WORKSHOP

# Tracing the Spread of Disease

EDVOTEK®

Designed for the Classroom

SINCE 1987

## Introduction

Respiratory viruses like Influenza or COVID can lead to worldwide pandemics. We'll discuss how diseases spread and perform experiments to explore how disease testing works.

## Tracing the Spread of Disease

In humans, plants, and animals, infectious agents can spread from individual to individual, causing sickness. Even bacteria are not immune from infection; small viruses called bacteriophage infect bacteria and use the cellular machinery to create more copies of the virus. Today, we are obviously hearing about disease spread in the news with the COVID-19 Pandemic, but other infections like the flu, chicken pox, and the measles are still problematic. In this workshop, we're going to talk about types of pathogens, how they spread, and how we can use testing (and a little bit of detective work) to identify infections.

## Background

The epidemiological triad is a model focusing on a host organism that is susceptible to an infectious agent. The environment brings the host and the agent together where they can interact. Most of the time the host, the infectious agent, and the environment are all in balance with one another, which is considered the normal, healthy state (Figure 1).

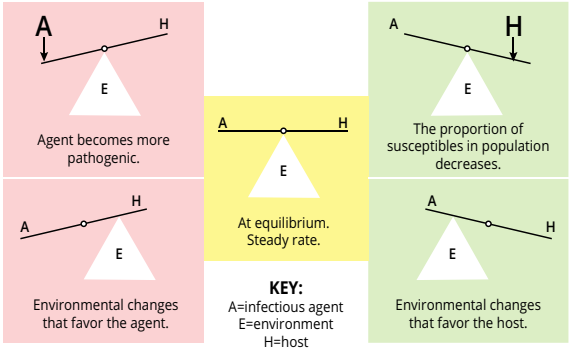



**Figure 1:** The Epidemiological triangle.

When the relationship between the host, agent, and environment shifts, the model projects the outcome. For example, if a virus acquires mutations that make it more pathogenic, the scale is tipped in favor of the agent and more of the hosts gets sick. If the environment changes, this can influence the host-agent interaction. We see cough and cold more often in the fall and winter when people move indoors because of the cold weather. Interventions like vaccines shift the balance in favor of the host since fewer people will be able to be infected and spread the disease.

Luckily, with proper precautions, we can prevent the spread of disease. For example, respiratory viruses rapidly spread person-to-person through liquid droplets that come out of your nose and mouth when you cough or sneeze, therefore we need to be careful to sneeze into a shoulder or a tissue. Gastrointestinal disease can spread through contaminated food or infected surfaces, but foodborne illnesses can be prevented through proper food storage and handling techniques. The most important thing is that if you are exhibiting symptoms of a serious illness, please reach out to your doctor for diagnosis and treatment.

#### DIAGNOSING COVID-19 INFECTIONS, PAST AND PRESENT

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. The COVID antibody test detects 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.

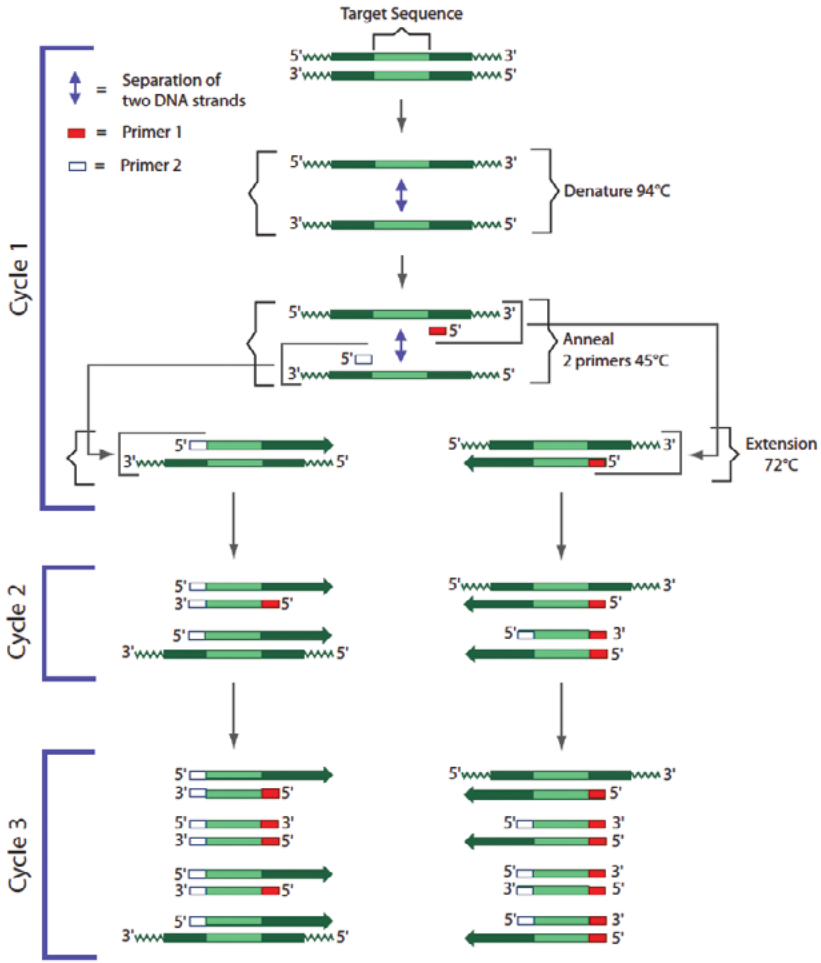



**Figure 2:** Polymerase Chain Reaction

## NUCLEIC ACID TESTING FOR SARS-COV-2

The Polymerase Chain Reaction, or PCR, replicates DNA in vitro using short, synthetic DNA oligonucleotides (known as primers) and DNA Polymerase I in a process like DNA replication in a cell's nucleus. Furthermore, because researchers can customize the primers to target a specific gene, this method would allow for the rapid amplification of a selected DNA sequence. Because of its ease of use and its ability to rapidly amplify DNA, PCR has become indispensable in medical diagnostic laboratories for the detection of pathogens.

To perform PCR, purified double-stranded DNA is mixed with primers, a thermostable DNA polymerase (Taq) and nucleotides (Figure 2). Then, the mixture is heated to 94°C to denature the DNA duplex (i.e., unzip it into single strands). Next, the sample is then cooled to 45°C-60°C, allowing the primers to base pair with the target DNA sequence (called "annealing"). Lastly, the temperature is raised to 72°C, the optimal temperature at which Taq polymerase will extend the primer to synthesize a new strand of DNA. Each "PCR cycle" (denaturation, annealing, extension) doubles the amount of the target DNA in less than five minutes (summarized in Figure 2). To produce enough DNA for analysis, twenty to forty cycles may be required. To simplify this process, a specialized machine, called a "thermal cycler" or a "PCR machine", was created to rapidly heat and cool the samples.

Unfortunately, since Taq polymerase is a DNA-dependent DNA polymerase, it cannot use the RNA genome of SARS-CoV2 as a template. To detect COVID-19 using PCR, Reverse Transcriptase (RT) is used to synthesize complementary DNA (cDNA) copies of the RNA genome. A small amount of the cDNA is mixed with Taq polymerase, dNTPs and primers for amplification by PCR. 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.

Commonly used RT-PCR tests combine three primer sets in one PCR test, which is known as multiplex PCR. The first two sets of primers target regions in the SARS-CoV-2 N protein. As an internal control, the third set of primers amplify the human housekeeping gene RNase P (RP). When combined in a PCR sample, the three sets of primers produce DNA fragments of different lengths depending upon whether the SARS-CoV-2 virus is present in a patient sample.

To analyze this sample, scientists may use a technique called agarose gel electrophoresis to separate DNA fragments according to size. The mixture of DNA molecules is added into depressions (or "wells") within a gel, and then an electrical current is passed through the gel. Because the sugar-phosphate backbone of DNA has a strong negative charge, the current drives the DNA through the gel towards the positive electrode (Figure 3A).

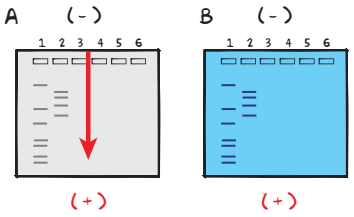
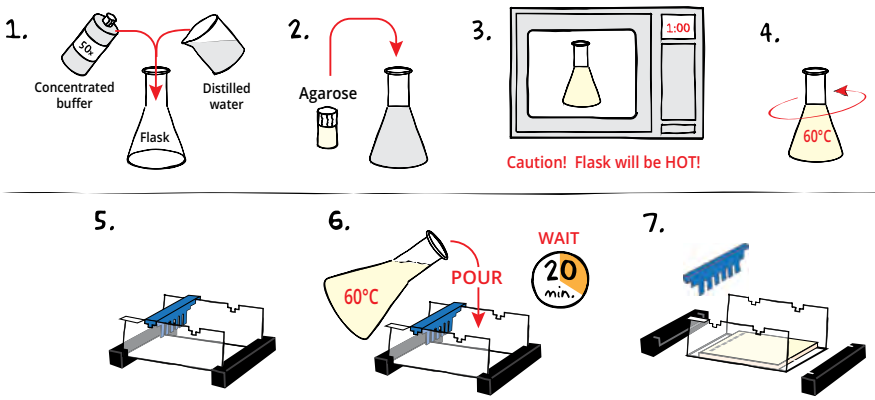



**Figure 3:** Overview of Agarose Gel Electrophoresis

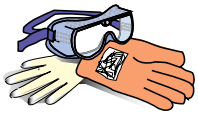
At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the DNA can pass. Small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be the bands can be visualized using a stain that sticks to DNA (Figure 3B).

# Experiment Procedures: Agarose Gel Electrophoresis




## CASTING THE AGAROSE GEL

1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60 °C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.



**Wear gloves and safety goggles**

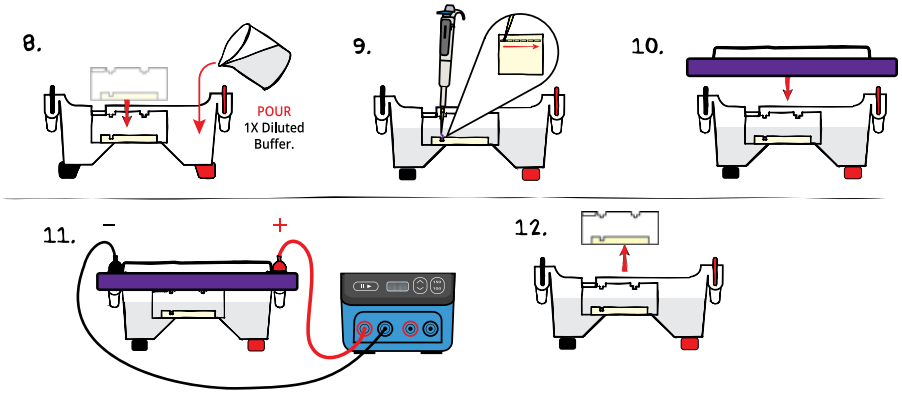
Table A — **Individual 0.8% UltraSpec-Agarose™ Gels**

| Size of Gel Casting Tray | Concentrated Buffer (50x) | + Distilled Water | + Amt of Agarose | = TOTAL Volume |
|---|---|---|---|---|
| 7 x 7 cm | 0.6 mL | 29.4 mL | 0.24 g | 30 mL |
| 10 x 7 cm* | 0.9 mL | 44.1 mL | 0.36 g | 45 mL |
| 14 x 7 cm | 1.2 mL | 58.8 mL | 0.48 g | 60 mL |

**REMINDER:** This experiment requires 0.8% agarose gels cast with 6 wells.

**Recommended gel volume for the EDGE™ Integrated Electrophoresis System. (Cat. #500).*

## Experiment Procedures: Agarose Gel Electrophoresis




**RUNNING THE GEL**

8. **PLACE** the gel (still on the tray*) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µL) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber.

**Table 1: Gel Loading**

| Lane | Tube | Sample |
|---|---|---|
| 1 | Tube A | DNA Standard Marker |
| 2 | Tube B | Negative Control |
| 3 | Tube C | Positive Control |
| 4 | Tube D | Patient 1 Sample |
| 5 | Tube E | Patient 2 Sample |
| 6 | Tube F | Patient 3 Sample |

**REMINDER:**
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Table B — **1x Electrophoresis Buffer (Chamber Buffer)**

| EDVOTEK Model # | Total Volume Required | Dilution: 50x Conc. Buffer | + Distilled Water |
|---|---|---|---|
| EDGE™ | 150 mL | 3 mL | 147 mL |
| M12 | 400 mL | 8 mL | 392 mL |
| M36 | 1000 mL | 20 mL | 980 mL |

Table C — **Time and Voltage Guidelines**
**(0.8% Agarose Gel)**

| | Electrophoresis Model: EDGE™ | M12 & M36 |
|---|---|---|
| Volts | Min/Max (minutes) | Min/Max (minutes) |
| 150 | 10/20 | 20/35 |
| 125 | N/A | 30/45 |
| 100 | 15/25 | 40/60 |

## The ELISA Assay for SARS-CoV-2

The ELISA for SARS-CoV-2 identifies two different antibodies in patient samples: IgM and IgB. 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 antibodies – one reagent that is bound to the plate to "capture" the antigen, and one that is used to detect it (Figure 4). First, the capture reagent added to the wells of a transparent plastic microtiter plate. This reagent is an antibody that 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.

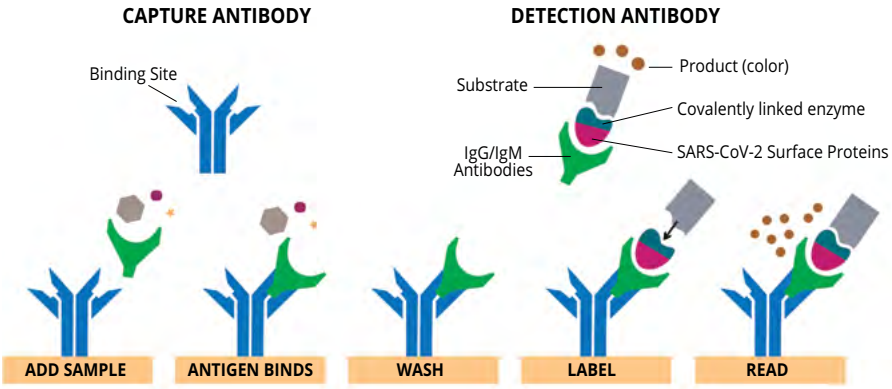
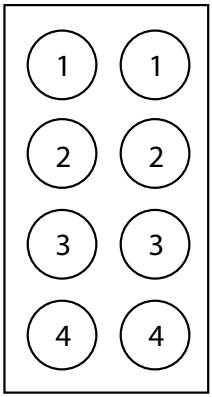



**Figure 4:** Optimized sandwich ELISA workflow.

## Experiment Procedures: ELISA TEST

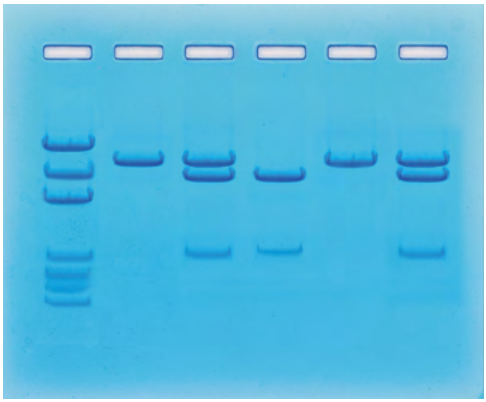



1. **LABEL** the bottom of wells according to the chart on the right.
2. **RINSE** a micropipet in a beaker of distilled or deionized (DI) water. **SQUEEZE** the pipet slowly to get one drop at a time. When you are comfortable with using the pipet, remove any remaining water before starting the experiment.
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.

## Experimental Results and Analysis

**RT-PCR**

In this simulated medical test, we will use RT-PCR to detect the presence of the SARS-CoV-2 virus in blood sample from three patients with COVID-19. In the patients that have been infected with the virus, the test will detect the viral genome and the control and three bands will be seen on the gel. In contrast, a patient who was not infected with SARS-CoV-2 will only have one band from the internal control.



| Lane | Tube | Sample | Result | Molecular Weights (MW) |
|---|---|---|---|---|
| 1 | A | DNA Standard Markers | --------- | 6751, 3652, 2827, 1568, 1118, 825, 630 |
| 2 | B | Negative Control | Negative (human control only) | 4282 |
| 3 | C | Positive Control | Positive (human control and viral proteins) | 4282, 3000, 1282 |
| 4 | D | Patient #1 | Indeterminant: test again | 3000, 1282 |
| 5 | E | Patient #2 | Negative for SARS-CoV-2 | 4282 |
| 6 | F | Patient #3 | Positive for SARS-CoV-2 | 4282, 3000, 1282 |

## Experimental Results and Analysis

### ELISA

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.

### RESULTS

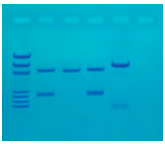
INTERPRETATION OF RESULTS:
Clear: Negative for SARS-CoV-2
Pink: Positive for SARS-CoV-2

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

# Related Products

Cat. #123

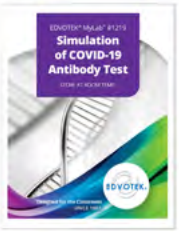
## Nucleic Acid Testing for COVID-19



*For 8 gels.* SARS-CoV-2 is a novel coronavirus that has caused a worldwide outbreak of respiratory disease beginning in 2019. In this simulated medical test, we will use electrophoresis to detect the presence of the SARS-CoV-2 virus in samples from patients with symptoms of COVID-19.

Cat. #1219

## Simulation of a COVID-19 Antibody Test




*Includes samples for 2-3 runs of the experiment.* Trace amounts of blood are often sufficient to identify the individual responsible for any number of crimes, including murder, burglary, or assault. Enhancement procedures can make a small stain of body fluid or tissue visible to the naked eye. In this experiment, students will act as detectives following the aftermath of a drug bust involving gang warfare over territory. Reagents that are routinely used as a first screen will be utilized to detect simulated blood and DNA. In addition, biological materials will be recovered from splatters, blood trajectory, and small droplets of simulated human materials.

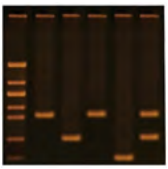
Cat. #263

## Expanding our Testing: Using ELISA to Detect COVID-19

*For 10 groups.* 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.

Cat. #363

## Detecting COVID-19 Using Reverse Transcription PCR



*For 25 students working in 5 groups.* Reverse-transcription PCR (RT-PCR) is the gold standard for the detection of SARS-CoV-2 due to the sensitivity, specificity, and feasibility of the test. In this simulated RT-PCR experiment, students will explore the diagnostic test used worldwide to diagnose and monitor the spread of COVID-19. This experiment requires a PCR thermal cycler.

Cat. #163

## Vax to the Future: Developing a Vaccine for a Novel Pathogen

*For 30 students.* Vaccines are a powerful tool for disease prevention. These medical miracles are designed to train the human body to fight pathogens without getting sick. In this experiment, students will follow the process of vaccine development, from the identification of a novel disease through the creation and clinical testing of a vaccine.

**Details for all these products and MORE can be found on our website!**

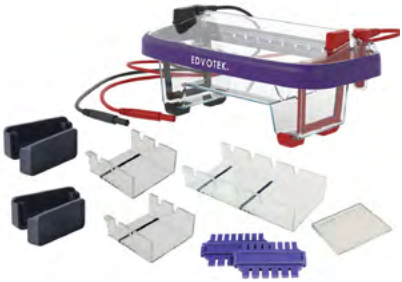
## Related Products

**EDGE™ Integrated Electrophoresis System**
*Cat# 500*


**EdvoCycler™ Jr. Personal PCR Machine**
Holds 16 x 0.2 mL Samples
*Cat# 540*

**EDVOTEK® Variable Micropipette**
5-50 µL Micropipette
*Cat. # 590*

**Fixed Volume MiniPipet™**
35 µL MiniPipet™
*Cat. # 587-2*



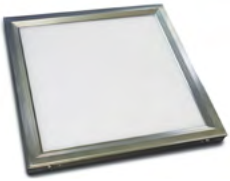

**M12 Complete™ Electrophoresis Package**
*Cat# 502-504*


**TruBlu™ Jr Blue Light Transilluminator**
*Cat# 555*

**TruBlu™ 2 Blue/White Transilluminator**
*Cat# 557*

**DuoSource™ 150**
75/150 V, for 1 or 2 units
*Cat# 509*


**QuadraSource™**
10-300 V, for up to 4 units
*Cat# 5010*



**White Light LED Transilluminator**
*Cat# 552*

**Details for all these products and MORE can be found on our website!**

Visit our website at **www.edvotek.com**