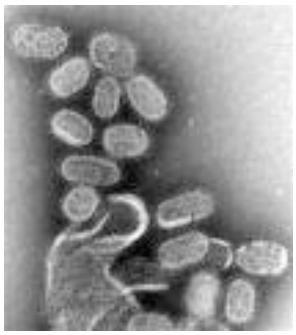
Name: _____

Outbreak!

An Influenza disease transmission and diagnosis activity

Adapted for use on the MdBioLab from Bio-Rad's *ELISA Immuno Explorer Kit*, Catalong #166-2400EDU

H1N1 - Swine Flu



Swine flu was first detected in people in the United States in April 2009. On June 11, 2009, the World Health Organization (WHO) signaled that a pandemic of 2009 H1N1 flu was underway.

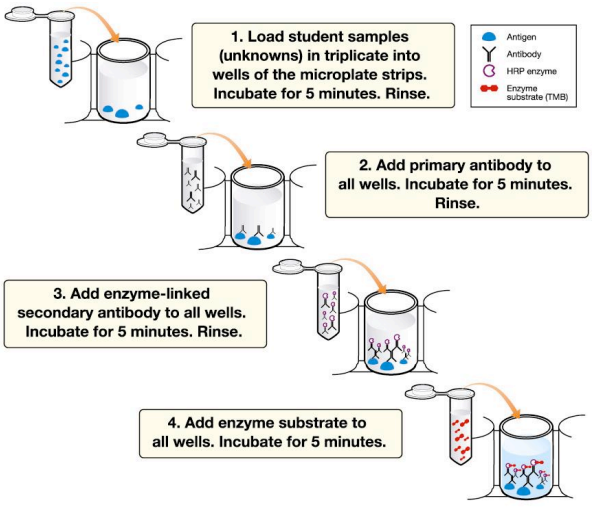
Spread of 2009 H1N1 virus is thought to occur in the same way that seasonal flu spreads. People with influenza spread flu viruses mainly from person to person through coughing or sneezing. Touching something –such as a surface or object – may infect sometimes people with flu viruses on it and then touching their mouth or nose.

People infected with seasonal and 2009 H1N1 continuously she the virus and may be able to infect others from 1 day before getting sick to 5 to 7 days after. This can be longer in some people, especially children and people with weakened immune systems and in people infected with the new H1N1 virus.

Source: http://www.cdc.gov/H1N1FLU/

Outbreak in the Air: Imagine the vacation of a lifetime. You and your friends have taken an overseas flight to visit another country. When you arrive at the airport, you are stopped by security. Apparently a few passengers on your flight are exhibiting the symptoms of swine flu. You must be quarantined and tested for the virus before you enter the country. **What are some of your concerns?**

ELISA



You are about to perform an experiment in which you will share simulated "body fluids" with your classmates. After sharing, you will perform an **enzyme-linked immunosorbent assay,** or **ELISA,** to determine if you have been exposed to a contagious "disease." The ELISA relies on antibodies to detect the presence of a disease agent, (for example, viruses, bacteria or parasites) in your blood or other body fluid. You will then track the disease back to its source.

Antigens & Antibodies: 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. Like magic bullets, antibodies seek out and attach themselves to their target antigens, flagging the invaders for destruction by other cells of the immune system.

Antibodies are important tools used to diagnose and treat disease. The number of different antibodies circulating in the blood has been estimated to be between 10,000,000 and 1,000,000,000,000, so there is usually an antibody ready to deal with any antigen. In fact, up to 15 percent of your total blood serum is antibodies that recognize a single antigen.

ELISA Protocol

You will simulate the passengers on the plane and have been given a simulated "body fluid" sample. One or two of the passengers have been "infected." You have also been given positive and negative control samples. You and the other students will assay your samples for the presence of the "disease agent" to track the spread of the disease through the airplane.

The following is a list of the materials you will be using for the experiment:

Test sample ("body fluid")	12-well microplate strip
(+) - Positive control	Micropipettes
(-) - Negative control	Transfer pipette
(PA) - Primary antibody	Paper towels/napkins
(SA) - Secondary antibody	Marking pen
(SUB) - Enzyme substrate	Wash Buffer

PART 1 – Share Body Fluids

☐ 1. Label a "body fluid" sample tube with your initials.

☐ 2. Use the micropipette to transfer 200 µL of "body fluid" sample into the tube of another student. Pipette up and down to mix the samples.

☐ 3. Write down the name of the student next to "Sharing Partner #1."

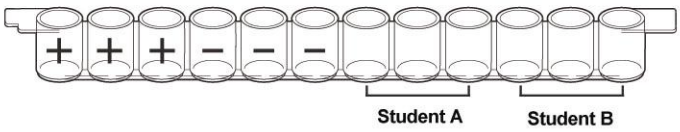
Sharing Partner #1
Sharing Partner #2
Sharing Partner #3

☐ 4. When told to do so, repeat the sharing protocol two more times with two other students so that you have shared your sample with three students total. Make sure that you record their names in the order in which you shared.

PART 2 – Perform ELISA

☐ 5. Label the outside wall of each well of your 12-well strip. Two students may share a strip of 12 wells. On each strip, label the first three wells (1, 2, and 3) with a "+" for the positive controls and the next three wells (4, 5, and 6) with a "-" for the negative controls.

☐ 6. On the remaining wells write your and your partner's initials.



☐ 7. Bind the antigen to the wells:

7.1 Use a **new** micropipette tip to transfer 50 µL of the positive control (+) into the three "+" wells in your 12-well strip.

7.2 Use a **new** micropipette tip to transfer 50 µL of the negative control (-) to the three "-" wells in your 12-well strip.

7.3 Use a **new** micropipette tip for each sample and transfer 50 µL of each of your team's samples into the appropriately initialed three wells.

☐ 8. Incubate (wait) five minutes while all of the proteins in the samples bind to the wells on the 12-well strip.

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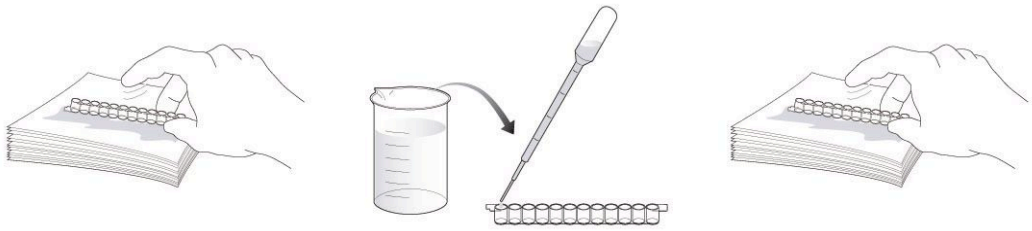
☐ 9. Wash the unbound sample out of the wells:



9.1 Tip the microplate strip upside down onto two paper towels so that the sample drains out, then gently tap the strip a few times upside down on the paper towels making sure not to tap on the same area each time. Make sure to avoid samples splashing back into wells.

9.2 Place two paper towels on top of the ones you just used.

9.3 Use a transfer pipette filled with wash buffer from the beaker to fill each well half-way with wash buffer taking care not to spill over into neighboring wells. The same transfer pipette will be used for all washing steps.

9.4 Tip the microplate strip upside down onto the paper towels so that the wash buffer drains out, and then gently tap the strip a few times upside down on the paper towels careful not to tap on the same area of paper towel each time.

9.5 Add two more paper towels onto the stack.

☐ 10. Wash the wells again by **repeating step 9**.

☐ 11. Use a **new** micropipette tip to transfer 50 µL of primary antibody (PA) into all 12 wells of the microplate strip. If the well contains the antigen then the primary antibody will **bind** to it.

☐ 12. Incubate five minutes to allow the primary antibody to bind.

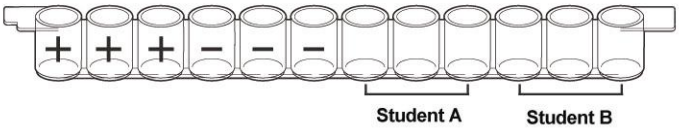
☐ 13. Wash the unbound primary antibody out of the wells by **repeating wash step 9 two times**.

☐ 14. Use a **new** micropipette tip to transfer 50 µL of secondary antibody (SA) into all 12 wells of the microplate strip. If the well contains the antigen then the secondary antibody will **bind** to the primary antibody.

☐ 15. Incubate five minutes for the secondary antibody to bind.

☐ 16. Washing the unbound secondary antibody out of the wells by **repeating wash step 9 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 – write your predictions on the figure below:



☐ 17. Use a **new** pipette tip to transfer 50 µL of enzyme substrate (SUB) into all 12 wells of the microplate strip. Incubate for five minutes. If the well contains the antigen then the substrate will **bind** to the secondary antibody.

PART 3 - Results

☐ 18. Label the figure below with the same labels you wrote on the wells in step five. In each of the wells, put a "+" if the well turned blue and a "-" if there was no color change.

Are you "infected" with the disease? YES NO (circle one)

PART 4 - Conclusion

Compare the entire class's results. With your instructor, track the progress of the disease through the class by having each student record whether they tested positive or negative next to the names of the people with whom they shared "body fluids." Can you track your results back to the first infected people in the class?

Think about the results of your ELISA and the rest of the class's. What conclusions can you make about the spread of the disease through the Mobile Laboratory? Assume you are sharing the results with your classmates and they do not know how the test works.

PART 5 – Discussion Questions 1.

A disease agent or foreign particle that triggers a response in the body is called a(n) _____.

The body responds to an antigen by producing _____.

What process does the ELISA acronym stand for?_____.

What does the secondary antibody bind two of the well contains the antigen?

The Assay

Draw what is happening during each step of the ELISA if the well contains antigen.