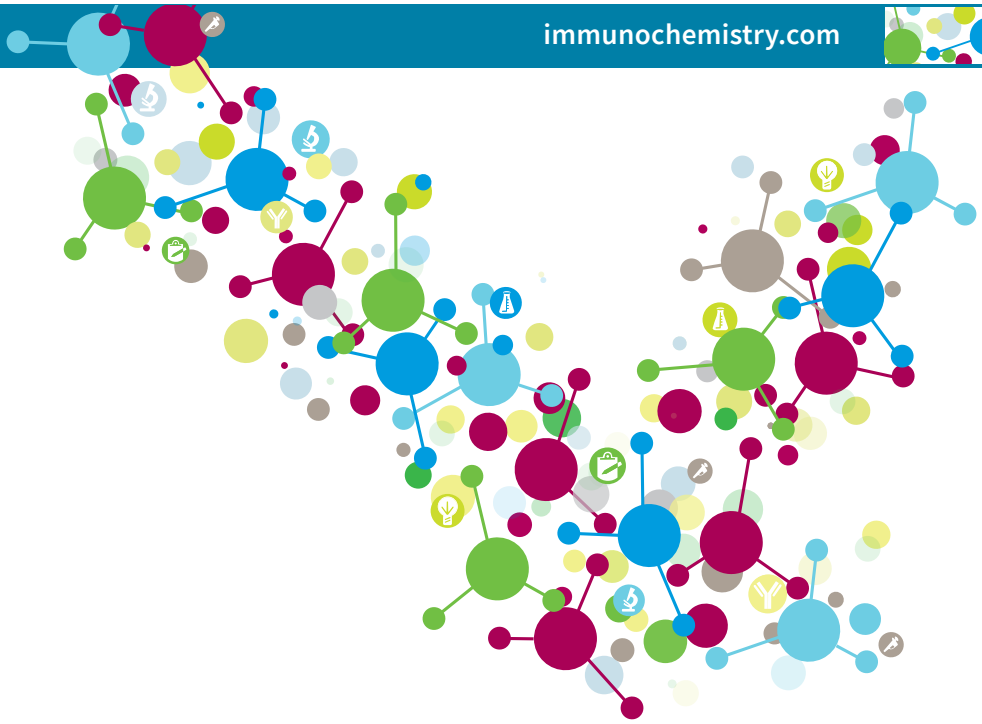

BRIGHT MINDS,
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Back to Basics: What is an immunoassay?

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What is an immunoassay or ELISA?

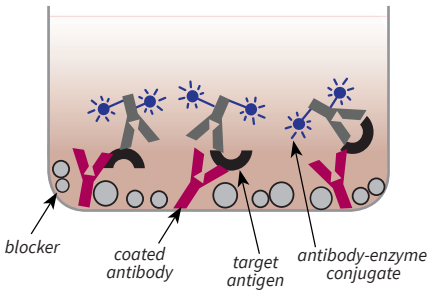
Immunoassays are quick and accurate tests that can be used on-site and in the laboratory to detect specific molecules. Immunoassays rely on the inherent ability of an antibody to bind to the specific structure of a molecule. Antibodies are proteins generated by animals in response to the invasion of a foreign molecule (antigen) into the body. Antibodies are found in blood and tissue fluids and will bind to the antigen whenever it is encountered. Because antibodies are developed to the specific three-dimensional structure of an antigen, or analyte, they are highly specific and will bind only to that structure. Once purified from the blood, monoclonal and polyclonal antibodies are ideal assay reagents to detect and monitor specific target molecules with limited interferences from other substances. Four typical ELISA formats are: antibody sandwich immunoassays, antigen-down immunoassays, competitive inhibition assays, and rapid assays.

Antibody Sandwich Immunoassay

In a typical antibody sandwich immunoassay, a monoclonal antibody is adsorbed onto a plastic microtiter plate. When the test sample is added to the plate, the antibody on the plate will bind the target antigen from the sample, and retain it in the plate. When a polyclonal antibody is added in the next step, it also binds to the target antigen (already bound to the monoclonal antibody on the plate), thereby forming an antigen 'sandwich' between the two different antibodies.

- **Step 1:** Monoclonal antibodies are adsorbed onto the well of a plastic microtiter plate with coating buffer (no sample added).
- **Step 2:** Addition of a sample (such as human blood, diluted appropriately) to the well of the microtiter plate. The target antigen binds to the antibody adsorbed on the plate, retaining the antigen in the well.
- **Step 3:** Binding of an enzyme-conjugated polyclonal antibody to the target antigen (bound to the monoclonal antibody on the plate), thereby forming an antigen 'sandwich' between the two different antibodies.
- **Step 4:** Addition of a colorimetric substrate for detection of the enzyme-conjugated polyclonal antibodies will generate a color signal proportional to the amount of target antigen present in the original sample added to the plate.

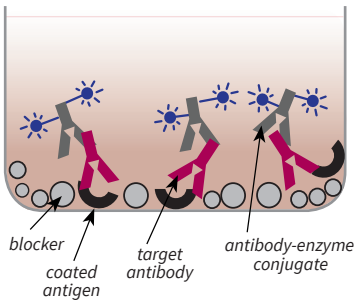
Antibody Sandwich ELISA



Antigen-Down (Immunity Test) Immunoassay

In an antigen-down (immunity test) immunoassay, the analyte is coated onto a 96-well microtiter plate (rather than an antibody) and used to bind antibodies found in a sample. When the sample is added (such as human serum), the antigen on the plate is bound by antibodies (IgE for example) from the sample, which are then retained in the well. A species-specific antibody (anti-human IgE for example) labeled with HRP is added next, which binds to the antibody bound to the antigen on the plate. The higher the signal, the more antibodies there are in the sample. Antigen-down (immunity test) assays can be configured as rapid tests and are often used to diagnose allergy conditions – routinely a patient's blood is tested against different allergens to see if the person has antibodies to that allergen.

Antigen-Down ELISA



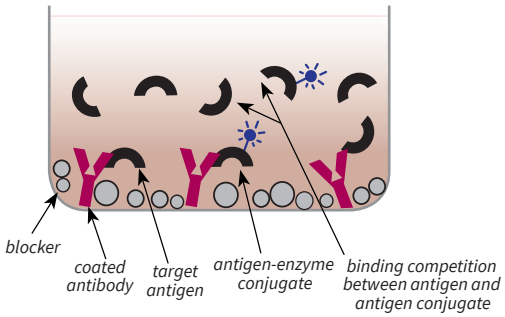
Competitive Inhibition Immunoassay

In addition to the Monoclonal-Polyclonal (Mo-Po) Antibody Sandwich format, many immunoassays are structured in a competitive inhibition format. Competitive inhibition assays are often used to measure small analytes because competitive inhibition assays only require the binding of one antibody rather than two, as in standard ELISA formats. Because of the high probability for steric hindrance occurring when two antibodies attempt to bind to a small molecule at the same time, a sandwich assay format may not be feasible. Therefore, a competitive inhibition assay would be preferable.

In a sequential competitive inhibition assay, the sample and conjugated analyte are added in steps like a sandwich assay, while in a classic competitive inhibition assay, these reagents are incubated together at the same time. In a sequential competitive inhibition assay format, a monoclonal antibody is coated onto a 96-well microtiter plate. When the sample is added, the MoAb captures free analyte out of the sample.

In the next step, a known amount of analyte labeled with either biotin or HRP is added. The labeled analyte will then also attempt to bind to the MoAb adsorbed onto the plate, however, the labeled analyte is inhibited from binding to the MoAb by the presence of previously bound analyte from the sample. This means that the labeled analyte will not be bound by the monoclonal on the plate if the monoclonal has already bound unlabeled analyte from the sample. The amount of unlabeled analyte in the sample is inversely proportional to the signal generated by the labeled analyte. The lower the signal, the more unlabeled analyte there is in the sample. A standard curve can be constructed using serial dilutions of an unlabeled analyte standard. Subsequent sample values can then be read off the standard curve as is done in the sandwich ELISA formats. The classic competitive inhibition assay format requires the simultaneous addition of labeled (conjugated analyte) and unlabeled analyte (from the sample). Both labeled and unlabeled analyte then compete simultaneously for the binding site on the monoclonal capture antibody on the plate. Like the sequential competitive inhibition format, the colored signal is inversely proportional to the concentration of unlabeled target analyte in the sample. Detection of labeled analyte may be made by using a peroxidase substrate such as TMB, which can be read on a microtiter plate reader.

Competitive ELISA

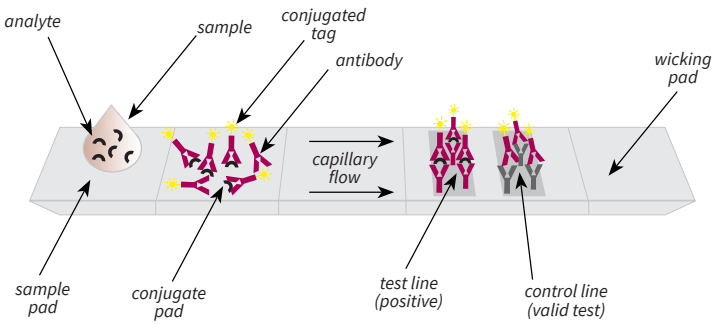


Rapid Immunoassay

In addition to microtiter plates, immunoassays are also configured as rapid tests, such as a home pregnancy test. Like microtiter plate assays, rapid tests use antibodies to react with antigens and can be developed as MoAb-PoAb sandwich formats, competitive inhibition formats, and antigen-down formats. With a rapid test, the antibody and antigen reagents are bound to porous membranes, which react with positive samples while channeling excess fluids to a non-reactive part of the membrane. Rapid immunoassays commonly come in 2 configurations: a lateral flow test where the sample is simply placed in a well and the results are read immediately; and a flow through system, which requires placing the sample in a well, washing the well, and then finally adding an analyte-colloidal gold conjugate and the result is read after a few minutes. One sample is tested per strip or cassette.

Because rapid tests are faster than microtiter plate assays, require little sample processing, are often cheaper, and generate yes/no answers without using an instrument, they are often used in the field by non-laboratory people testing whole samples. However, rapid immunoassays are not as sensitive nor can they be used to accurately quantitate an analyte (Self-monitoring of blood glucose levels by diabetics is considered quantitative rapid testing, however, immunoassay technology is not used for these tests). All rapid immunoassay tests can be converted to a microtiter plate assay, but not all microtiter plate assays can be converted to a rapid test.

Rapid Immunoassay (Lateral Flow)



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