Antigen Detection ELISA LAB
Adapted from: BIORAD ELISA Immuno Explorer Kit

Background
When you are exposed to a disease agent, your body mounts an immune response. Molecules that cause your body to mount an immune response are called antigens, and may include components of infectious agents like bacteria, viruses, and fungi. Within days, millions of antibodies — proteins that recognize the antigen and bind very tightly to it — are circulating in your bloodstream. Antibodies seek out and attach themselves to their target antigens, flagging the invaders for destruction by the immune system.

Antibodies have become vital scientific tools, used in biotechnology research and to diagnose and treat disease. The number of different antibodies circulating in the blood has been estimated to be between 106 and 1011. In fact, antibodies make up to 15% of your total blood serum protein. Antibodies are very specific; each antibody recognizes only a single antigen.

How are antibodies made?
Animals such as chickens, goats, rabbits, and sheep can be injected with an antigen and, after a period of time, their serum will contain antibodies that specifically recognize that antigen. These antibodies used to recognize antigens are called primary antibodies. Secondary antibodies recognize and bind to the primary antibodies, which are antibodies from another species. Secondary antibodies are prepared by injecting antibodies made in one species into another species. It turns out that antibodies from different species are different enough from each other that they will be recognized as foreign proteins and provoke an immune response.

For example, to make a secondary antibody that will recognize a human primary antibody, human antibodies can be injected into an animal like a rabbit. After the rabbit mounts an immune response, the rabbit serum will contain antibodies that recognize and bind to human antibodies. The secondary antibodies used in this experiment are conjugated to the enzyme horseradish peroxidase (HRP) which produces a blue color in the presence of its substrate, TMB. These antibody and enzyme tools are the basis for the ELISA.

Where are ELISAs used in the real world?
With its rapid test results, the ELISA has had a major impact on many aspects of medicine and agriculture. ELISA is used for such diverse purposes as pregnancy tests, disease detection in people, animals, and plants, detecting illegal drug use, testing indoor air quality, and determining if food is labeled accurately.

For new and emerging diseases, one of the highest priorities of the US Centers for Disease Control (CDC) and the World Health Organization (WHO) has been to develop an ELISA that can quickly and easily verify whether patients have been exposed to a virus.

Why Do We Need Controls?
Positive and negative controls are critical to any diagnostic test. Control samples are necessary to be sure your ELISA is working correctly. A positive control is a sample known to be positive for the disease agent, and a negative control is a sample that does not contain the disease agent.

Pre-Lab Questions:
1. How does the immune system protect us from disease?

2. How are primary antibodies formed?

3. How are secondary antibodies formed?

4. How are ELISAs used?
5. Why are enzymes used in this immunoassay?

6. Why do you need to assay positive and negative control samples as well as your experimental samples?

Introduction:
After the biotechnology fieldtrip to the CDC, it is later determined that a health care worker who rode on Marta has Ebola, and one or two of the students may have contracted the disease. The remainder of the class may also have been exposed. Some have had contact with several of their classmates since their exposure. Perform an ELISA to determine the spread of Ebola within your classroom. It is important to determine as soon as possible which students have been exposed so that they may be isolated. (Quarantine of exposed individuals is currently the best way to prevent the spread of this very infectious virus.) To determine which students have been exposed, perform an ELISA to detect the Ebola virus in samples of their body fluid.

Materials:
yellow tubes, violet tube (+ control), blue tube (- control), green tube (primary antibody), orange tube (secondary antibody), brown tube (enzyme substrate), microplate strip, micropipette, tips, plastic pipets, wash buffer, beaker

Procedure:
1. Label a yellow tube with your initials.
2. Label the outside wall of each well of your 12-well strip as indicated in the diagram.

3. Use a pipet to transfer 50 µl of the positive control (+) from the positive tube into the three “+” wells.
4. Use a fresh pipet tip to transfer 50 µl of the negative control (–) from the negative tube into the three “–” wells.
5. Use a fresh pipet tip for each sample and transfer 50 µl of each of your sample into the appropriately initialed three wells.
6. Wait 5 minutes while all the proteins (antigen) in the samples bind to the plastic wells.
7. Wash the unbound sample out of the wells:
   a. Tip the microplate strip upside down onto the paper towels, and gently tap the strip a few times upside down. Make sure to avoid samples splashing back into wells.
   b. Discard the top paper towel.
   c. Use a fresh transfer pipet to fill each well with wash buffer, taking care not to spill over into neighboring wells. The same transfer pipet will be used for all washing steps. **Do not touch the well with the wash buffer pipet.**
   d. Tip the microplate strip upside down onto the paper towels and tap.
   e. Discard the top 2–3 paper towels.
8. Repeat wash step 7.
9. Use a fresh pipet tip to transfer 50 µl of primary antibody (PA) into all 12 wells of the microplate strip. **Do not touch the well. If you touch the well, get a new tip.**
10. Wait 5 minutes for the primary antibody to bind to the antigens.
11. Wash the unbound primary antibody out of the wells by repeating wash step 7 - two times.
12. Use a fresh pipet tip to transfer 50 µl of secondary antibody (SA) into all 12 wells of the microplate strip. **Do not touch the well. If you touch the well, get a new tip.**
13. Wait 5 minutes for the secondary antibody to bind.
14. Wash the unbound secondary antibody out of the wells by repeating wash step 7 - three times.
The secondary antibody is attached to an enzyme (HRP) that chemically changes the enzyme substrate, turning it from a colorless solution to a blue solution.

Predict which wells of your experiment should turn blue and which should remain colorless and which wells you are not sure about.

15. Use a fresh pipet tip to transfer 50 µl of enzyme substrate (SUB) into all 12 wells of the microplate strip.
16. Wait 5 minutes. Observe and record your results.

Results:
Label the figure below with the same labels you wrote on the wells in step 2. Put a “+” if the well turned blue and a “–” if there is no color change.

Are you “infected” with the disease? __________

Post-Lab Questions:
1. The samples that you added to the microplate strip contain many proteins and may or may not contain the disease antigen. What happened to the proteins in the plastic well if the sample contained the antigen? ____________________________________________________________ What if it did not contain the antigen? ____________________________________________________________
2. Why did you need to wash the wells after every step? ____________________________________________________________
3. When you added primary antibody to the wells, what happened if your sample contained the antigen? ____________________________________________________________ What if it did not contain the antigen? ____________________________________________________________
4. When you added secondary antibody to the wells, what happened if your sample contained the antigen? ____________________________________________________________ What if it did not contain the antigen? ____________________________________________________________
5. Why did you assay your samples in triplicate? ____________________________________________________________

In this lab, the antigen is chicken gamma-globin, the 1° antibody is a polyclonal rabbit antibody, the 2° antibody is rabbit IgG and the enzyme is horseradish peroxidase (HRP).

Teacher Prep:
Prepare buffers; Rehydrate antigen, 1° antibody, 2° antibody; Dilute 50x stock solutions; Dispense reagents (pages 41-43)