EDVOTEK® MyLab™ #1219

Simulation of COVID-19 Antibody Test

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Updated to reflect innovations in COVID testing!

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OBJECTIVE

In this simulated medical test, the ELISA is used to detect the presence of anti-SARS-CoV-2 antibodies in a patient's blood sample.

PLEASE NOTE

This experiment is a simulation of medical tests. It does NOT include the SARS-CoV-2 virus, nor can it identify viral infection in patient samples. If you are exhibiting symptoms of COVID-19, please contact your healthcare professional as soon as possible.

COMPONENTS

This experiment contains reagents and disposables for three (3) experiments. All reagents are simulations. None of the components have been prepared from human or viral sources.

A Anti-Human SARS-CoV-2 IgG/IgM Antibody
B Negative Control
C Positive Control
D Patient 1 Sample
E Patient 2 Sample
F Enzyme-linked SARS-CoV-2 Antigen
G Substrate
- Plastic Strips of Microtiter wells
- Micropipets

REQUIREMENTS

- Distilled or deionized water
- Small cup
- Permanent marker
- Timer or clock
- Gloves and Goggles

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INTRODUCTION

SARS-CoV-2 is a novel coronavirus that has caused a worldwide outbreak of respiratory disease. Coronaviruses are not rare. Each year, experts estimate that they 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).

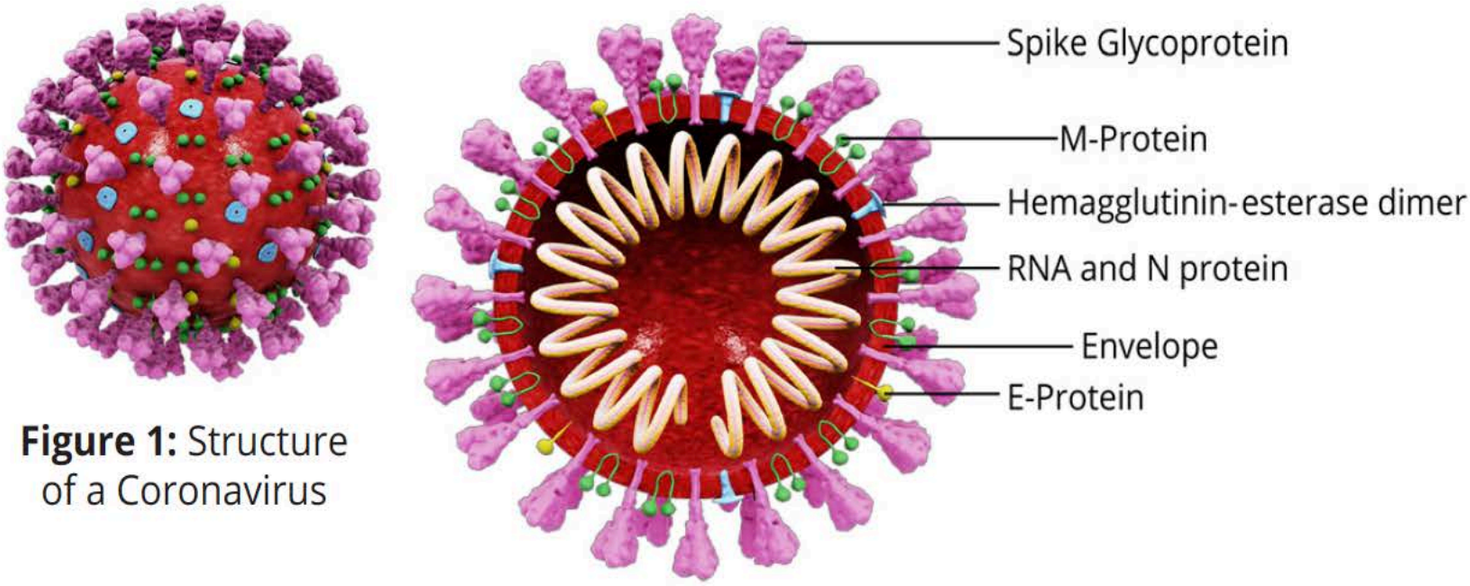


Figure 1: Structure of a Coronavirus

In December 2019, SARS-CoV-2 emerged and spread worldwide in a very short period of time. Symptoms of COVID-19, the disease caused by the SARS-CoV-2 virus, include fever, cough, and shortness of breath. In severe cases, patients may have pneumonia, respiratory distress, kidney failure, or even death. Treatment for COVID-19 includes rest, fluids, and over-the-counter cold medications. If you are exhibiting symptoms of COVID-19, seek medical attention from your doctor to be tested for the virus.

With proper precautions, we can prevent the spread of COVID-19. 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.

Testing for SARS-CoV-2

There are two diagnostic tests to confirm COVID-19 – Reverse Transcription PCR (RT-PCR) and Enzyme-Linked Immunosorbent Assay (ELISA). RT-PCR tests detect the viral genome, signifying active infection. Because RT-PCR is extremely sensitive and can detect minute amounts of the virus, it is an ideal assay to diagnose SARS-CoV-2 infections. The COVID Antigen test is a highly sensitive ELISA that uses antibodies to detect the presence of SARS-CoV-2 surface proteins in patient samples. 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.

Once the patient's immune system has cleared the SARS-CoV-2 infection, no viral nucleic acid or protein remains in the body, making the RT-PCR and antigen tests ineffective. However, the antibodies generated to fight off the infection remain in the body after the patient has healed. A second ELISA, the COVID antibody test, 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 ELISA Assay for SARS-CoV-2 Antibodies

The ELISA for SARS-CoV-2 identifies two different antibodies in patient samples: IgM and IgG. 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.

One of the most sensitive ELISA techniques is the sandwich ELISA, in which two separate reagents are used to detect the IgM and IgG antibodies – one reagent that is bound to the plate to "capture" the antigen, and one that is used to detect it (Figure 2). First, the capture reagent added to the wells of a transparent plastic microtiter plate. This reagent recognizes human IgG/IgM antibodies, which makes it an anti-antibody. It non-specifically adheres to the plastic through hydrophobic and electrostatic interactions. Any unbound antibody is washed out with buffer.

Next, the wells are "blocked" with a protein-containing buffer to prevent non-specific interactions between the sample and the plastic wells. Following the blocking step, the patient samples are added to the wells. The bound antibody recognizes a specific

area of the antigen (called an epitope) and binds. In this experiment, the antibodies recognize regions within the human IgG and IgM antibodies in the patient samples.

After the incubation period, the wells are washed to remove excess sample that did not bind. Next, the purified detection reagent is added and allowed to bind with the patient antibodies. The detection reagent is a recombinant SARS-CoV-2 antigen. The antigen 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 enzyme-linked antigen is present, the enzyme turns the clear substrate solution to pink.

In this simulated medical test, we will use the ELISA to detect the presence of anti-SARS-CoV-2 antibodies in a patient's 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.

Figure 1: Picture of a coronavirus: https://en.wikipedia.org/wiki/Coronavirus#/media/File:3D_medical_animation_coronavirus_structure.jpg

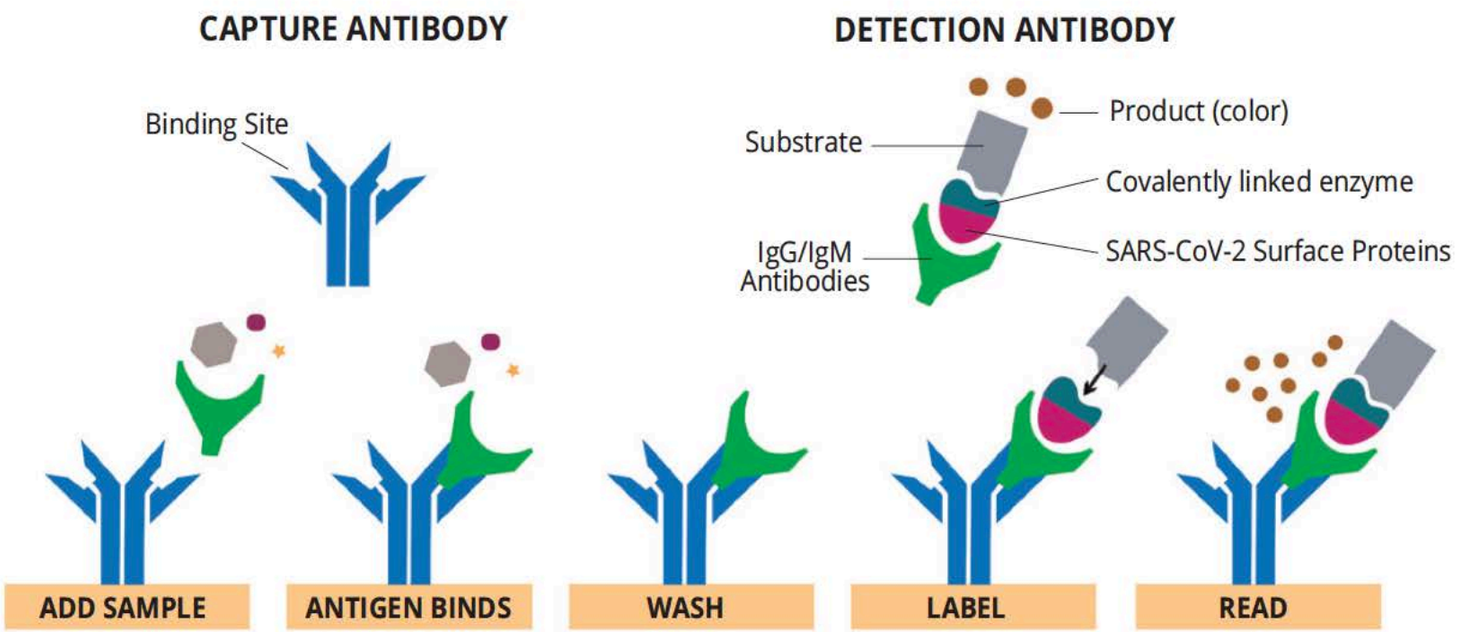
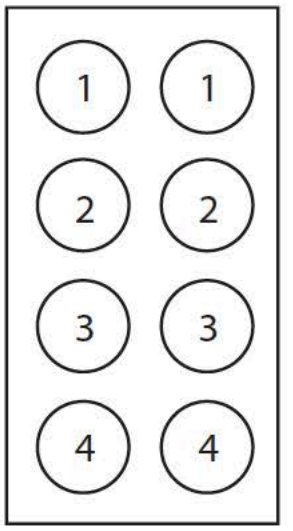


Figure 2: Optimized sandwich ELISA workflow.

EXPERIMENTAL PROCEDURES



1. **LABEL** the bottom of wells according to the chart on the right.
2. **RINSE** a micropipet in a beaker of distilled or deionized (DI) water. **SQUEEZE** the pipet slowly to get one drop at a time. When you are comfortable with using the pipet, remove any remaining water before starting the experiment.
3. Carefully **ADD** two drops of Anti-Human IgG/IgM antibody (A) into each of the eight wells of the microtiter strip. **RETURN** unused sample to the tube. **RINSE** the pipet several times in distilled or DI water. **DISCARD** and **REPLACE** the water used for washing the pipet.
4. **INCUBATE** the plate for five minutes at room temperature.
5. **ADD** two drops of the Negative Control sample (B) into each of the two negative control wells (1). **RETURN** unused sample to the tube. **RINSE** the pipet several times in distilled or DI water. **DISCARD** and **REPLACE** the water used for washing the pipet.
6. **ADD** two drops of the Positive Control sample (C) into each of the two positive control wells (2). **RETURN** unused sample to the tube. **RINSE** the pipet several times in distilled or DI water. **DISCARD** and **REPLACE** the water used for washing the pipet.
7. **ADD** two drops of the Patient 1 sample (D) into each of the two Patient 1 wells (3). **RETURN** unused sample to the tube. **RINSE** the pipet several times in distilled or DI water. **DISCARD** and **REPLACE** the water used for washing the pipet.
8. **ADD** two drops of the Patient 2 sample (E) into each of the two Patient 2 wells (4). **RETURN** unused sample to the tube. **RINSE** the pipet several times in distilled or DI water. **DISCARD** and **REPLACE** the water used for washing the pipet.
9. **INCUBATE** the plate for five minutes at room temperature. ***(NOTE: This is a simplified version of an ELISA. Normally, this step would be followed by a step to wash off any unbound primary antibodies.)***
10. Using a new pipet, **ADD** two drops of the Detection Reagent (Enzyme-linked SARS-CoV-2 Antigen) (F) into all wells. **RETURN** unused sample to the tube. **RINSE** the pipet several times in distilled or DI water. **DISCARD** and **REPLACE** the water used for washing the pipet.
11. **INCUBATE** the plate for five minutes at room temperature. ***(NOTE: This is a simplified version of an ELISA. Normally, this step would be followed by a step to wash off any unbound secondary antibodies.)***
12. **ADD** two drops of Substrate (G) into all wells.
13. **OBSERVE** and **RECORD** results in your laboratory notebook.

RESULTS

INTERPRETATION OF RESULTS:
Clear: Negative for SARS-CoV-2
Pink: Positive for SARS-CoV-2

EXPECTED RESULTS:
Negative Control: Clear
Positive Control: Pink
Patient 1: Pink
Patient 2: Clear

STUDY QUESTIONS

1. Name and define some distinguishing features of coronaviruses.

2. How do coronaviruses spread? How can we prevent the spread of SARS-CoV-2?

3. What tests are used to identify COVID-19? What are the advantages and disadvantages?

GENERAL SAFETY PRECAUTIONS

Parental or adult supervision required.

1. Designate a clean and uncluttered area for performing experiments.
2. Read all instructions before you begin.
3. Do not eat or drink. Do not apply make-up or contact lenses. Adult(s) should not smoke while performing experiments.
4. Wash your hands before and after performing the experiment.
5. Gloves and goggles should be worn routinely as good laboratory practice.
6. Disinfect the counter top or bench with 70% isopropyl alcohol (rubbing alcohol, or place clean newspaper over the area to be used.

WARNING

Choking hazard. Product may contain small parts. Not appropriate for children under 5 years old. No human or animal products are used in any experiments.

Answers to Study Questions:

1. Single-stranded RNA genome: the virus' genetic code
Helical capsid: surrounds and protects the genome
Membrane envelope: surrounds the capsid, studded with proteins that help the virus infect cells.

2. Coronaviruses like SARS-CoV-2 spread person-to-person through liquid droplets that come out from the nose and mouth when you cough or sneeze.

<u>We can prevent the spread of the virus by:</u>
- *Frequent washing of hands, since soap, hand sanitizer and other disinfectants kill coronaviruses.*
- *Keep hands away from your eyes, nose and mouth, since touching your face with contaminated hands can introduce the virus to your mucus membranes*
- *Wearing cloth masks to cover the mouth and nose, which prevent our respiratory droplets from spreading via cough or sneeze.*
- *Social distancing reduces the likelihood of infecting those around us, slowing the spread of the disease.*

3. Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) identifies SARS-CoV-2 based on the molecular sequence of the viral RNA genome.
- *Advantage: RT-PCR is extremely sensitive and can detect very low levels of the virus.*
- *Disadvantage: The viral genome can only be detected during an active infection, meaning that this test cannot be used to determine whether people were infected after the symptoms have subsided.*

The Enzyme Linked ImmunoSorbent Assay (ELISA) is a highly sensitive test that uses antibodies to detect the presence of specific biomolecules (i.e.peptides, proteins, antigens and hormones) in a complex sample. There are two ELISA methods used for COVID detection.

COVID Antigen Test
- *Advantage: The ELISA identifies the presence of SARS-CoV-2 surface proteins in patients, signifying that a person has been infected by the virus.*
- *Disadvantage: The surface proteins can only be detected during an active infection, meaning that this test can not be used to determine whether people were infected after the symptoms have subsided.*

COVID Antibody Test
- *Advantage: The ELISA identifies the presence of antibodies to SARS-CoV-2 in patients, signifying that a person had been previously infected by the virus, even after the virus itself has been cleared from the body.*
- *Disadvantage: Since the immune system takes several days to produce these antibodies, the ELISA may not be able to detect infected people before clinical symptoms arise.*

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