Edvo-Kit # 263

Edvo-Kit #263

Expanding Our Testing: Using ELISA to Detect COVID-19

Experiment Objective:

Due to the worldwide spread of the respiratory disease COVID-19, scientists developed diagnostic tests in order to identify and monitor the disease. In this simulated medical test, students will perform the ELISA to detect the presence of COVID-19 antibodies in simulated patient samples.

See page 3 for storage instructions.

Version 263.201022

EDVOTEK®

Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

	Components	Storage	Check (✓)
A	10X ELISA Wash Buffer	Refrigerator	❑
B	ELISA Dilution Buffer	Refrigerator	❑
C	Antigen (lyophilized)	Refrigerator	❑
D	Primary Antibody (lyophilized)	Refrigerator	❑
E	Secondary Antibody (lyophilized)	Refrigerator	❑
F	ABTS Substrate (lyophilized)	Refrigerator	❑
G	ABTS Reaction Buffer	Refrigerator	❑

Experiment #263 contains enough reagents for 10 lab groups.

REAGENTS & SUPPLIES

Store all components below at room temperature.

- Microtiter plate ❑
- Transfer pipets ❑
- Snap-top Microcentrifuge tubes ❑
- 15 mL conical tubes ❑

Experiment Requirements *(NOT included in this experiment)*

- Distilled or deionized water
- Beakers
- Pipet pump or bulb
- Lab glassware
- Disposable lab gloves
- Safety goggles
- Recommended: Automatic micropipettes (0-50 µL, 100-1000 µL) and tips

Make sure glassware is clean, dry and free of soap residue.

For convenience, additional disposable transfer pipets can be purchased for liquid removal and washing steps.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

None of the experiment components are derived from human sources..

Background Information

SARS-CoV-2 is a novel coronavirus that caused a worldwide outbreak of the respiratory disease known as COVID-19. In December 2019, SARS-CoV-2 emerged and spread worldwide in a very short period of time. Symptoms of COVID-19 include fever, cough, and shortness of breath. In severe cases, patients may develop pneumonia, respiratory distress, kidney failure, or even death. Treatment for COVID-19 includes rest, fluids, and over-the-counter cold medications.

Coronaviruses are not rare. Each year, experts estimate that Coronaviruses cause 15-30% of all common cold cases. These symptoms are generally mild and include fever and sore throat. Coronaviruses have a single-stranded RNA genome wrapped in a helical capsid. A membrane envelope surrounds the capsid. The envelope is studded with proteins that help the virus infect cells. By electron microscopy, the envelope proteins create a hazy halo around the virus particle. Scientists described them with the Latin word *corona*, which means "crown" or "halo" (Figure 1).

BOX 1: Important Definitions

- **SARS-CoV-2, or severe acute respiratory syndrome coronavirus 2:** the name of the novel coronavirus responsible for the current pandemic.
- **COVID-19, or coronavirus disease 2019:** this is the disease caused by SARS-CoV-2, characterized by fever, cough, and shortness of breath.
- **Outbreak:** a rapid increase in the number of cases of a disease at a specific time and place
- **Pandemic:** an outbreak of a disease in many different geographic areas that affects a significant proportion of the population

With proper precautions, the spread of COVID-19 can be prevented. Coronaviruses spread person-to-person through liquid droplets that come out when you cough or sneeze. Soap, hand sanitizer and other disinfectants kill coronaviruses, so frequent washing of hands can limit its spread. Touching your face with contaminated hands can introduce the virus to your mucus membranes, so it is important to keep hands away from your eyes, nose and mouth. Cloth masks that cover the mouth and nose prevent our respiratory droplets from spreading via cough or sneeze. Furthermore, while the disease is spreading, actions like social distancing can reduce the likelihood of infecting those around us, which limits the spread of the disease.

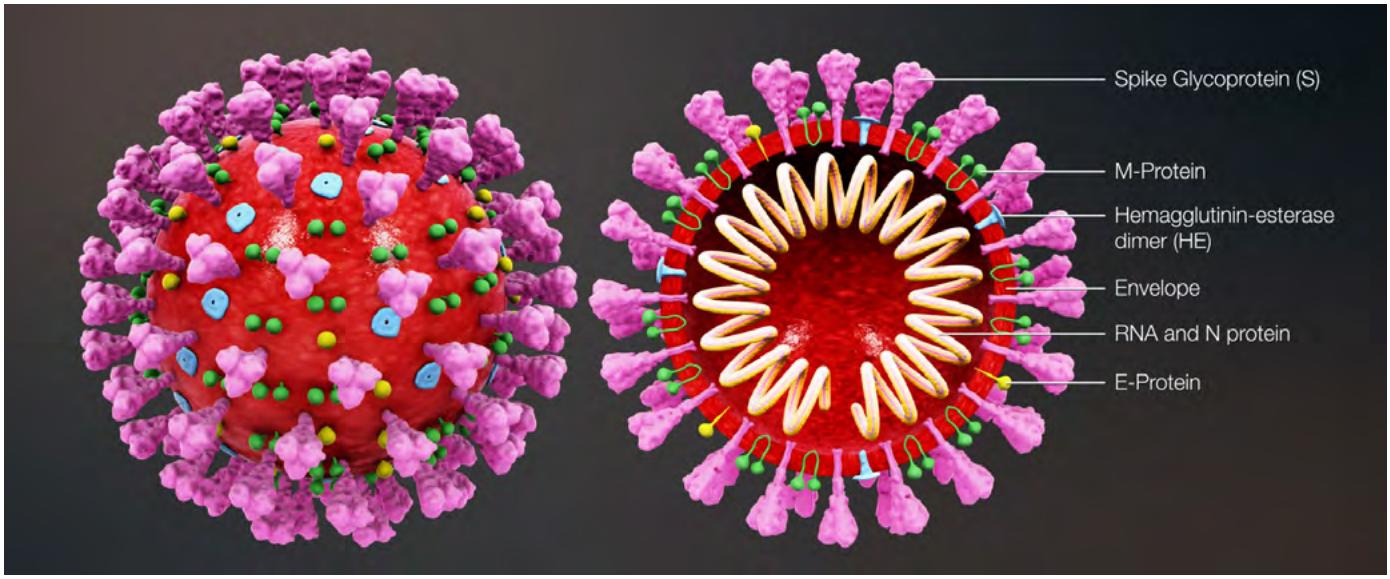


Figure 1: Anatomy of a coronavirus.

TESTING FOR COVID-19

One of the most powerful tools in the mission to reduce the spread of SARS-CoV-2 is widespread testing for the virus. There are two diagnostic tests to confirm a diagnosis of COVID-19: Reverse Transcription PCR (RT-PCR) and Enzyme-Linked Immunosorbent Assay (ELISA). A positive test does not mean that a patient will become seriously ill; however, these diagnoses are important as they allow epidemiologists to trace the spread of COVID-19.

RT-PCR detects the presence of viral genome in patient samples, signifying active infection. The assay targets unique sequences that can only be found in SARS-CoV-2. RT-PCR is extremely sensitive and as such can detect minute amounts of the virus. In these ways, it is an ideal assay to diagnose SARS-CoV-2 infections. Because RT-PCR is extremely sensitive and can detect very low levels of the virus, it is considered the "gold standard" for SARS-CoV-2 detection. However, since RT-PCR tests are performed in a medical diagnostic laboratory, it may take several days to get the results, even though the actual test takes a few hours.

The virus responsible for COVID-19, SARS-CoV-2, does not integrate itself into the human genome during infection (e.g. HIV, HSV). This means that once the patient's immune system has cleared the SARS-CoV-2 infection, no viral nucleic acid remains in the body, making the RT-PCR test ineffective. However, the antibodies generated by the human body to fight off the infection remain in the body after the patient has healed (Figure 2).

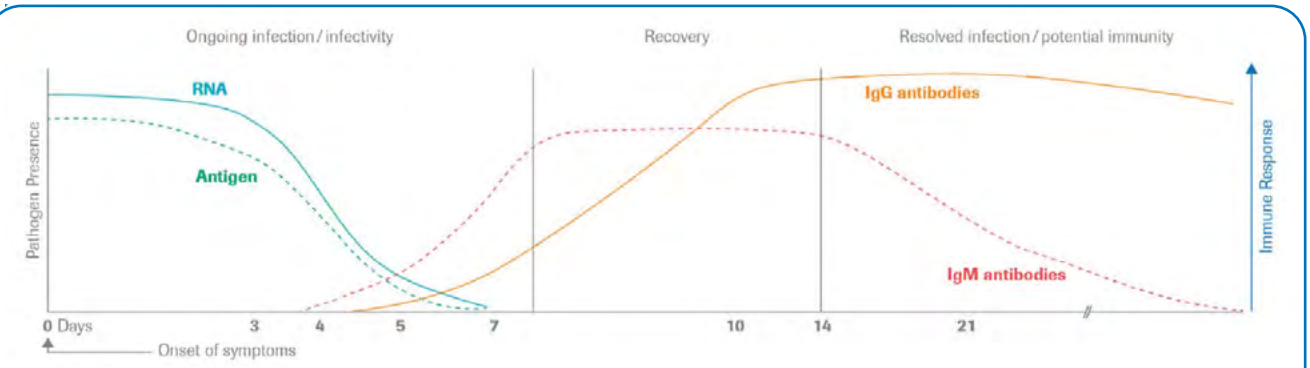


Figure 2: Changes in patient SARS-CoV-2 antibody levels over time.

The ELISA is a highly sensitive test that uses antibodies to detect the presence of specific biomolecules (i.e. peptides, proteins, antibodies and hormones). This immunoassay can detect the presence of antibodies to SARS-CoV-2 in patients, signifying that a person had been previously infected by the virus. By using this assay, researchers will be able to identify individuals affected by this disease who were not tested while ill. However, since the body takes several days to produce these antibodies, the ELISA cannot diagnose infection before clinical symptoms arise.

THE COVID-19 IMMUNOASSAY

Antibodies are specialized proteins that allow the immune system to distinguish between "self" and "non-self" molecules, known as antigens. Each antibody is a Y-shaped molecule composed of four linked protein chains: two identical "heavy chains" and two identical "light chains" (Figure 3). The antigen binding sites are located at the ends of the short arms of the Y. The amino acid sequence in this region is variable, allowing for each antibody to recognize a unique epitope (a particular location within an antigen).

IgM and IgG are antibodies in patient samples that form the immune response to SARS-CoV-2. The IgM antibody serves as the first line of defense against SARS-CoV-2 by binding to pathogens and labeling them for inactivation by the immune system. As the body creates long-term immunity to the virus, IgG antibodies are produced in the plasma B-cells. Only individuals whose immune systems have been exposed to the SARS-CoV-2 virus would contain these antibodies.

The ELISA for SARS-CoV-2 uses antibodies to detect both IgM and IgG in patient blood samples. One of the most sensitive ELISA techniques is the sandwich ELISA, in which two separate antibodies are used to detect the antigen – one antibody that is bound to the plate to "capture" the antigen, and one that is used to detect it (Figure 3). First, the capture antibody is added to the wells of a transparent plastic microtiter plate. The antibody non-specifically adheres to the plastic through hydrophobic and electrostatic interactions. Any unbound antibody is washed out with buffer.

Next, the patient samples are added to the wells. The bound antibody recognizes a specific area of the antigen (called an epitope) and non-covalently binds. After the incubation period, the wells are washed to remove excess sample that did not bind. The purified detection antibody is added and allowed to bind with the antigen. After a short incubation period, any unbound antibody is washed away with a buffer.

The detection antibody is covalently linked to an enzyme that allows for the detection of the antibody-antigen complex. A clear, colorless substrate solution is added to each well. In wells where the secondary antibody is present, the enzyme turns the clear substrate solution to green. Since most enzymes have a high catalytic activity, with its substrate turnover rates exceeding 10^6 per second, this assay allows us to quickly detect even the smallest amount of antibody.

In this **simulated medical test**, we will use the ELISA to detect the presence of anti-SARS-CoV-2 antibodies in a patient blood sample. In patients that have been infected with the virus, the ELISA will detect the anti-SARS-CoV-2 antibodies and a color change reaction will be seen. In contrast, a sample from a patient who was not infected with SARS-CoV-2 will not have a color change.

Please note, this experiment is a simulation. There is no virus or human blood products included with this experiment. The experiment will not detect SARS-CoV-2 antibodies in human blood samples.

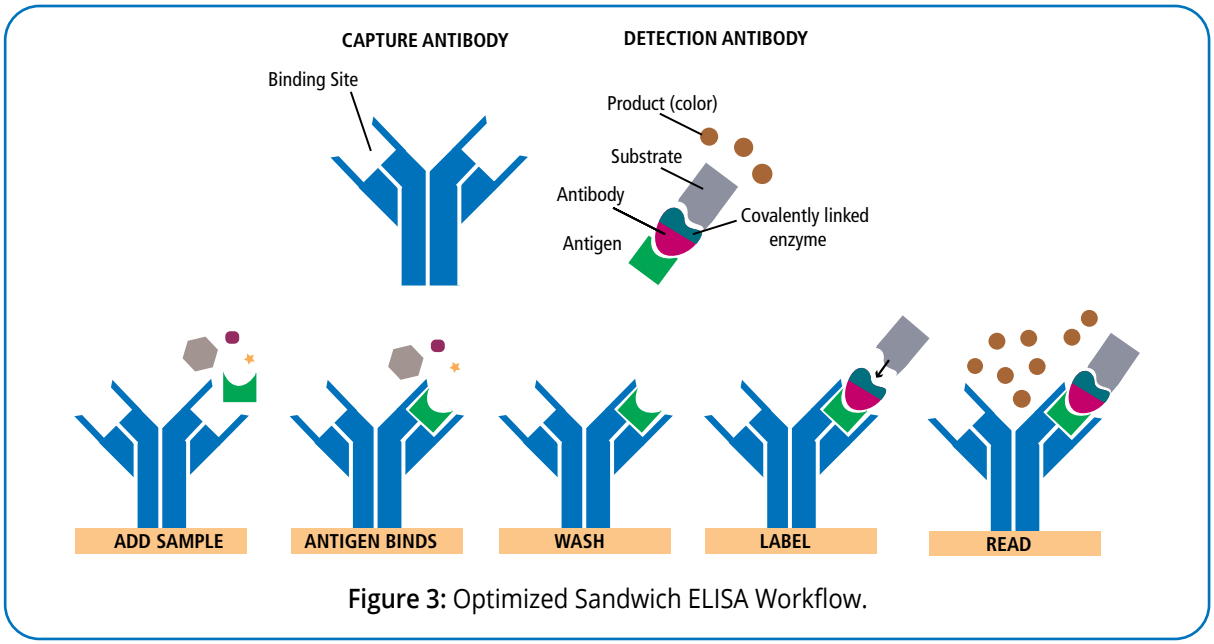


Figure 3: Optimized Sandwich ELISA Workflow.

Figure 1 by https://www.scientificanimations.com - https://www.scientificanimations.com/wiki-images/, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=86436446
Figure 2 from https://diagnostics.roche.com/
Figure 2 Sources:
Long, Q. et al. (2020). medRxiv. preprint doi: https://doi.org/10.1101/2020.03.18.20038018
Lou, B. et al. (2020). medRxiv. preprint doi: https://doi.org/10.1101/2020.03.23.20041707
Zhao, J. et al. (2020). Clin Infect Dis. pii: ciaa344. doi: 10.1093/cid/ciaa344. [Epub ahead of print]
Liu, W. et al. (2020). J Clin Microbiol. pii: JCM.00461-20. doi: 10.1128/JCM.00461-20. [Epub ahead of print]
To ,K. et al. (2020). Lancet Infect Dis. pii: S1473-3099(20)30196-1. doi: 10.1016/S1473-3099(20)30196-1 [Epub ahead of print] Xiao, D.A.T. et al. (2020). J Infect., S0163-4453(20)30138-9. doi:10.1016/j.jinf.2020.03.012. [Epub ahead of print]
Zhang, B. et al. (2020). medRxiv. preprint doi: https://doi.org/10.1101/2020.03.12.20035048
Wölfel, R. et al. (2020). Nature. Apr 1. doi: 10.1038/s41586-020-2196-x. [Epub ahead of print]
Tan, W. et al. (2020). medRxiv. preprint doi: https://doi.org/10.1101/2020.03.24.20042382

Experiment Overview

EXPERIMENT OBJECTIVE:

Due to the worldwide spread of the respiratory disease COVID-19, scientists developed diagnostic tests in order to identify and monitor the disease. In this simulated medical test, students will perform the ELISA to detect the presence of COVID-19 antibodies in simulated patient samples.

LABORATORY SAFETY

Wear gloves and safety goggles

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOKS

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

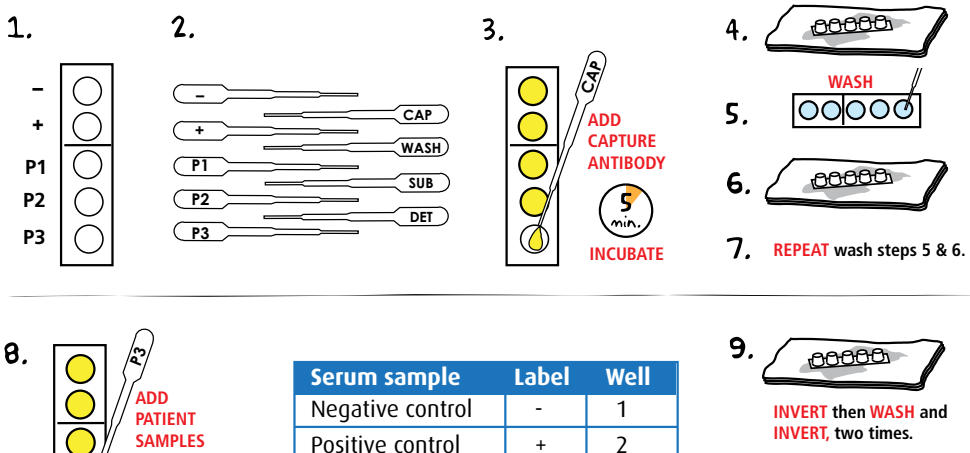
During the Experiment:

- Record (draw) your observations, or photograph the results.

After the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

COVID-19 Immunoassay



Serum sample	Label	Well
Negative control	-	1
Positive control	+	2
Patient 1	P1	3
Patient 2	P2	4
Patient 3	P3	5

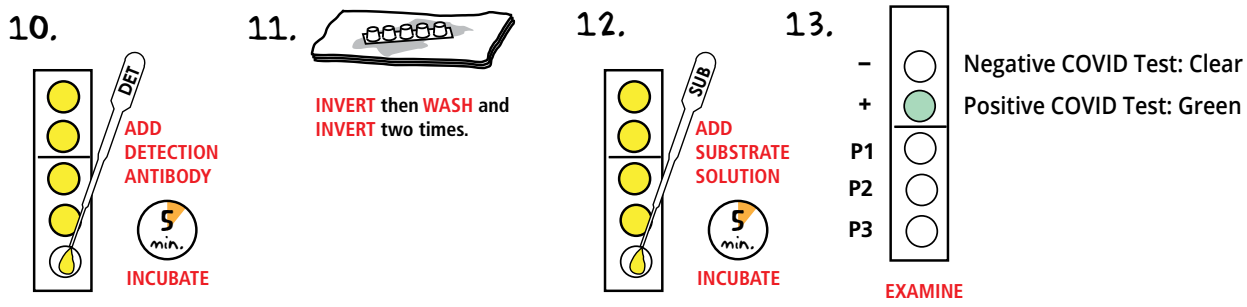
1. **LABEL** the wells of the microtiter plate as shown in diagram.
2. **LABEL** the transfer pipets as follows. These pipets will be used to add and remove liquid from the wells.

(–)	Negative Control	(CAP)	used to add/remove the capture antibody
(+)	Positive Control	(WASH)	used to add wash buffer to each well
(P1)	Patient 1	(SUB)	used to add substrate to each well
(P2)	Patient 2	(DET)	used to add detection antibody to each well
(P3)	Patient 3		

3. Using the transfer pipet for CAP, **ADD** 50 µL of the capture antibody solution (CAP) to all of the wells. (If using transfer pipets, three drops is approximately 50 µL). **INCUBATE** the plate at room temperature for 5 minutes.
4. **INVERT** the strip over the sink or a stack of paper towels to remove the samples. Gently **TAP** the strip 4-5 times onto a fresh paper towel. **DISCARD** the wet paper towels.
5. Using the WASH transfer pipet, **ADD** wash buffer to fill each well, being careful not to overfill.
   NOTE: To minimize cross-contamination it is important that you avoid spilling buffer into neighboring wells.
6. **REPEAT** step 4 to **REMOVE** the wash buffer.
7. Using the same transfer pipet, **REPEAT** the wash a second time. **INVERT** strip onto fresh paper towels and **TAP.**
8. Using the appropriately labeled transfer pipet, **ADD** 50 µL of each of the controls and patient samples to the appropriate well (see above table). **INCUBATE** the plate for 5 minutes at room temperature.
9. **INVERT** onto paper towels and **TAP**. **WASH** the wells twice as in steps 4-7.

OPTIONAL STOPPING POINT: For overnight storage, **ADD** 200 µL of wash buffer to each well. Carefully **COVER** the samples and leave the plate at room temperature. The experiment should be resumed during the next lab period. **REMOVE** the wash buffer before continuing with step 10.

COVID-19 Immunoassay, continued



10. **ADD** 50 µL of the detection antibody solution (DET) to each well. **INCUBATE** the plate for 5 minutes at room temperature.
11. **INVERT** onto paper towels and **TAP**. **WASH** the wells twice as in steps 4-7.
12. **ADD** 50 µL of the substrate solution (SUB) to each well. **INCUBATE** the plate for 5 minutes at room temperature.
13. **EXAMINE** your results:
    Negative COVID Test - Clear
    Positive COVID Test - Green
14. **RECORD** your results in the table, below.

Sample	Color	Interpretation
Negative Control (-)		
Positive Control (+)		
Patient 1 (P1)		
Patient 2 (P2)		
Patient 3 (P3)		

Study Questions

1. Describe the relationship between an outbreak and a pandemic.

2. What is the ELISA test and how does it diagnose COVID-19?

3. Describe two of the antibodies involved in the body's response to viral infection.

Instructor's Guide

NOTES TO THE INSTRUCTOR

This lab is designed for 10 lab groups. Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site. In addition, Technical Service is available from 8:00 am to 5:30 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity. If preparing reagents ahead of time, they should be stored at 4 °C until needed.

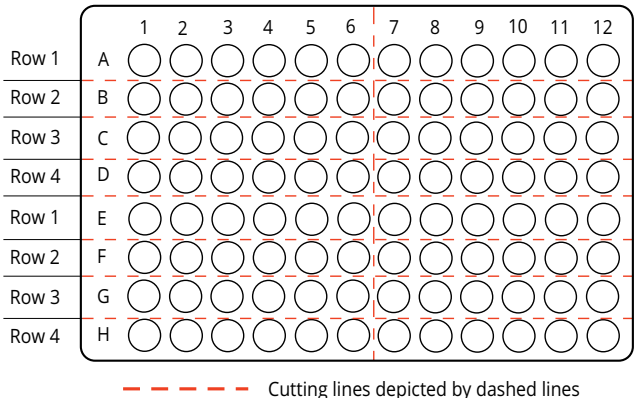
Preparation for:	What to do:	When?	Time Required:
COVID-19 Immunoassay	Divide microtiter plate.	Before the class period.	15 min.
	Dilute 10X ELISA Wash Buffer to 1X solution and aliquot.	Anytime before the experiment. Cover and store in the refrigerator.	5 min.
	Aliquot ELISA Dilution Buffer for negative control and patient samples.	Anytime before the experiment. Store tubes in the refrigerator.	5 min.
	Rehydrate and aliquot the Antigen.	Up to 1 week before performing the experiment.	10 min.
	Rehydrate and aliquot the Primary Antibody.	Up to 1 week before performing the experiment.	5 min.
	Rehydrate and aliquot the Secondary Antibody.	Up to 3 days before performing the experiment.	5 min.
	Rehydrate and aliquot the ABTS Substrate.	Up to 1 week before performing the experiment.	5 min.

Red = Prepare immediately before module. Yellow = Prepare shortly before module. Green = Flexible / prepare up to a week before the module.

Pre-Lab Preparations: COVID-19 Immunoassay

MICROTITER PLATES

1. As shown in the figure at right, orient the microtiter plates so that the numbers 1-12 are at the top and the letters A-H are on your left.
2. Cut each plate on the dotted lines as shown in the figure. Each piece will have six wells on one axis and one on the other axis. Each lab group will receive one piece.



COMPONENT SAMPLES

This simulated medical test uses real antibodies and antigens to replicate the steps in the COVID detection ELISA. Some student samples are labeled differently than the component tubes. Be careful to follow the directions outlined in the preparation directions when labeling the student samples.

COMPONENT TABLE

Component Letter	Component Label	Experimental Label	
B	ELISA Dilution BUffer	"Patient or Negative Control Sample"	This reagent is used to dilute the antibodies and antigens. It is also used to simulate the negative control sample and as patient samples 1 and 3.
C	Antigen (lyophilized)	"Capture Antibody"	After dilution with component B, this reagent will be used to simulate the capture antibody in all wells.
D	Primary Antibody (lyophilized)	"Patient or Positive Control Sample"	This reagent will be used to simulate the positive control sample and patient sample 2.
E	Secondary Antibody (lyophilized)	"Detection Antibody"	This reagent will be used in all wells to detect the formation of the antibody-antigen complex.

PREPARATION OF THE "CAPTURE ANTIBODY"

1. Transfer 7 mL of ELISA Dilution Buffer (Component B) to a 15 mL conical tube. Label the tube "Antigen".
2. Carefully remove the stopper from the vial of lyophilized Antigen (Component C) and transfer approximately 0.5 mL of the ELISA Dilution Buffer from the tube in step 1. Close the stopper and gently shake the vial to mix.
3. Transfer the entire contents of reconstituted Antigen back to the 15 mL tube from step 1. Mix well.
4. Label 10 microcentrifuge tubes "CAP" and dispense 650 µL into each tube.

Pre-Lab Preparations: COVID-19 Immunoassay

PREPARATION OF THE WASH BUFFER

1. Add all of the 10x ELISA Wash Buffer (Component A) to 180 mL of distilled water and mix well. Label as "Wash Buffer".
2. Dispense 18 mL into small beakers for each lab group.

PREPARATION OF THE PATIENT AND THE CONTROL SAMPLES

1. Label fifty 1.5 mL snap-top microcentrifuge tubes as follows:
   a. 10 – Positive Control (+)
   b. 10 – Negative Control (-)
   c. 10 – Patient 1 (P1)
   d. 10 – Patient 2 (P2)
   e. 10 – Patient 3 (P3)
2. Dispense 100 µL ELISA Dilution Buffer (Component B) into the negative control (-), Patient 1 (P1), and Patient 3 (P3) tubes.
3. Transfer 7 mL of ELISA Dilution Buffer to a 15 mL conical tube. Label the tube "1°AB".
4. Carefully remove the stopper from the vial of lyophilized Primary Antibody (Component D) and transfer approximately 0.5 mL of the ELISA Dilution Buffer from the tube in step 3. Close the stopper and gently shake the vial to mix.
5. Transfer the entire contents of reconstituted Primary Antibody back to the 15 mL tube from step 3. Mix well.
6. Dispense 100 µL of the primary antibody into the positive control (+) and Patient 2 (P2).

Each Student Group Should Receive:

1	6-well microtiter plate
1	Beaker containing approximately 18 mL wash buffer
5	Snap-top microcentrifuge tubes containing 100 µL of the control and the patient samples (+, -, P1, P2, P3)
1	Snap-top microcentrifuge tube containing 650 µL of the Capture Antibody (CAP)
1	Snap-top microcentrifuge tube containing 650 µL of the Detection Antibody (DET) prepared on the day of the experiment
1	Snap-top microcentrifuge tube containing 650 µL of the ABTS Substrate (SUB)
9	Small transfer pipets
1	Automatic micropipette and tips (optional)
1	Empty beaker or tube labeled "waste"

PREPARATION OF DETECTION ANTIBODY
(Prepare on the same day as needed for the experiment.)

1. Transfer 7 mL of ELISA Dilution Buffer (Component B) to a 15 mL conical tube. Label the tube "2°AB".
2. Carefully remove the stopper from the vial of lyophilized Secondary Antibody (Component E) and transfer approximately 0.5 mL of the ELISA Dilution Buffer from the tube in step 1. Close the stopper and gently shake the vial to mix.
3. Transfer the entire contents of reconstituted Secondary Antibody back to the 15 mL tube from step 1. Mix well.
4. Label 10 microcentrifuge tubes "DET". Dispense 650 µL per tube.

PREPARATION OF ABTS SUBSTRATE

1. Transfer 10 mL ABTS Reaction Buffer (Component G) into a 15 mL conical tube. Label the tube "ABTS".
2. Carefully remove the stopper from the vial of lyophilized ABTS (Component F) and transfer approximately 0.5 mL of the ABTS from the tube in step 1. Close the stopper and gently shake the vial to mix.
3. Add the reconstituted ABTS mixture back to the 15 mL conical tube in step 1.
4. Label 10 microcentrifuge tubes "SUB". Dispense 650 µL per tube.

Experiment Results and Analysis

Sample	Color	Interpretation
Negative Control (-)	Clear	Negative for anti-SARS-CoV-2 antibodies
Positive Control (+)	Green	Positive for anti-SARS-CoV-2 antibodies
Patient 1 (P1)	Clear	Negative for anti-SARS-CoV-2 antibodies
Patient 2 (P2)	Green	Positive for anti-SARS-CoV-2 antibodies
Patient 3 (P3)	Clear	Negative for anti-SARS-CoV-2 antibodies

The presence of the antibodies in the patient's blood, as indicated by the green color, suggests that the patient is producing antibodies to SARS-CoV-2. A positive response suggests that the person is infected or has been infected by the disease.

Please refer to the kit insert for the Answers to Study Questions

Appendix A

EDVOTEK® Troubleshooting Guides

TROUBLESHOOTING GUIDE FOR ELISA

Cross-contamination: Color develops in negative controls	Used wrong transfer pipet.	Be careful to use the correct transfer pipet. Alternatively, use an adjustable-volume micropipet and change tips between samples.
	Too much force was used to wash out wells.	Wash the wells gently and slowly.
	Wash buffer overflowed into neighboring wells.	Only add the wash buffer until wells are 80% full, around 200 µL.
Color doesn't develop or is slow to develop	Incubation time too short.	Incubate ELISA at room temperature for five more minutes.
	The secondary antibody was prepared too far in advance.	The secondary antibody should be prepared no more than three days before use. The diluted antibody should be stored at 4°C. For best results, the secondary antibody should be prepared no more than 24 hours before the experiment.