We have had a thorough introduction to antibody structure and formation in developing B-cells. We should now have a better understanding of how a given antibody is formed to have specificity for one or a few related antigens and to have one of 5 different constant regions which will give the antibody different effector functions.

 Today’s lab will be the third that shows the utility of Mother Nature’s design on antibodies. We will perform an assay called an **e**nzyme-**l**inked **i**mmuno**s**orbent **a**ssay (**ELISA**). The assay is used in medical laboratories for diagnosis, in research laboratories, in drug testing and many other applications in which the goal is to test for the presence of a specific substance (referred to as an antigen) in a variety of sample types (e.g. blood, urine, water air). One or more of the pregnancy tests we used, may have used ELISA technology, but we did not spend much time on the details of ELISA last week.

 The ELISA procedure relies on specific antigen-antibody interactions followed by an enzymatic detection method. In the one type of ELISA, a specific antibody (called the primary antibody) is added to a special type of tube or plate, which will allow stable binding (adsorption) to the wells of the plate.\* Next, a liquid sample (e.g. urine, blood, water to be tested) is introduced to the same well of the plate. If the antigen is present, it will bind to the antigen binding site of the adsorbed antibody. \* A second antibody (called the secondary antibody) that is specific for different epitope of the antigen of interest is then added. The secondary antibody is covalently linked to an enzyme. If the antigen of interest is present, it will be immobilized on the primary antibody and retained in the well. The secondary, enzyme-linked antibody will be retained in the well through the second antigen-antibody interaction. The final step in ELISA is addition of a substrate for the enzyme. The enzymatic reaction must alter the substrate to make it detectable in some way (the substrate is often chromogenic or color-generating). At the end of an ELISA one should be able to “see” either by eye or by other detection systems, color development, only in the samples that had the antigen of interest.

 \* The wells of an ELISA plate are washed with gentle buffers between each of the additions of new reagents. (See step by step description of ELISA, next page.)

There are many, many variations and applications of ELISA (see attached and animations). <http://www.immunospot.com/index.php?id=164>

<http://www.sumanasinc.com/webcontent/animations/content/ELISA.html>

In our lab today we will **simulate**ELISA used to detect an HIV antigen in mock student samples. Early detection of HIV infection is essential for life-prolonging therapies to be effective. Early detection of HIV is also key in preventing the spread of the virus to others. ELISA-based detection methods are easy to perform (given the availability of a primary antibody for the antigen) and rapid, and are common tools in detection of HIV antigens (or HIV antibodies produced as a result of HIV infection).

Today we will simulate a typical ELISA assay to detect the HIV capsid p24 protein in mock serum samples from each student.

**Materials (tube colors may vary---check with Dr. Super)**

* Student test samples (yellow tubes labeled 1-8) **take 1/student**
* Purified HIV capsid protein (+ control) (violet tubes) **take 1/pair**
* Some other purified protein (- control) ( blue tubes) **take 1/pair**
* Primary mouse anti-HIV capsid p24 antibody (green tubes) **take 1/pair**
* Enzyme-linked secondary anti-mouse antibody (orange tubes) **take 1/pair**
* Substrate for enzyme (Horseradish peroxidase) (brown tube) **take 1/pair**
* 12-well ELISA microplate strips **take 1/pair**
* Racks for reagents
* Wash buffer
* Stack of paper towels
* Micropipettor/tips

**See attached protocol.** Each student will check their own sample in triplicate using one 12-well microplate strip. Skip a well between your + control, your – control and your sample wells. You should use 11 of the wells.

+ + + skip - - -skip sample, sample, sample