# Antibody Diagnostics Kit

This kit has been designed to show how monoclonal antibodies are used in diagnostic tests. Components have been included to model:

- A Lateral Flow Rapid Antigen test (such as a Covid Test)
- Several forms of an ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay), including
    - Direct ELISA,
    - Indirect Antigen ELISA,
    - Indirect Antibody ELISA, and
    - Sandwich ELISA tests.

**Lateral Flow Rapid Antigen Test**

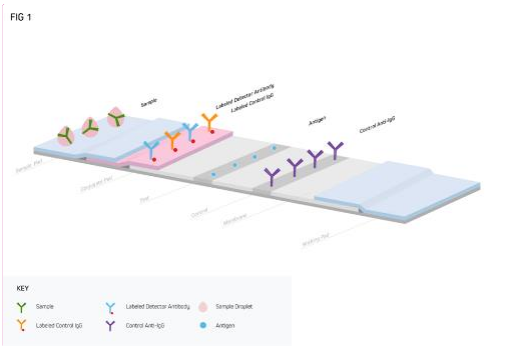



Figure 1: Schematic of a Lateral Flow Strip from Jackson ImmunoResearch

**Commented [MA1]:** Lateral Flow Immunoassays - Jackson ImmunoResearch

Figure 2: A Rapid Antigen Test Card showing a positive test from AdobeStock

**Commented [MA2]:** AdobeStock_408892963_WP-1536x1136.jpg (1536×1136) (mforum.com.au)

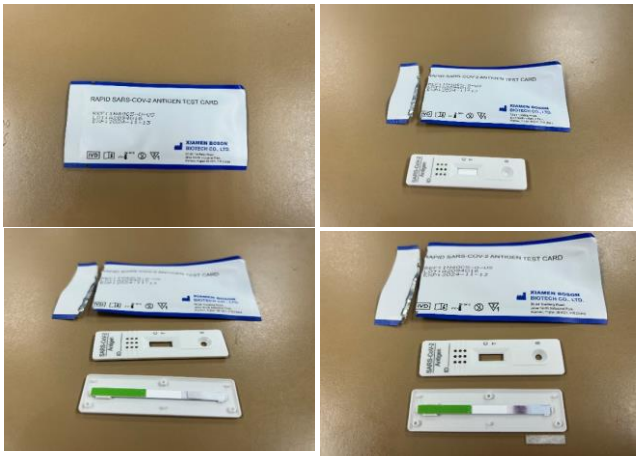
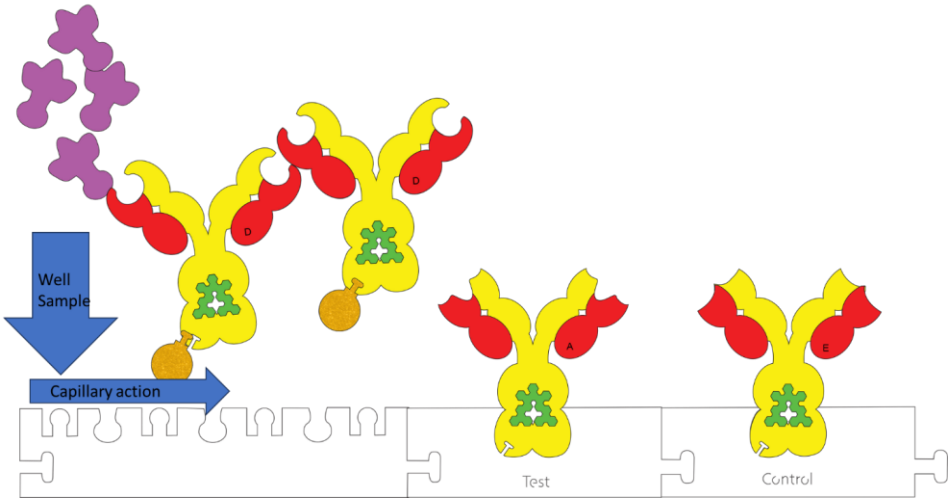
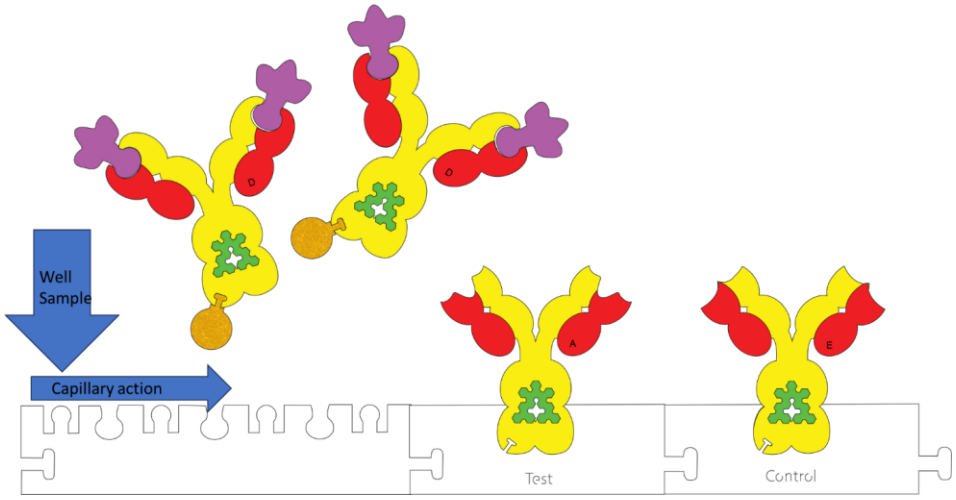



Figure 3: Internal Components of a Rapid SARS-CoV-2 Antigen Test Card

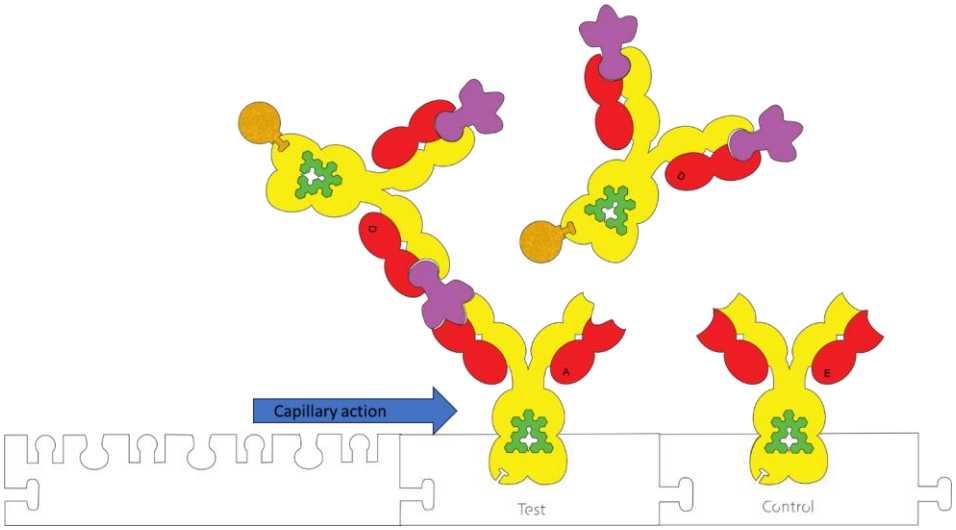
Setting up:



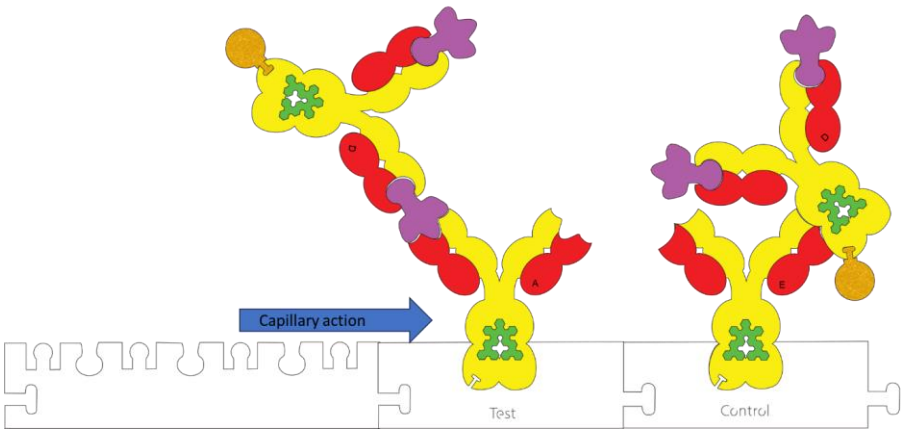

Antigen-specific antibodies (D) with a gold conjugate are concentrated near the site to add your sample. The test line has a bound Antibody A, while the control line has a bound Antibody E. In this example, we are using the influenza (purple) antigens. If you would like to use HIV (orange), switch the Antibody B at the test line. If using Coronavirus (red), switch to Antibody C at the test line.




Antigens from the patient sample will interact with the antibodies, binding to the variable region of the antibody. Capillary action will move these antibodies towards the test line.



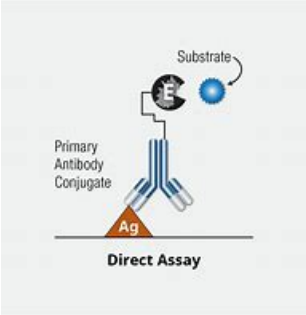

If the correct antigen was present, these antibodies will have the other side of the antigen react with the antibodies (A) already fixed at the test line. This will concentrate the gold at the test line and form a visible band. If the antibody does not bind to the antigen, it will not react at the test line.



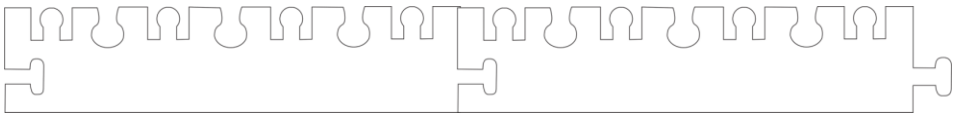

Antibodies that did not react at the test line will continue to the control line. Here, the fixed antibodies (E) will interact with the constant regions of antibodies, causing them to become trapped. The gold color will be concentrated here, resulting in the appearance of a visible line.

**ELISA – Direct Test**

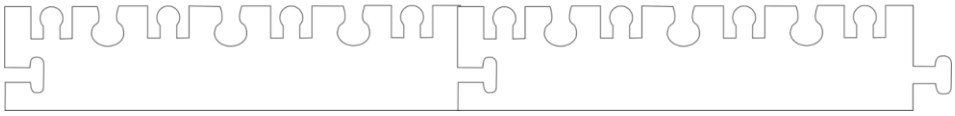
A Direct ELISA test would be used to determine the presence of a specific pathogen by antigen detection.



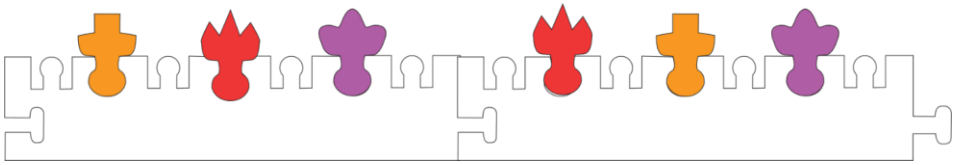

Setup:



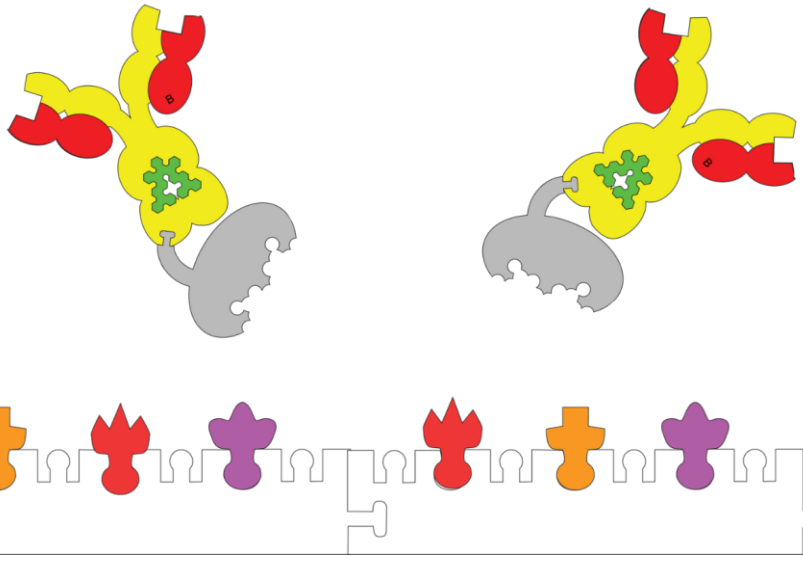
Start by setting up a" well plate" .. This represents a single well.



Add the sample from the patient. The sample will contain many different antigens.

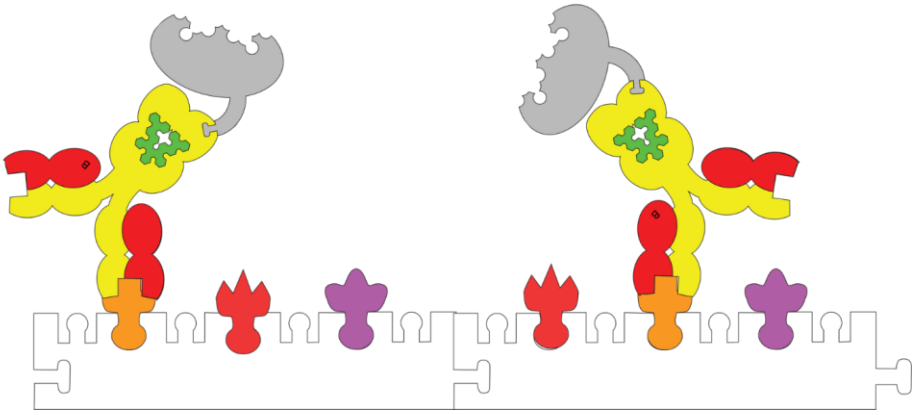


After a short incubation period, the antigens from the sample will bind to the well. A wash buffer is added to remove any unbound antigens.

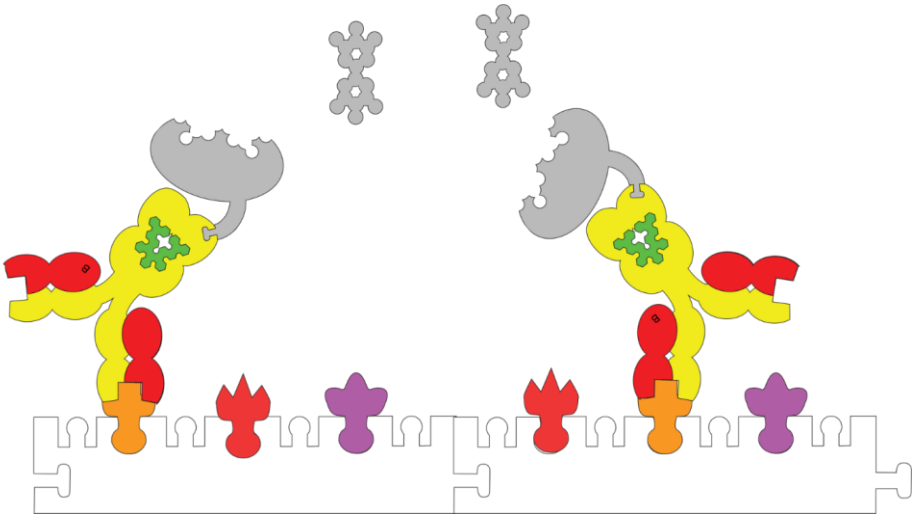




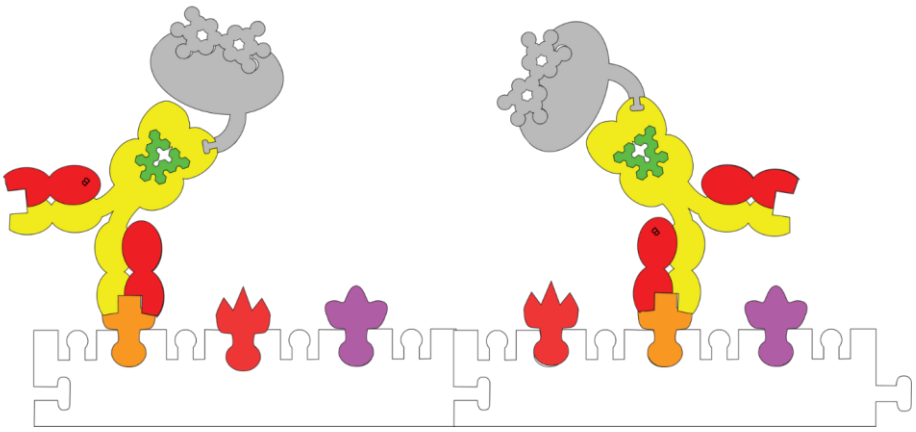

Next, antibodies that have been conjugated with an enzyme are added to the well plate. In our example, we have used Antibody B.



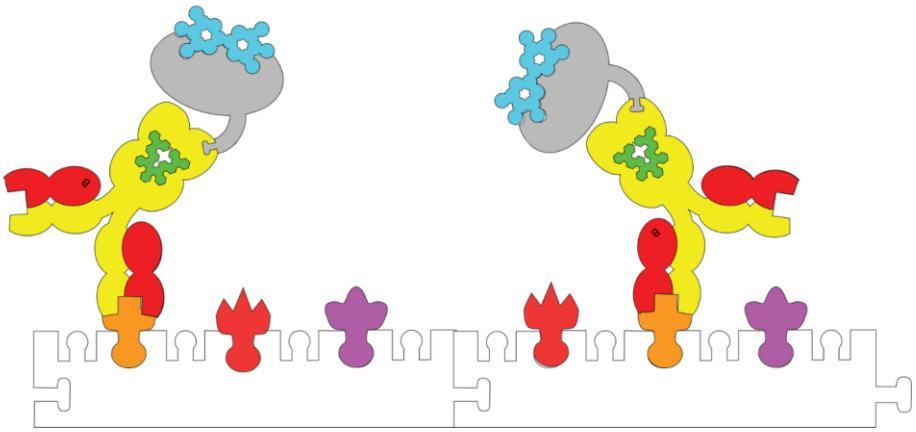
After a short incubation period, the antibodies will interact with a specific antigen. Another wash buffer is added to remove any antibodies that did not interact.



In the next step, we add a substrate and give it a short incubation period.



The substrate interacts with the enzyme, resulting in oxidation.

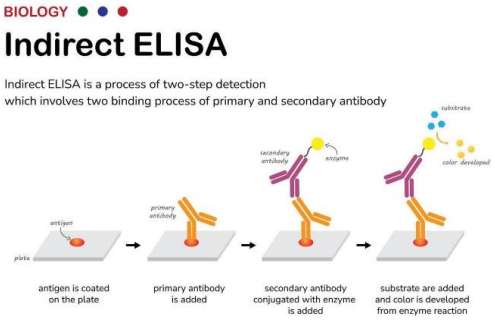


The oxidized substrate will change from colorless to blue. Spectroscopy or other methods (like a control dilution series) can be used to determine the amount of antigen present in the sample.

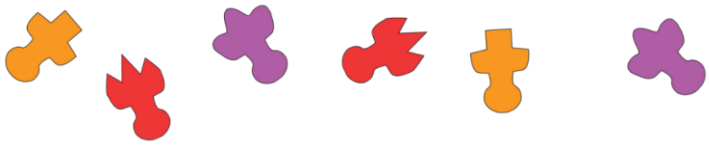
**ELISA – Indirect Antigen Test**

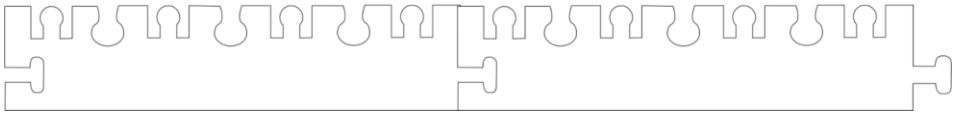
Commented [MA3]: biological-diagram-explain-process-of-indirect-elisa-as-the-two-step-detection-of-antibody-and-antigen-vector.jpg (1386×980) (vecteezy.com)

Indirect ELISA testing allows for some flexibility. Using two antibodies allows for the detection and quantification of not only the presence of an antigen (pathogen) but can also be used to detect the presence of an immune response in the patient. This procedure detects a specific pathogen by the presence of an antigen.

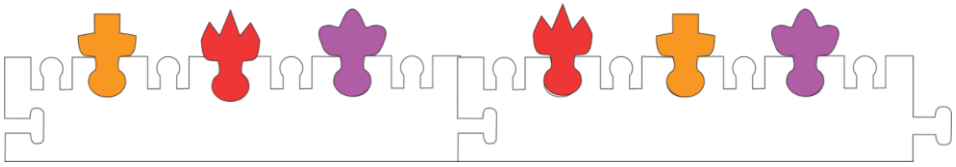



Set up:

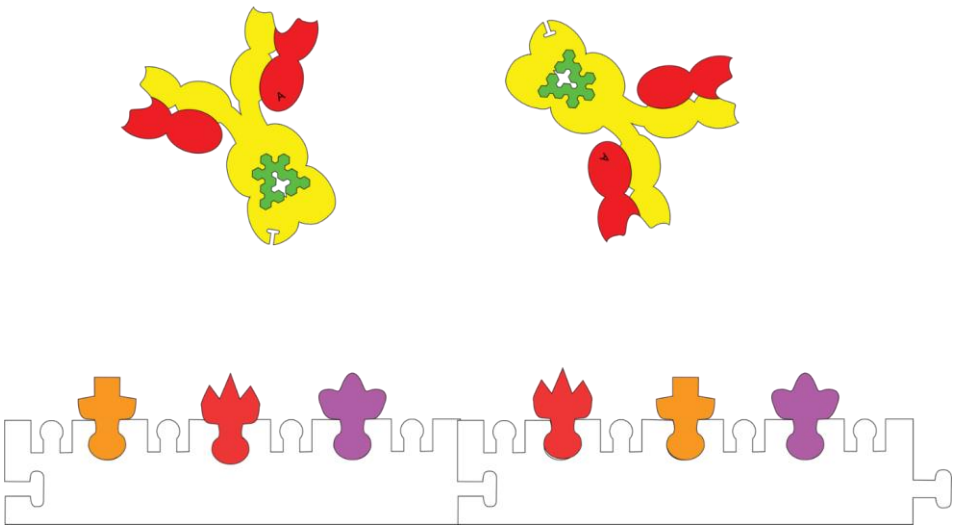




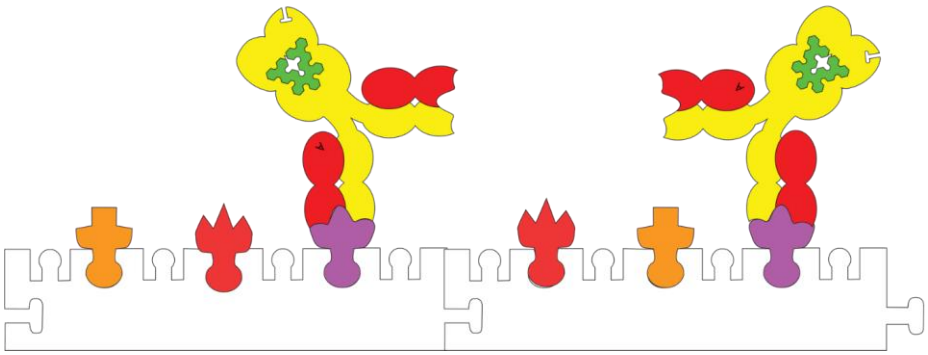
Add the sample from the patient. The sample will contain many different antigens.



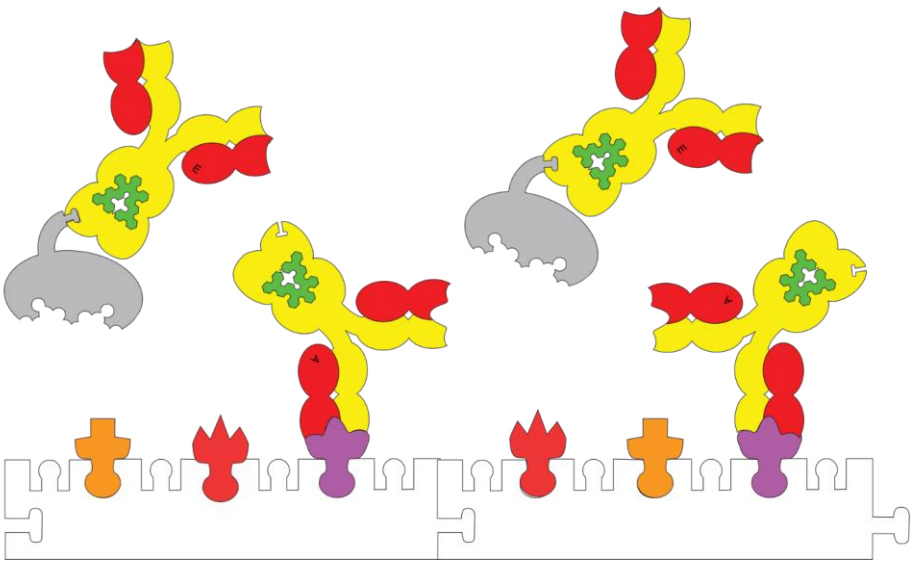
After a short incubation period, all antigens from the sample will bind nonspecifically to the well. A wash buffer is added to remove any unbound antigens.



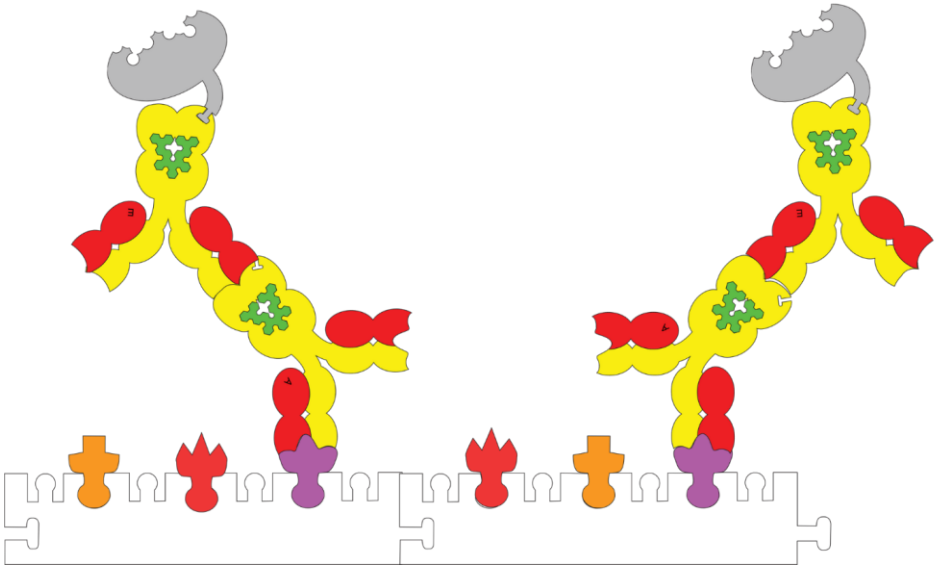
Primary antibodies (A) are added to our well plate. This antibody will only interact with one specific antigen.



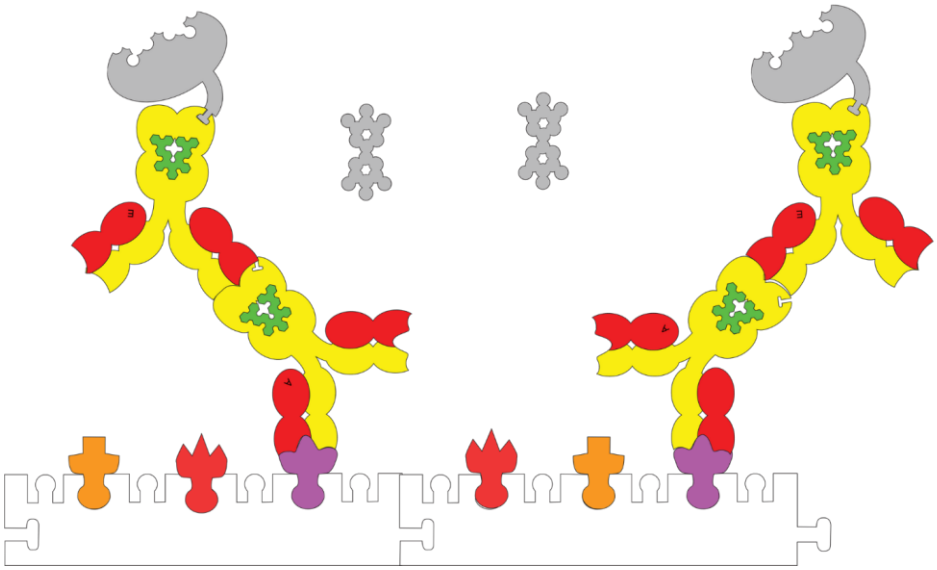
After a short incubation period, a wash buffer is added to remove any unbound primary antibodies.



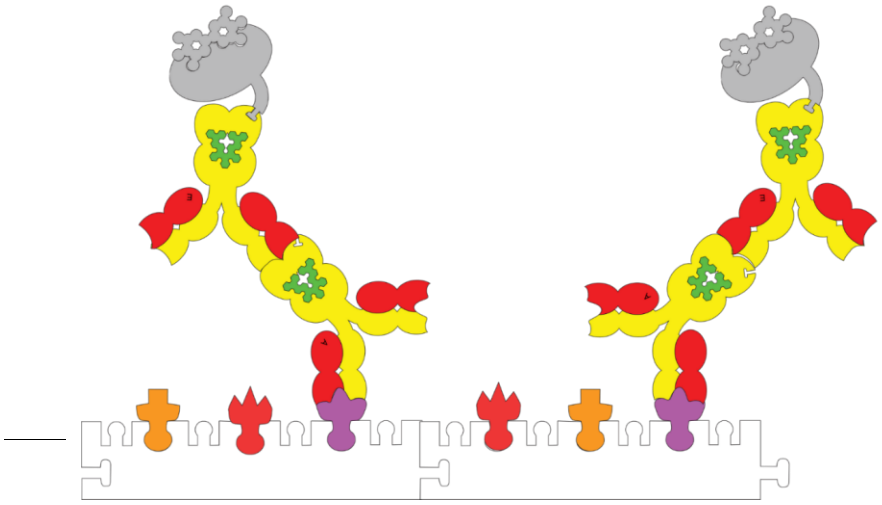
Secondary antibodies (E) that have been conjugated with an enzyme are added to the well plate.



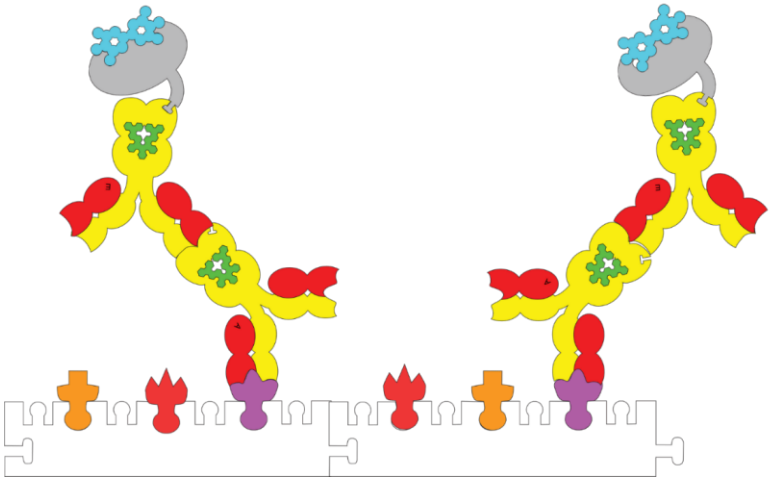
After a short incubation period, secondary antibodies will interact and bind to the primary antibodies. A wash buffer is added to remove any unbound secondary antibodies.



Add substrate to the well plate.



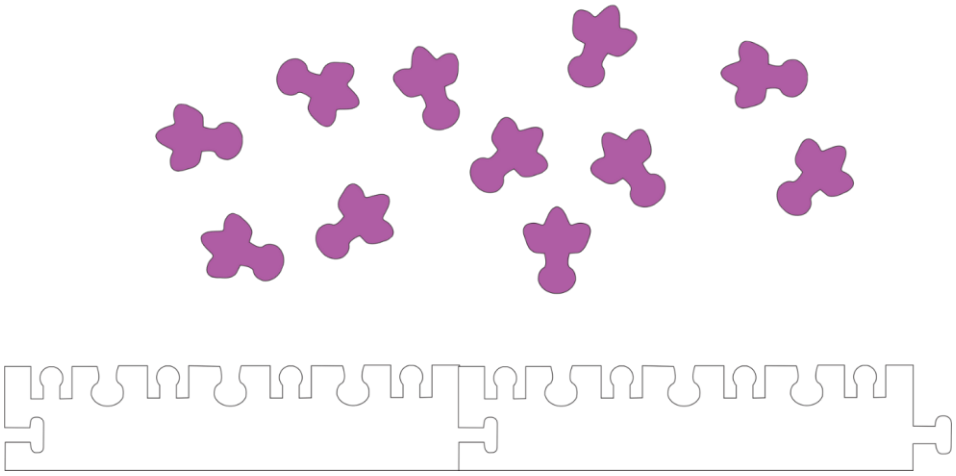
The substrate interacts with the enzyme, resulting in oxidation.



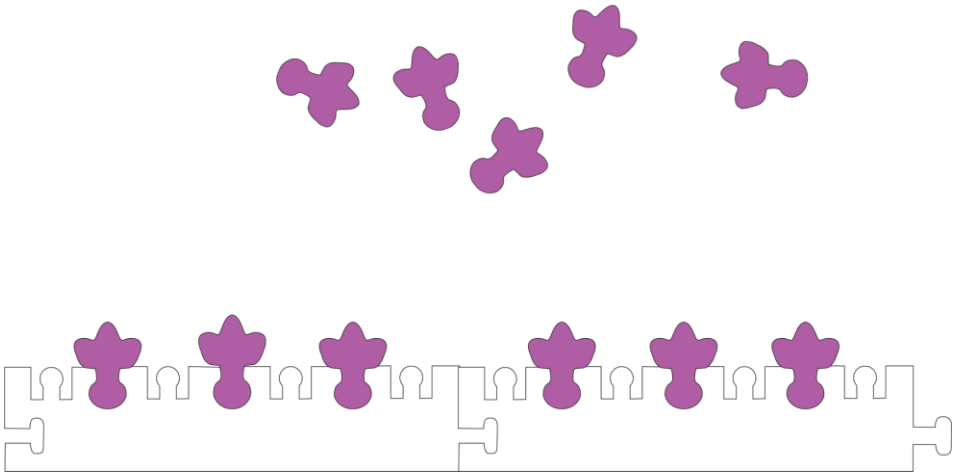
The oxidized substrate will change from colorless to blue. Spectroscopy or other methods (like a control dilution series) can be used to determine the amount of antigen present in the sample.

**ELISA – Indirect Antibody Test**

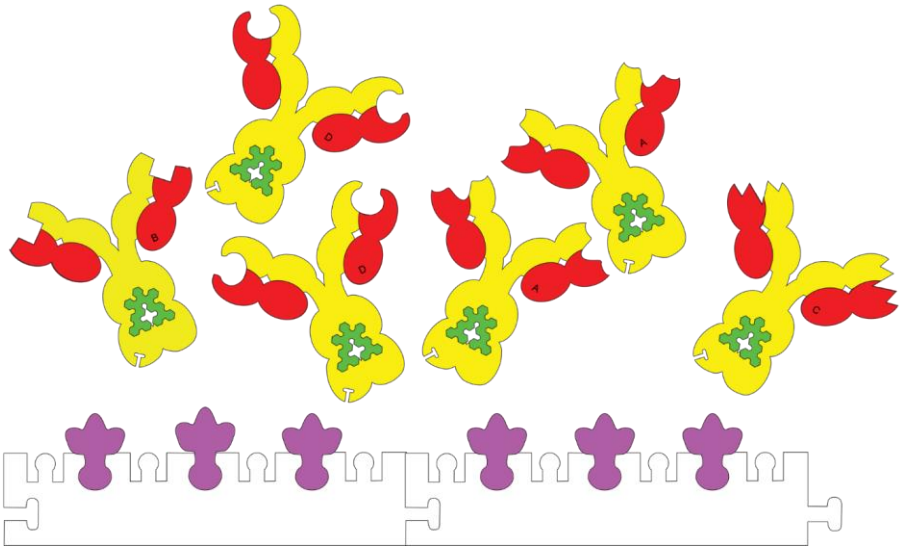
Let's see how this changes when looking for an immune response from a patient.



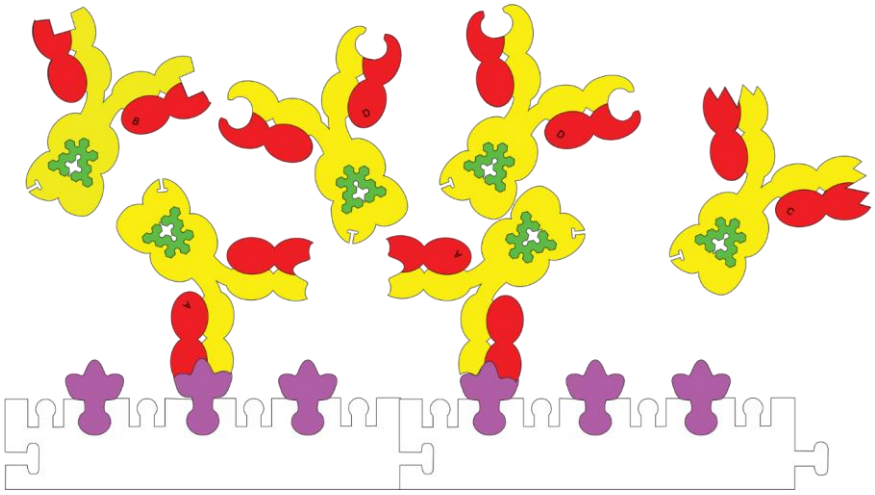
A prepared sample is added to the wells that contains one specific antigen.



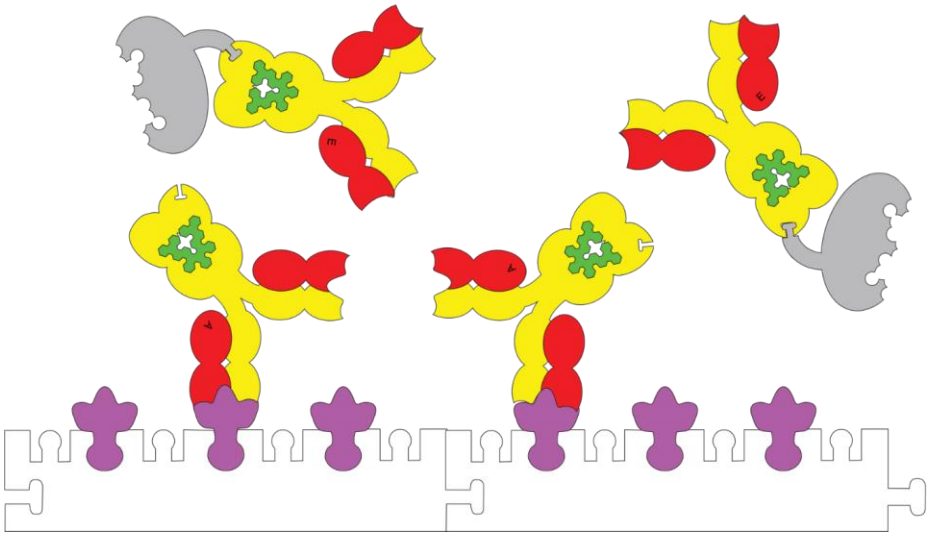
The antigens interact with the well plate. Any unbound antigens are removed with a wash buffer.



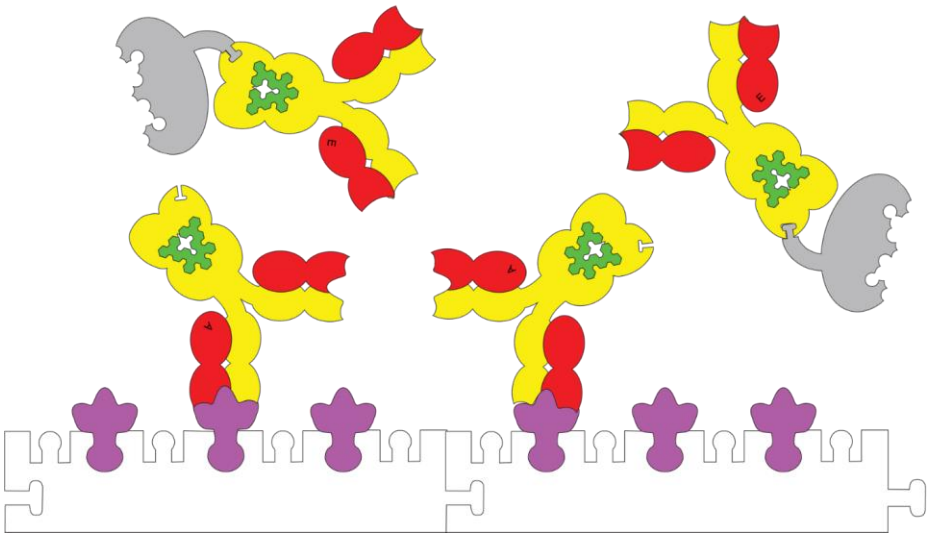
The patient sample (blood plasma) contains lots of different antibodies (add A-D). When added to the plate, only the antibody (A in our example) specific to the antigen will bind.



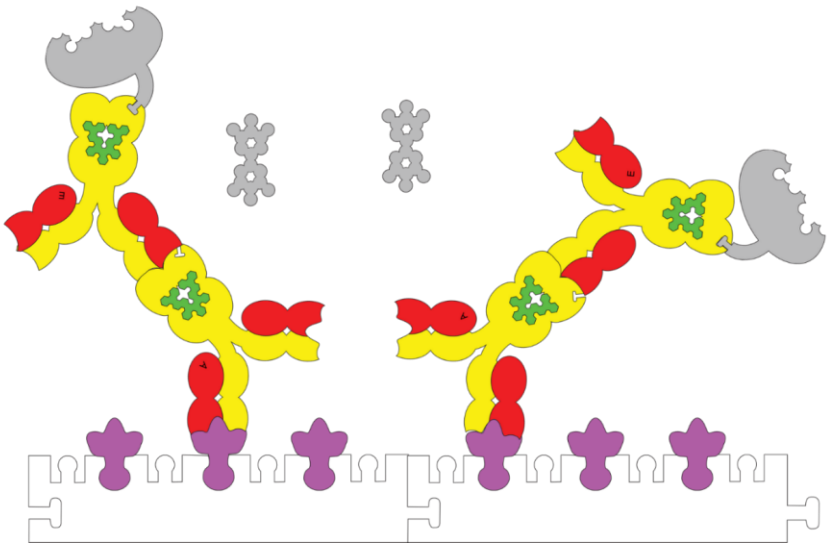
A wash buffer is added to remove any other antibodies.



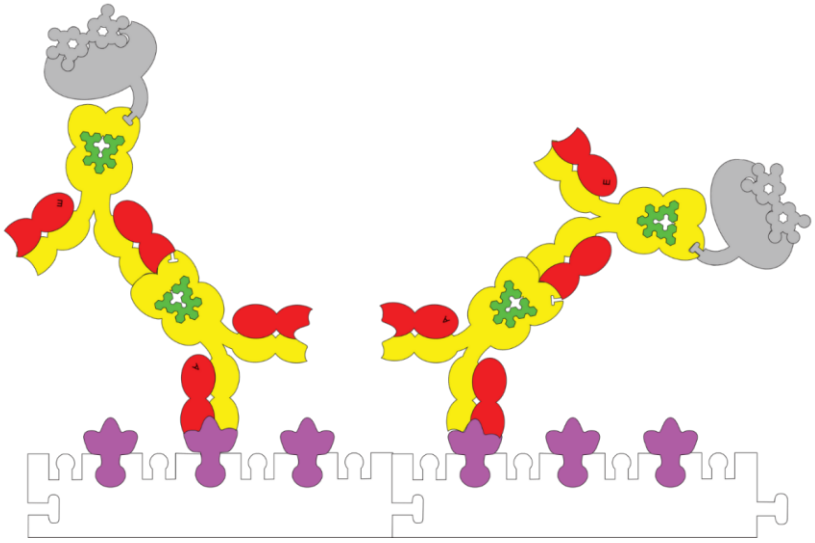
Secondary antibodies conjugated to an enzyme are added to the well plate.



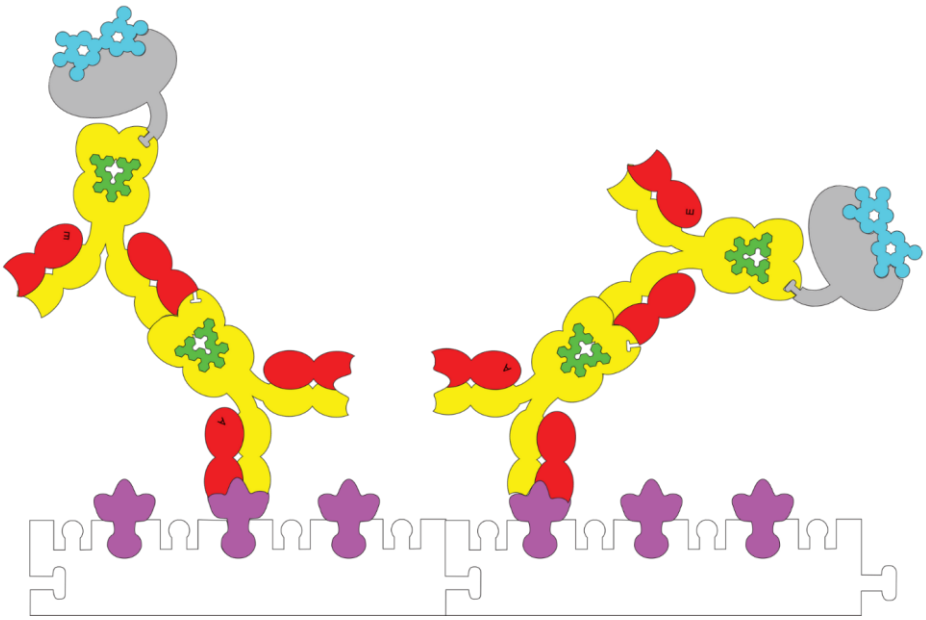
The secondary antibodies will interact with and bind the constant domain of the antibodies from the patient sample.



Add your substrate.

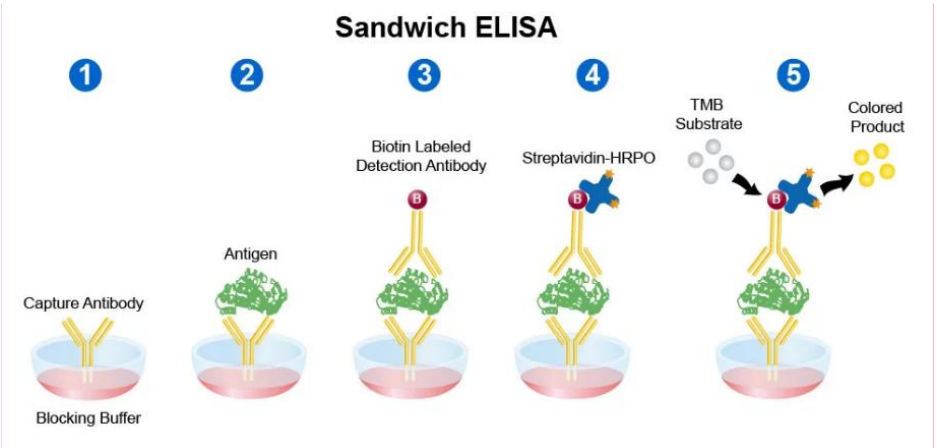


The substrate will interact with the conjugated enzyme, causing it to oxidize.



The oxidized substrate will change from colorless to blue. Spectroscopy or other methods (like a control dilution series) can be used to determine the number of antibodies present in the sample.

**ELISA – Sandwich test**




Commented [MA4]: Sandiwich-ELISA-illustration-02.jpg (983×471) (leinco.com)

Challenge your students to model a Sandwich ELISA Test.