassay (RIA)

- **Used to test antigens (e.g., hormone levels in the blood) without the need to use a bioassay.**
- **Involves mixing known quantities of radioactive antigen (frequently labeled with gamma-radioactive isotopes of iodine attached to tyrosine) with antibody to that antigen, then adding unlabeled or "cold" antigen and measuring the amount of labeled antigen displaced.**
- **Initially, the radioactive antigen is bound to the antibodies. When "cold" (unlabeled, quest) antigen is added, the two compete for antibody binding sites - at higher concentrations of "cold" antigen, more of it binds to the antibody, displacing the radioactive variant. The bound antigens are separated from the unbound ones. The latter stay in the supernatant, the radioactivity of which is measured and a binding curve is plotted.**
- **The technique is both extremely sensitive and specific, but it requires special precautions, requires sophisticated apparatus and is expensive.**

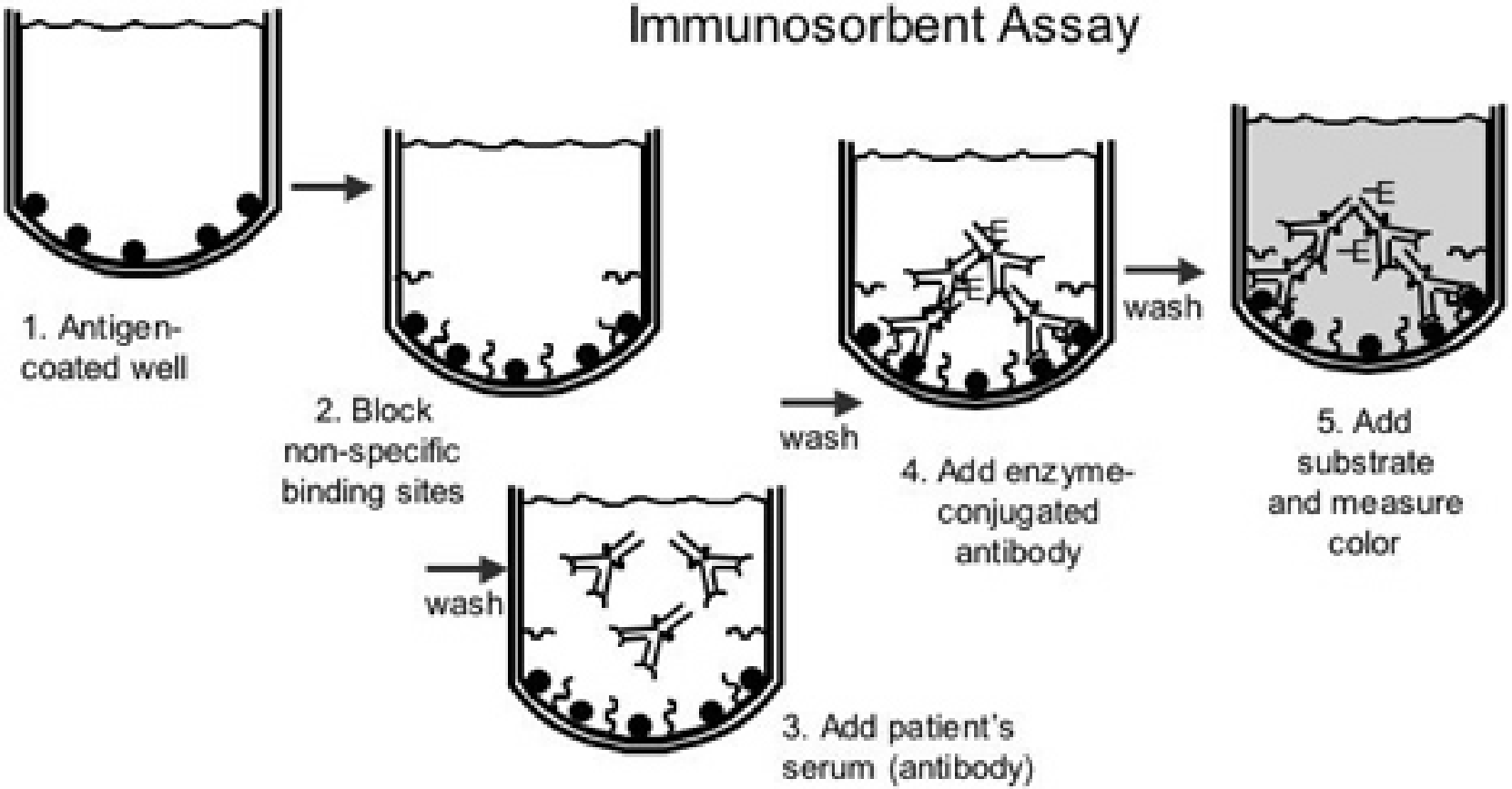
Application of RIA

- In medicine it is especially useful in
- diagnosing and treating autoimmune diseases such as
  - Hashimoto's thyroiditis and
  - Systemic Lupus Erythematosus
- in diagnosing hormonal abnormalities
- In determining the levels of plasma proteins, viral markers, cancer markers etc in blood

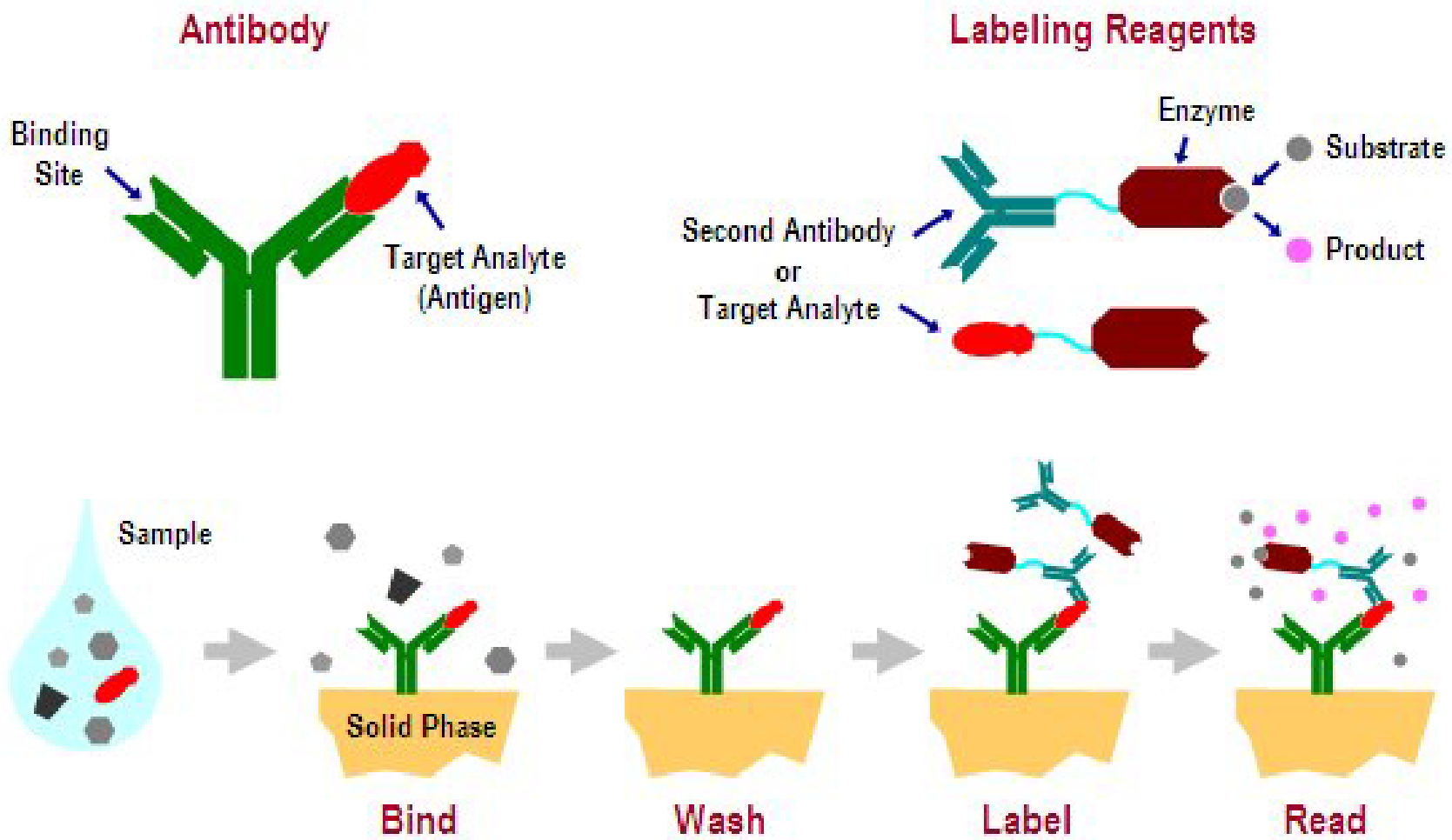
Enzyme Linked Immunosorbent Assays (ELISA)

- **In ELISAs, antibodies are used to selectively recognize and tightly bind to a specific target molecule,**
- **and are combined with enzymes that produces a colored or fluorescent product from a colorless substrate molecule,**
- **to amplify the signal from a binding event to enable specific target measurement down to a level of picograms.**

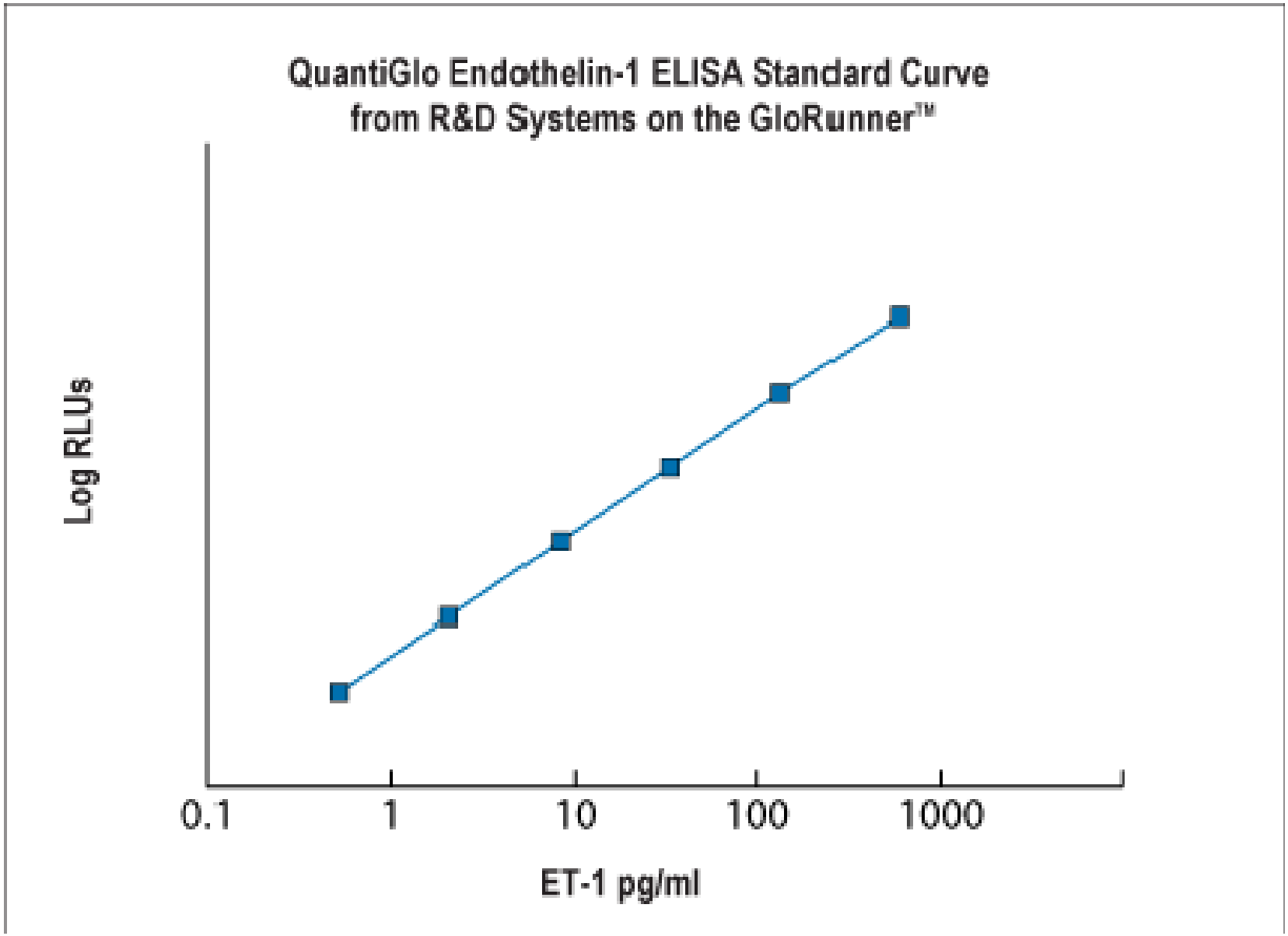
ELISA:
Enzyme-Linked Immunosorbent Assay



ELISA



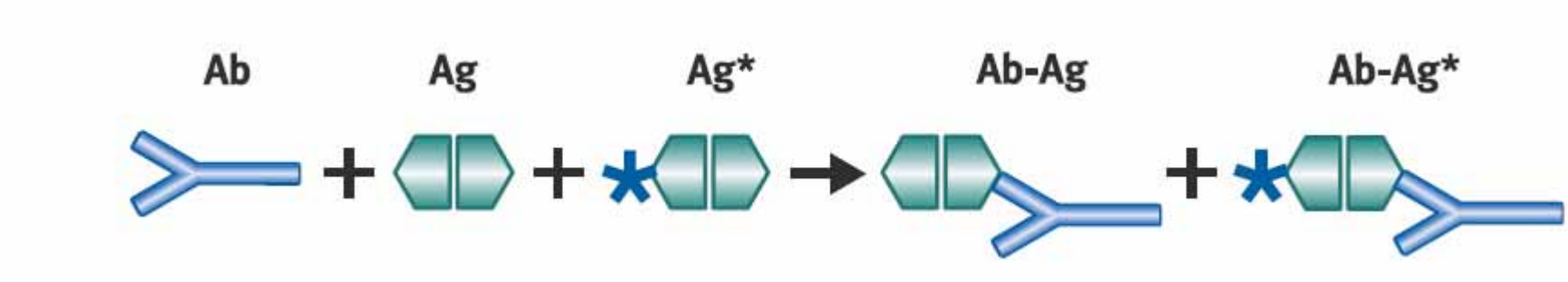
A.S.Warsy


QuantiGlo Endothelin-1 ELISA Standard Curve
from R&D Systems on the GloRunner™
Log RLUs
0.1
1
10
100
1000
ET-1 pg/ml

A.S.Warsy

One step competitive immunoassay

In the one step competitive format, both the labeled antigen reagent (Ag*) and the unlabeled specimen (or test sample analyte) compete for a limited amount of antibody.



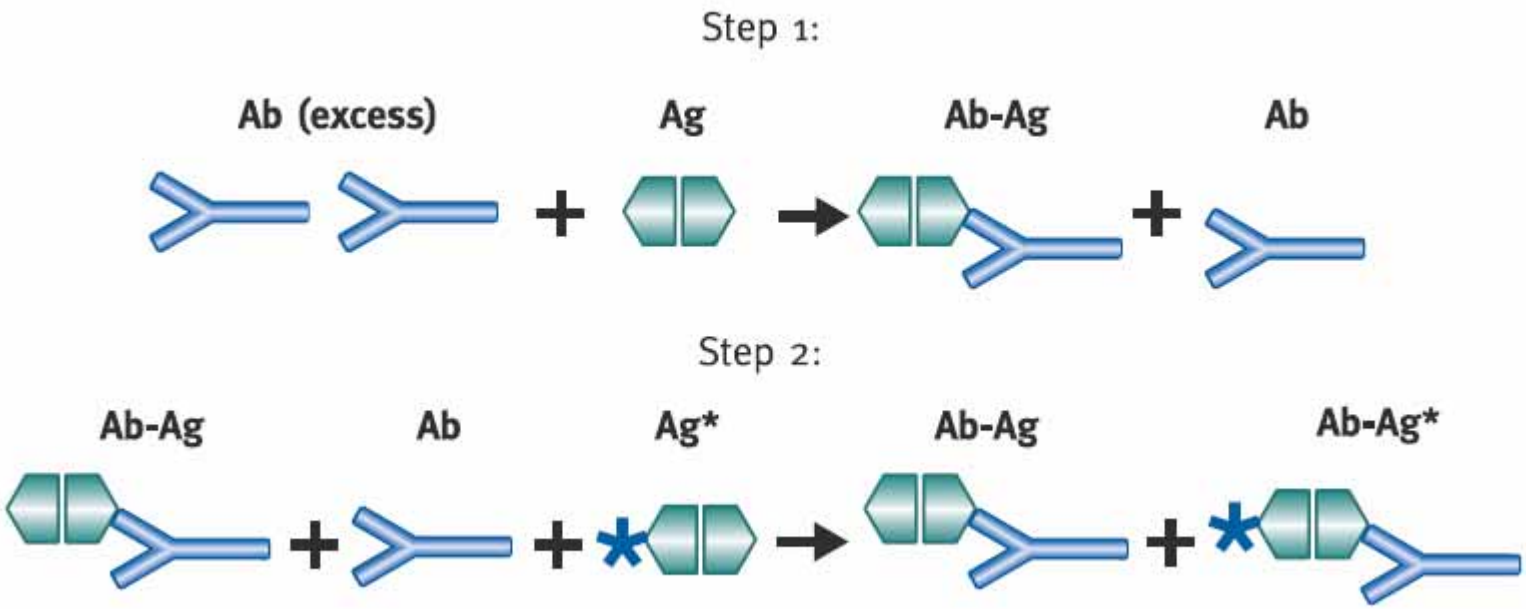
Two step competitive immunoassay

In the two step competitive format, the antibody concentration of the reaction solution is present in excess in comparison to the concentration of antigen.

Antibody reagent is first incubated with specimen containing antigens of interest; then in the second step, labeled antigen is added.

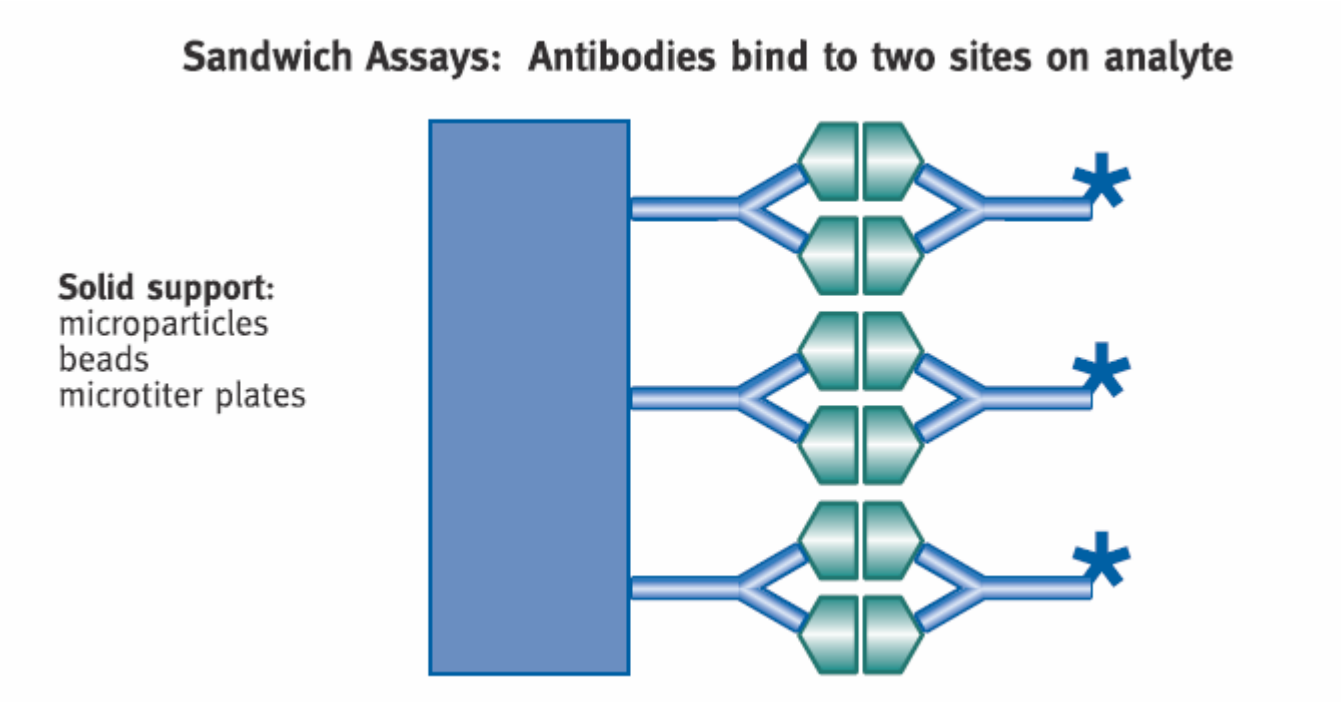
In the competitive format, less bound labeled antigen indicates more antigen present in the test sample.

Two step competitive assay formats provide several fold improved assay sensitivity compared to one step assay formats.

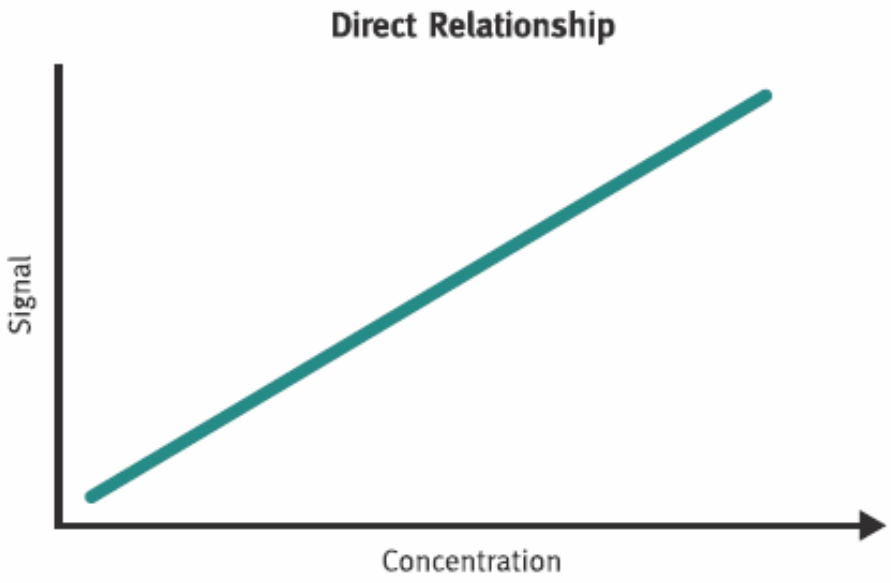


Noncompetitive (Sandwich) Method

Noncompetitive assay generally provide the highest level of assay sensitivity and specificity and are applied to the measurement of critical analytes such as cardiac and hepatitis markers. This assay is referred to as a "sandwich" assay because analyte is bound (sandwiched) between two highly specific antibody reagents



- **Noncompetitive assay can also utilize either one step or two step methods, as with the competitive assay. The two step assay employs wash steps in which the sandwich binding complex is isolated and washed to remove excess unbound labeled reagent and any other interfering substances.**
- **The two step noncompetitive assays usually offers the highest specificity and sensitivity of all the assay methods.**



Amount of antigen is directly related to the amount of label (signal) in competitive assays

In noncompetitive assays, the measurement of labeled analyte, usually antibody, is directly proportional to the amount of antigen present in the sample.

This can be represented by a dose response curve. The X-axis plots concentration of an antigen. The Y-axis plots response, which in this case is signal.

Thus, the more antigen that is present, the more labeled antibody that will bind.

This direct proportionality is in contrast with the indirect proportionality of competitive immunoassays.

Homogeneous and Heterogeneous Immunoassay Methods

- **Immunoassay methods that require separation of bound Ab-Ag* complex are referred to as heterogeneous immunoassays.**
- **Those that do not require separation are referred to as homogeneous immunoassays.**
- **Homogeneous methods have been generally applied to the measurement of small analytes such as abused and therapeutic drugs.**
- **Since homogeneous methods do not require the separation of the bound Ab-Ag* from the free Ag*, they are generally much easier and faster to perform.**

Homogeneous and heterogeneous immunoassays