Enzyme-linked Immunosorbent Assay (ELISA)

Testing for Measles, Streptococcus, and Mononucleosis

*PROTOCOL ADAPTED FROM THE BIOTECHNOLOGY EXPLORER, ELISA IMMUNO EXPLORER KIT (BIO-RAD) INSTRUCTION MANUAL

Teacher's Guide

Materials

Note: harmless antigens and simulated blood serum are used; nothing is harmful to your health.

ELISA Immuno Explorer Kit (Bio-Rad, Cat.#166-2400
Gloves
Lab Coats
Fixed 50 uL pipets, or adjustable 20-200 uL pipets and tips
Clear 1.5 mL microcentrifuge tubes
1.5 mL tube racks (1 per student pair)
Large stack of paper towels
Disposable plastic transfer pipets
50 mL conical tubes

Activity Preparation

1. Follow the Instruction Manual provided with the kit to prepare 1X PBS, wash solution, antigen, primary and secondary antibodies.
2. For each pair of students, label the yellow tubes "POS" for the positive control antigen, the violet tubes "1" for Measles antigen, the blue tubes "2" for Streptococcus antigen, the clear tube "3" for Mononucleosis antigen, green tubes "PA-X" for primary antibody with X representing the patient ID of A-E, etc, orange tubes "SA" for secondary antibody, and brown tubes "SUB" for substrate.
3. Aliquot 50 mL of Wash Buffer into a 50 mL conical tube for each pair of students.
4. Aliquot 1 mL of primary antibody into the labeled green tubes, one per group.
5. Aliquot 1 mL of secondary antibody into the labeled orange tubes, one per group.
6. Aliquot 1 mL of substrate into the brown tubes, one per group.
7. Aliquot 200 uL of 1X antigen or 1X PBS into the colored tubes for each pair of students as outlined in the table below. This table depicts the set up for 20 students working in 10 pairs.

Patient	Yellow- "POS"	Violet- "1" Measles	Blue- "2" Strep	Clear- "3" Mono
A	ANTIGEN	ANTIGEN	1X PBS	1X PBS
B	ANTIGEN	ANTIGEN	ANTIGEN	1X PBS
C	ANTIGEN	ANTIGEN	ANTIGEN	1X PBS
D	ANTIGEN	ANTIGEN	1X PBS	1X PBS
E	ANTIGEN	ANTIGEN	ANTIGEN	ANTIGEN
F	ANTIGEN	ANTIGEN	ANTIGEN	1X PBS
G	ANTIGEN	ANTIGEN	1X PBS	1X PBS
H	ANTIGEN	ANTIGEN	1X PBS	1X PBS
I	ANTIGEN	ANTIGEN	1X PBS	1X PBS
J	ANTIGEN	ANTIGEN	ANTIGEN	1X PBS

Protocol

1. Having students work in pairs, label a 12-well strip tube in triplicate for the positive control, and antigens 1, 2, and 3 as depicted in the photo below.

2. Add 50 uL of the appropriate antigen in each well. The positive control in the '+' wells, antigen for Measles in Sample 1, Streptococcus for Sample 2, and Mononucleosis for Sample 3. Use fresh pipet tips for each antigen samples. Do not cross-contaminate! Incubate at room temperature for 5 minutes.
3. Remove the solution by tapping the strip tube over a stack of paper towels. Fill each well with wash buffer using a 200 uL pipet or disposable transfer pipet and remove the solution by tapping the strip tube over a fresh region of the paper towel stack. Avoid cross-contamination by not over filling the wells. Repeat with one more wash. Remove the top layer of the stack if it is saturated.
4. Each group will be given the blood serum (green tubes) from a different patient (Patient A, B, C, D, etc...). These tubes are labeled "PA-X" with X representing the patient ID. The patient blood serum will contain antibodies specific to the diseases the patient has had exposure to. Remove the final wash solution from each well by inverting the strip tube over the stack of paper towels. Add 50 uL of the patient serum to each of the 12 wells. Incubate at room temperature for 5 minutes. Only antibodies specific for the antigen will bind.
5. Wash wells 2 times with wash buffer, draining on the paper towel stack.
6. Add 50 uL of the enzyme-labeled secondary antibody (orange tubes labeled "SA") to each well. This antibody binds to any primary antibody bound to the antigen. Incubate 5 minutes at room temperature.
7. Wash the wells 3 times with wash buffer, draining well after each wash.
8. Add 50 uL of the chromogenic enzyme substrate (brown tubes labeled "SUB") into all 12 wells of the tube strip. If a patient's blood serum contains antibodies for a specific antigen, then that patient has had the disease. When a "positive" reaction occurs, a blue color will appear within 5 minutes. Negative wells remain colorless.

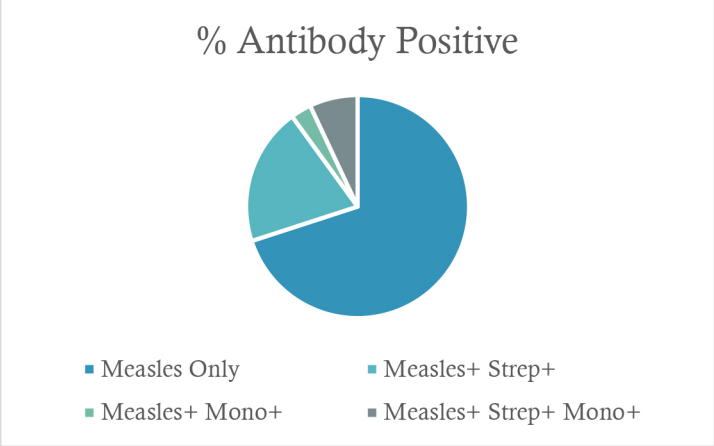
9. Record your results and share with the class.

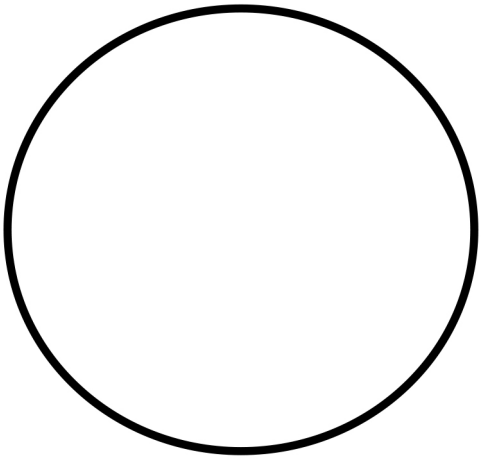
ELISA Test Results

	(none)	Streptococcus +	Mononucleosis +	Strep and Mono +
#Measles +	5	4	0	1
Percentage=	50	40	0	10

Create a pie chart expressing the results

Example:





Thought Questions

1. How can you explain the frequency of positive reactions for measles? (hint: have *you* ever had measles? Why not?)
2. What does this assay tell you about the specificity of antibodies? (hint: do measles antibodies bind to strep antigens?)
3. Some patients did not just recently have their disease(s). What does this tell you about antibody protection from disease?

Crime Scene Investigators

Agglutination Reactions using simulated blood and antibodies

Teacher's Guide

The Situation

A crime has been committed! The victim has Type O blood. A small trace of Type AB blood was found at the scene of the crime and the CSI Unit believes it to be from the perpetrator. They are holding 3 key suspects on unrelated charges and need further evidence to detain or clear them of this crime.

Course of Action

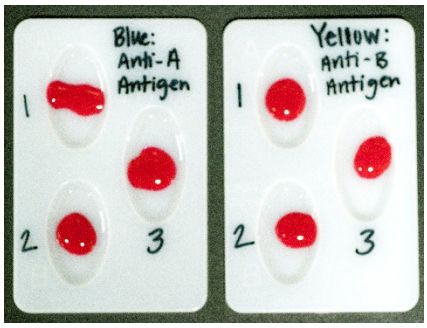
It is up to you to test the blood of the 3 suspects to see if any of them match the AB Blood Type found at the scene of the crime. You will perform a standard blood typing agglutination reaction to determine the blood type of the 3 suspects.

Materials

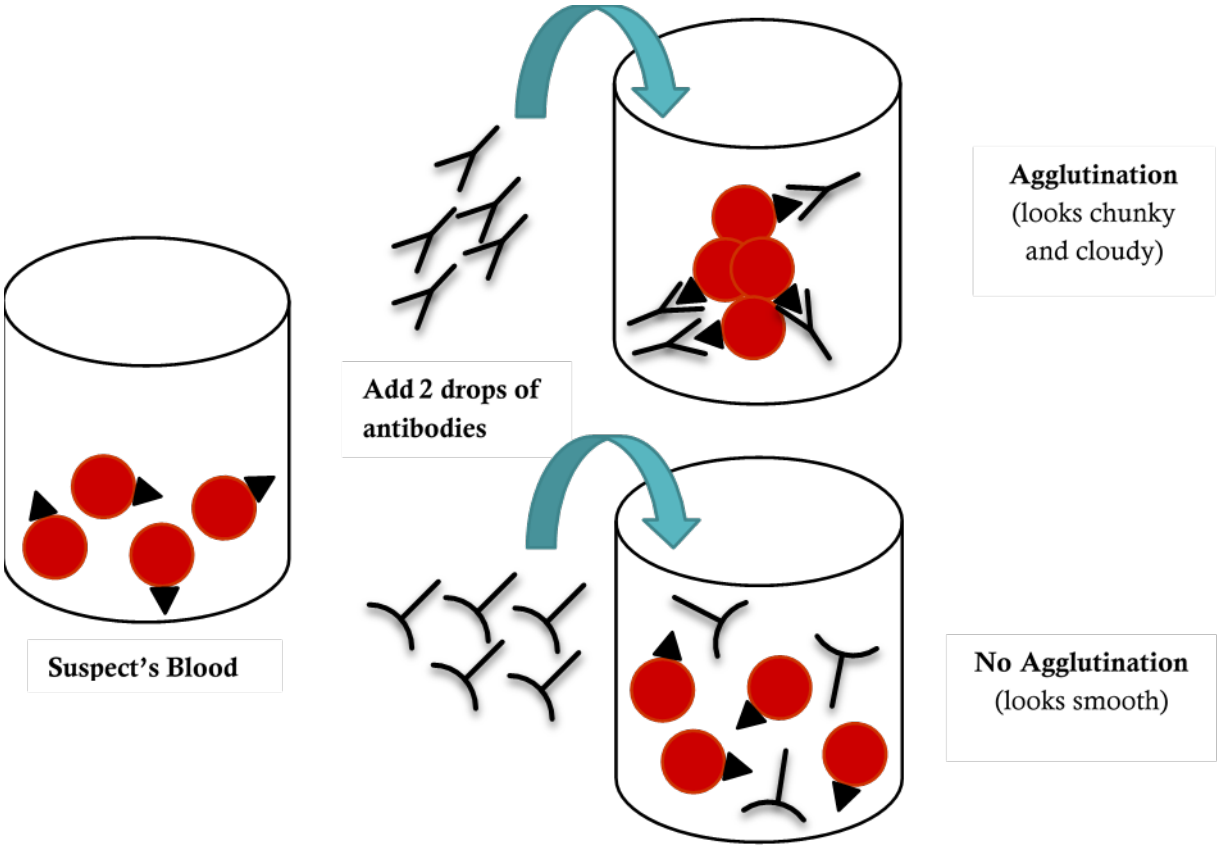
Carolina ABO-Rh Typing with Synthetic Blood Kit (www.carolina.com, part number 70-0101)
Gloves
Lab Coat
Table Diapers

Plate Set Up

Prepare two plates for each group of 2 students. Label one plate "Blue: Anti-A Antigen" and the other "Yellow: Anti-B Antigen". Label the wells of each plate 1, 2 and 3 for the three suspects in the scenario. Add two drops of Sample 4 to well 1 in each plate. Add two drops of Sample 3 to well 2 and two drops of Sample 1 to well 3.



Schematic of Agglutination



Protocol

1. Form groups containing 2 people
2. Obtain 2 trays with suspect blood: One tray labeled "Blue: Anti-A antigen" and the other labeled "Yellow: Anti-B Antigen". Gather three blue toothpicks and three yellow toothpicks.
3. Carefully add 1 drop of the correct antibody to each well.
4. Very gently stir each well with a clean toothpick (don't cross-contaminate by using the same toothpick to stir different wells).
5. Watch for a cloudy, clumpy appearance to appear in the wells.
6. Compare the results seen in Anti-A antigen Tray to the Anti-B antigen within your group to determine the blood type of each suspect.

Agglutination Results

	Anti-A Antigen: Did it agglutinate?	Anti-B Antigen: Did it agglutinate?	Blood Type
Suspect 1	No	No	O
Suspect 2	Yes	Yes	AB
Suspect 3	Yes	No	A

Based on these results, which suspect can NOT be ruled out as a possible suspect? Suspect 2

Thought Questions

1. If a person was born without B cells (this can happen and is called X-linked agammaglobulinemia) do you think that you would have a good agglutination reaction? (hint: think of what cell makes antibodies)
2. Why do you think antigens are important? (hint: think of "self" versus "microbe".....)
3. Can you think of a disease where "self" antigens are NOT ignored, but attacked, and the person gets sick?
4. Do you think the same problem faces patients who receive organ transplants? Why or why not?

Fill in the chart below

Blood Type	Antigens on Red Blood Cells	Antibodies in Blood	Can give blood to:	Can receive blood from:
Type A	A	Anti-B Antigens	A and AB	A and O
Type B	B	Anti-A Antigens	B and AB	B and O
Type AB	A and B	None to A or B	AB only	AB and O
Type O	Neither A nor B	Bind to A and B antigens	All blood groups	O only