Edvo-Kit # 163

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

Experiment Objective:

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.

See page 3 for storage instructions.

Version 163.220106

EDVOTEK®

Table of Contents

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

Experiment Components

Store all components at room temperature.

COMPONENTS		CHECK (✓)
Module I/IV		
A	Sodium Hydroxide Pellets	❑
B	Color Indicator	❑
C	Vaccine Buffer	❑
•	15 mL conical tube	❑
•	50 mL conical tube	❑
•	5 mL snap top tubes	❑
•	1.7 mL snap top tubes	❑
•	Plastic Strips of Microtiter wells	❑
•	Transfer Pipets (large and small)	❑
•	Labels	❑
Module II		
•	Printables (see Appendix B)	❑
Module III		
D	Negative Control	❑
E	Positive Control	❑
F	Positive Patient Sample	❑
G	Negative Patient Sample	❑
H	Primary Antibody (1° Ab)	❑
I	Enzyme-linked Secondary Antibody (2° Ab)	❑
J	Substrate	❑

Experiment #163 is designed for 30 students.

No infectious agents or pharmaceuticals are included in this kit.

Requirements *(not included with this kit)*

- Distilled or deionized water
- Small cup
- Permanent marker
- Timer or clock
- Paper
- Tape
- Scissors
- Gloves
- Goggles

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.

Background Information

The human body is constantly being challenged by bacteria, viruses, fungi, and other infectious organisms. Many of these exist in and on your body at any given time, but when conditions change, they can invade the body and cause disease. Luckily, the human body is protected by a series of defenses, or else we would always be sick! This protective network of tissues, cells, and proteins known as the immune system targets and neutralizes pathogens. In vertebrates, the immune system has two main sets of defenses: innate immunity and adaptive immunity (Fig 1).

INNATE IMMUNITY • a general response that attacks and neutralizes any pathogens.	**Barriers:** • Skin • Secretions (mucus, enzymes, acid) **Non-specific defenses:** • Phagocytosis • Inflammation • Antimicrobial chemicals and proteins
ADAPTIVE IMMUNITY • a specific response to attack and neutralize a single pathogen.	**Defenses:** • Cell membrane embedded antigen receptors target pathogens and secrete antibodies. • Antibodies bind with pathogens to target and neutralize them.

Figure 1: Overview of the Vertebrate Immune System.

Innate immunity is a general response that attacks and neutralizes any pathogens regardless of what they are. First, there are physical barriers that prevent pathogens from getting into our bodies. This includes the skin barrier, which is largely impermeable. The skin keeps water and body fluids in the body, while keeping bacteria and other pathogens out. Next, secretions like the mucus in our sinuses traps microorganisms and moves them out of the body through blowing one's nose or coughing. Enzymes like lysozyme and lactoferrin are present in this secretion; these enzymes break down the microbial cell wall. Furthermore, we have areas of the body that are largely inhospitable to microbes, like the highly acidic environments of the stomach (from stomach acid) and the skin (from oil and sweat glands).

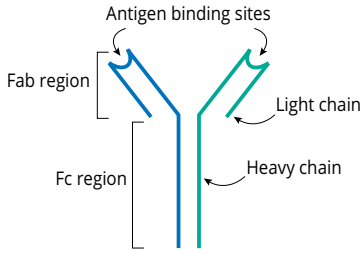
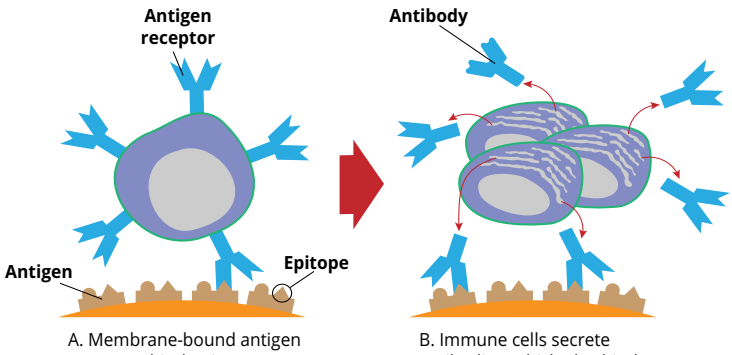


Figure 2: General structure of an antibody.

If a microbe is able to breach this first level of defense, there are additional non-specific defenses that the immune system mobilizes to neutralize pathogens. Membrane-embedded receptor proteins (Toll-like receptors) found on the surface of specific immune cells bind with common microbial proteins (like flagellin or lipopolysaccharide) and target the cells for phagocytosis. In this process, an immune cell engulfs and digests the invading microbe. Other immune cells, when they encounter pathogens, release enzymes and chemicals that lead to cell death. Additional cells near the site of infection release chemical signals which both increase blood flow to an affected area and recruit immune cells. This process, called inflammation, may cause pain and swelling in the affected area as the immune system is working.

In adaptive immunity, the body mounts a response that targets specific pathogens. Specialized proteins, called antibodies, allow the immune system to differentiate between self and non-self-molecules. We call these non-self molecules antibody generators, or antigens. These antigens stimulate the immune system to turn on.

An antibody is a Y-shaped molecule composed of four polypeptide chains: two "heavy chains" and two "light chains" (Fig 2). The polypeptides are linked



A. Membrane-bound antigen receptors bind epitopes on a pathogen's surface.

B. Immune cells secrete antibodies, which also bind with epitopes.

Figure 3: Adaptive Immunity.

together by disulfide bonds. When we compare the amino acid sequence of two receptors, most of the sequence is the same. However, the amino acid sequence of the antigen-binding site (the little pocket at the end of the Y) is variable, allowing each antibody to recognize a unique epitope (a particular location within an antigen). Since the sequence can be so variable, these proteins can recognize a lot of different molecules.

DID YOU KNOW?

Antibodies and **Antigen Receptors** are basically the same protein, but they differ in how the body uses them. **Antigen Receptors** are anchored to the cell membrane of white blood cells, meaning that they remain with the cell that produced them. In contrast, **Antibodies** are secreted and soluble, meaning that they are free to circulate through body fluids like blood, milk, and mucus.

The immune system first detects the presence of pathogens using antibody proteins that stud the surface of white blood cells (Fig 3A) called antigen receptors. During the first infection, the immune system creates many cells with antigen receptors, each with a different sequence in the variable region. Some are able to target and neutralize a specific pathogen. When these immune cells bind, they turn on production of soluble antibodies (or immunoglobulins), which are secreted into the blood and lymph (Fig 3B). The antibodies bind to the pathogen, neutralizing its ability to target a host cell while also labeling it for phagocytosis by white blood cells. This is a primary immune response.

After the infection, the memory immune cells store the antibody's sequence to "remember" the antigen. If the specific antigen enters the body again, the code for antibodies already exists so the body can produce the antibodies and attack the pathogen more quickly. In many cases, the host won't get sick at all, or the symptoms will be mild. For example, most people will only get the chickenpox (*varicella-zoster*) once. This is known as the secondary immune response, which is characteristic of the adaptive immune system.

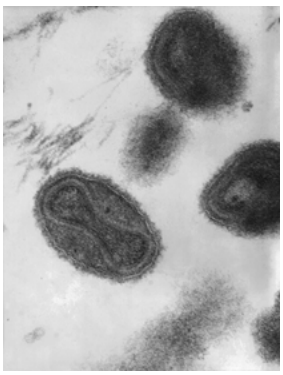


Figure 4: The smallpox virus.

Photo Credit:Content Providers(s): CDC/ Dr. Fred Murphy; Sylvia Whitfield, Public domain, via Wikimedia Commons

WHAT ARE VACCINES AND HOW DO THEY WORK?

Vaccines are a powerful tool for disease prevention. These medical miracles are designed to train the human body to fight pathogens without getting sick. A vaccine introduces specific antigens from a pathogen into a person so that their body can mount a primary immune response. Later, when a vaccinated person comes in contact with the pathogen, the immune system can quickly mount a response, which eliminates or reduces the severity of illness.

Smallpox is a disease caused by the *Variola* virus which causes fever, vomiting, and eventually a characteristic skin rash all over the body (Fig 4). About 30% of people infected with smallpox died from the infection [1], making the disease a huge public health problem. Evidence for the first inoculations against disease have been found in Asia, where historical records show that physicians deliberately infected patients with the smallpox virus to create an immune response. Smallpox scabs were powdered and rubbed into small wounds in the skin. When the pathogen was introduced to the body in this way, it was not as virulent, and so the immune system was able to fight the infection and establish memory immune cells. This practice, called variolation, was advocated for in Britain by Lady Mary Wortley Montagu in the early 1700's. Although there was a risk – patients could contract a serious case of the disease and die – most would acquire immunity and be safe from severe infection.

The first "vaccine" is attributed to Edward Jenner, an English physician. During his apprenticeship, Jenner had heard the rumor that milkmaids were protected from smallpox because they had already had cowpox. In 1796, Jenner decided to perform a controlled experiment to determine whether the cowpox infection was in fact protective. He took a small amount of matter from the wound of Sarah Nelms, a dairy maid who had an active case of cowpox, and used it to inoculate an 8-year-old boy, James Phipps. Over the next two weeks, Phipps exhibited mild symptoms of cowpox – fever, achiness, and general malaise. After a few months, Jenner returned, this time to inoculate the boy with smallpox. Phipps never contracted smallpox, and thus Jenner determined that his hypothesis was correct. Jenner was met with rejection and disbelief when it came to the effectiveness of "vaccination," the term coined to describe the introduction of antigens to the body to create immunity. Over time, the smallpox vaccine became accepted worldwide, leading to the eventual eradication of the disease.

Today, vaccines are a critical public health tool for preventing the spread of infectious disease. Safe and effective vaccines have been developed for thirty human diseases, and many more are in the development pipeline. The World Health Organization (WHO) estimates that vaccines prevent 4-5 million deaths per year and could prevent close to 1.5 million more deaths per year when global vaccination rates increase.

TYPES OF VACCINES

There are many types of vaccines in use today. One type isn't necessarily better than another – the best type of vaccine to prevent a disease is the one that creates the strongest immune response! After vaccination, the body recognizes the antigens and mounts an immune response. The main types are:

1) **Live, attenuated virus vaccines:** In the lab, researchers can identify versions of a pathogen that are attenuated. This means that the pathogen is still "live," but it becomes harmless. An example of this type of vaccine is the MMR, which contains attenuated versions of the measles, mumps, and rubella viruses.

2) **Inactivated virus vaccines:** These vaccines contain cultured pathogens that are inactivated, or killed, so that they can no longer cause disease. For example, the vaccine against rabies is made by purifying an attenuated strain of the virus and killing it using heat or chemicals.

3) **Toxoid vaccines:** Many pathogens cause sickness by secreting toxins into the host organism. A toxoid vaccine is created by inactivating the toxin using heat or chemical reagents. For example, *Clostridium tetani*, the bacteria that causes tetanus/lockjaw, secretes the tetanus toxin into the human body where it causes fever, headache, and painful muscle spasms. The vaccine uses the inactivated toxin (creating a toxoid) to stimulate immunity.

4) **Subunit vaccines:** This class of vaccine uses a piece of a pathogen to cause an immune response, rather than an entire inactivated or attenuated virus. These pieces can be full-length or fragments of proteins or polysaccharides. An example of a subunit vaccine is the Hepatitis B vaccine. For this vaccine, the viral surface protein is made in yeast cells and then purified for use.

5) **Messenger RNA (mRNA) vaccines:** These vaccines package a synthetic mRNA within a fatty lipid droplet that fuses with the cell membrane, depositing the nucleic acid into the cell's cytoplasm. The mRNA is recognized by the ribosome and translated into a viral protein. The immune system is then able to form antibodies against the antigen. Although in development for over 30 years for diseases like Zika, cytomegalovirus, and rabies. The SARS-CoV-2 vaccine is the first mRNA vaccine to be highly effective in clinical trials.

6) **Viral vector vaccines:** For this class of vaccine, scientists re-program attenuated viruses to produce an antigen from a different pathogen once within a cell. For example, several viral vector vaccines have been developed to deliver the sequence for an Ebola cell surface protein, which creates an immunity to the virus.

VACCINE DEVELOPMENT AND TESTING

In the United States, the Food and Drug Administration (FDA) reviews data from pharmaceutical companies before they are authorized for use in people. This includes extensive safety testing to ensure that the vaccine is free from manufacturing contamination and a full examination of the manufacturing facilities to ensure that they comply with current good manufacturing practice regulations (or cGMP). Companies perform clinical trials to test the vaccine in volunteers to determine both the safety and the effectiveness of the vaccine,

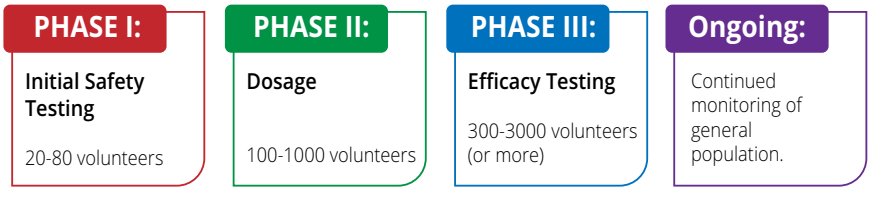


Figure 5: Steps in a Clinical Trial.

and any relative risks from side-effects. Only after the vaccine is proven to be safe and effective, and the benefit of the vaccine outweighs any risks, is the vaccine approved for use in humans.

Safety testing begins with pre-clinical evaluation in the laboratory. This includes tests to determine whether the vaccine produces an immune response, whether the vaccine is toxic, and how quickly the vaccine is broken down in the body. Next, the vaccine moves to clinical trials, where the vaccine is tested for safety and efficacy in people (Fig 5). This happens in three phases:

- **Phase I trials** are usually in small groups of healthy adult volunteers (20-80 people). The data collected tells the researchers whether the vaccine is safe, well-tolerated, and if it produces an immune response.
- **Phase II trials** expand the group of volunteers enrolled in the trial (100-1000 people). In this phase, researchers continue to monitor safety, but the main focus is on determining the appropriate dosage of the vaccine to cause an immune response.
- **Phase III trials** focus on how effective the vaccine is (300-3000 people or more). These studies are double blind, meaning that neither the researchers nor the patients know who gets the vaccine or an inactive substance called a placebo. By comparing the experimental group (those receiving the vaccine) to the control group (those receiving the placebo), researchers can evaluate the effectiveness of the vaccine, while also observing if the side effects are caused by the vaccine.

But authorization is not the end of a vaccine's safety journey. Once a vaccine is in widespread use, doctors will monitor their patients for unusual side-effects from vaccines. Furthermore, people can submit any symptoms and side-effects they may have had through the Vaccine Adverse Event Reporting System, or VAERS. This online database is a useful tool for vaccine researchers because it allows them to discover extremely rare side effects. However, anyone can submit to VAERS at any time, regardless of whether they had a recent vaccine, and the data is not always verified. For example, one physician successfully submitted a report to VAERS stating that after a flu shot, he was transformed into The Incredible Hulk [2]. Erroneous submissions aside, the database helps researchers identify potential rare "adverse events" for further study.

PREVENTING THE SPREAD OF DISEASE

There are many models for the spread of disease, but one of the most common is the epidemiological triad. In this model, the host is an organism that is susceptible to an infectious agent (Fig 6). 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.

When the relationship between the host, agent, and environment shifts, the epidemiologist uses their training to determine the effects. For example, if a virus acquires mutations that make it more pathogenic, the scale is tipped in favor of the agent and more hosts get sick. If the environment changes, this can influence the host-agent interaction. We see coughs and colds more often in the fall and winter when people move indoors because of the cold weather. Interventions like vaccines shift the

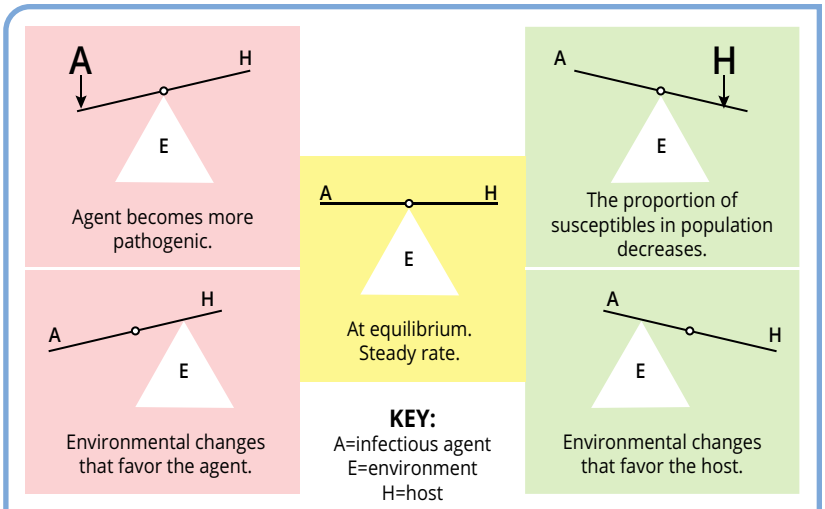


Figure 6: The Epidemiological triangle.

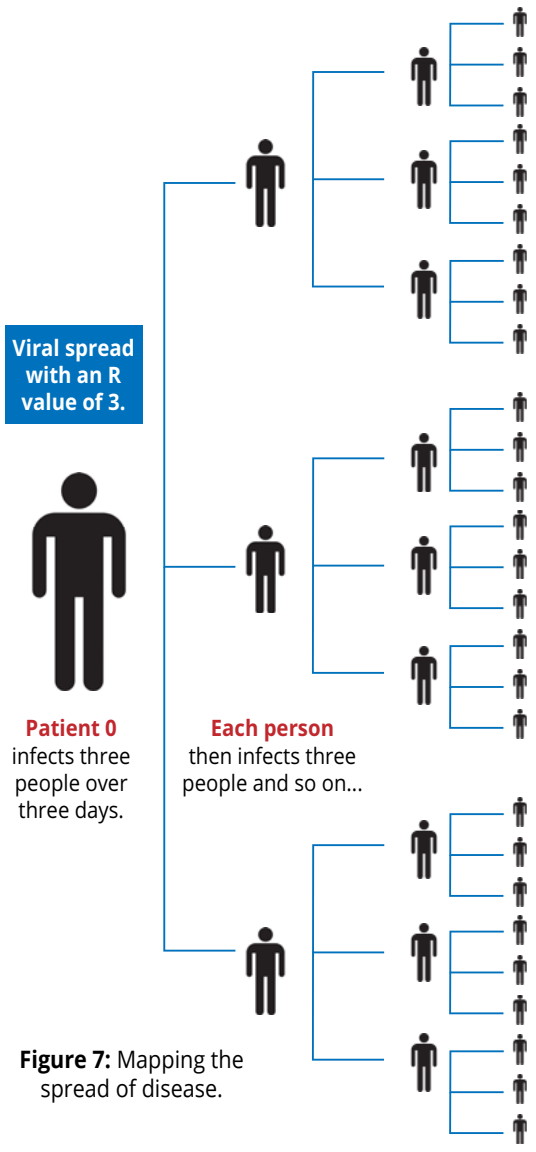


Figure 7: Mapping the spread of disease.

balance in favor of the host since fewer people will be able to be infected and spread the disease. To best control the spread of disease, all three of these factors must be considered.

Researchers evaluate the spread of disease using the R_0 (or R-naught), which is an average of how many people will contract a pathogen from one infected person during their infectious period. There are many variables that factor into this number, but the three most important are the number of people the infected person contacts, the likelihood of infecting that secondary contact, and the length of the infectious period. So, if one person had contact with three people per day, they had a 1 in 3 chance of being infected, and the infectious period was three days, we'd have an R_0 of 3 (Fig 7). In general, a R_0 above 1 means that the virus can quickly spread through a community unchecked, and the number of infected people will increase exponentially. An R_0 below 1 means that the spread of the virus is slowing and can likely be contained. This metric helps scientists, doctors, and public health professionals make recommendations for policies to contain disease locally and regionally.

Many times, the R_0 of an infectious agent is an average based on data from past outbreaks (Fig 8). However, public health interventions can reduce the spread of disease, which changes the R_0. For example, measures like hand washing, social distancing, and masking prevent the spread of disease physically. Medical interventions, like vaccinations, reduce the number of people susceptible to a disease by creating immunity against it. A high percentage of immune individuals in a community, either from infection or vaccination, results in population immunity (or herd immunity). This means that there are fewer people to infect, so the infectious agent cannot spread. Through these interventions, a community can stop the spread of disease.

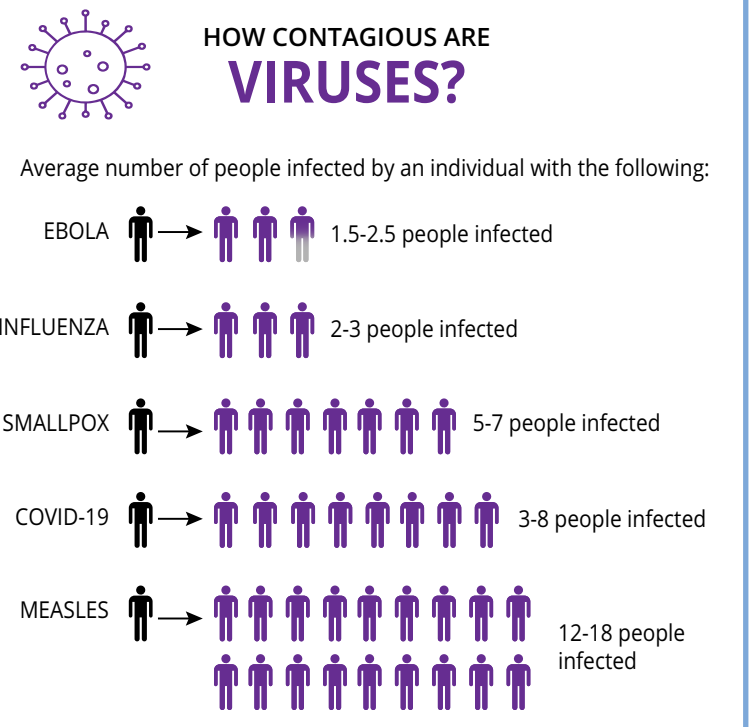


Figure 8: R_0 values of some common viruses

(1) For more information on the history of smallpox variolation and vaccination: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1200696/

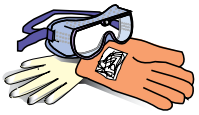
(2) VAERS "Hulk" Report (background): https://web.archive.org/web/20130419004549/http://neurodiversity.com/weblog/article/14/chelation-autism

Experiment Overview

EXPERIMENT OBJECTIVE

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.

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

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I: Transmission of a Novel Disease

Respiratory viruses like the common cold can be rapidly transmitted among humans. These can be spared through contact by handshakes or by aerosols generated during sneezing or coughing.

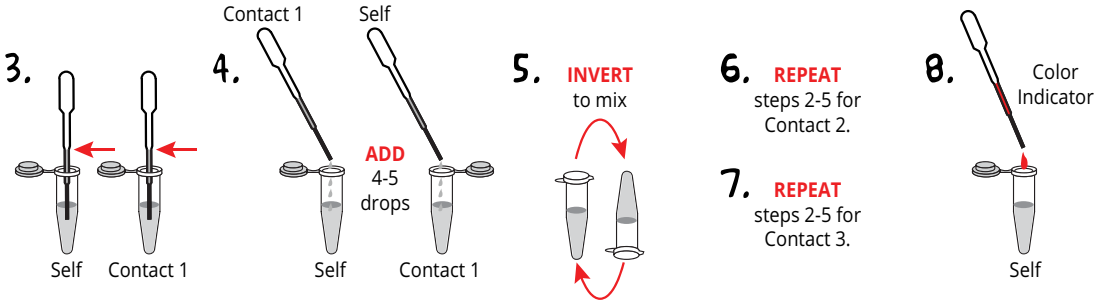
Each respiratory virus has a different incubation period that can range from two to twenty-one days or longer. Often, infected individuals may not be aware that they are carriers of a disease; however, they can transmit the infection to others prior to becoming symptomatic.

This experiment simulates the spread of a novel disease through a community. Since the disease was not present in the community prior to this introduction, there is no existing immunity from prior infection or from a vaccine.

1. **OBTAIN** the necessary reagents for the experiment:
   a. plastic transfer pipet
   b. numbered conical tube containing 4 mL of a clear solution.
   c. a printout of Table A.1 (See Appendix A)

   The tube represents your simulated biological "self". The liquid represents saliva, blood, or any other body fluid that can be responsible for disease transmission. **LABEL** the tube with your name and **RECORD** your code in the first column of Table A.1. At this point, you are not aware if you are a carrier of the infection.

2. Randomly **SELECT** a classmate for your first contact. **RECORD** this person's code as "Contact 1" in Table A.1.



3. Using the transfer pipet, **REMOVE** enough solution from your tube to reach the second line on the pipet. Your contact should do the same.

4. **ADD** 4-5 large drops of the solution in your dropper to your partner's tube. They will **ADD** 4-5 large drops of their solution to your tube. **RETURN** any solution remaining in your pipet to your tube.

5. **CAP** the tube tightly and **INVERT** 3-4 times to mix.

6. **REPEAT** for a second time (steps 2 through 5) by randomly selecting a second student and exchanging fluids.

7. **REPEAT** for a third time (steps 2 through 5) by randomly selecting a third student and exchanging fluids. At this point, you should have close to the same volume that you started with. However, the composition will be different – it will be a mixture of your "self" and the fluids added by your three contacts.

8. **ADD** one drop of color indicator to your tube. If your solution remains clear, then you have not been infected. If your solution turns pink, you have been infected. **NOTE** this result in Table A.1.

Module I: Transmission of a Novel Disease

9. With your class, **COMPLETE** and **ANALYZE** Table A.2. (Table A.2 can be found in Appendix A.)

    a. How many students have a pink sample?

    b. What percentage of the students does that cover?

    c. Using the data, create a flowchart that describes the community spread. Who are the suspected carriers? (They would be common among the groups whose fluid turned pink.)

Module II: Building an mRNA Vaccine

After the novel disease had spread through the community, researchers took samples from patients, isolated the virus, and sequenced its RNA genome. This means that they identified the precise order of the nucleotide building blocks (adenine, cytosine, uracil, and guanine) that build the viral genome. The order of the nucleotides creates genes, which are discrete units of genetic information that contain the instructions to build and maintain the virus.

To combat the virus, we are going to develop an mRNA vaccine. We will start with the sequence of the viral genome and select a gene that will be translated into protein in the body and become the antigen. There are many genes to choose from, but we will want to select one that is on the outside surface of the virus, as opposed to within its core. This means that any virus particles that get into the body will be quickly identified by the immune system and targeted for destruction.

In order for the viral protein to be properly made once immunized, our synthetic mRNA must include the following elements (summarized in Figure 9). These pieces let the body's ribosomes read the mRNA and produce proteins in a process called translation.

- **The 5' cap** – a special nucleotide called 7-methylguanosine is linked to the beginning of the mRNA. It helps make an mRNA stable and it encourages its translation.
- **The 5' Untranslated Region (UTR)** – a sequence that begins at the transcriptional start site and ends at the start codon. This region may contain special sequences that regulate translation.
- **Start codon** – a sequence (AUG) that signals the start of translation.
- **The protein coding sequence for the gene of interest** - this DNA sequence will be translated into the protein antigen.
- **Stop codon** – a sequence (UAG, UAA, UGA) that signals the end of translation.
- **The 3' Untranslated Region (UTR)** – a sequence that continues after the stop codon and ends with a poly adenylation signal. This region features elements that turn on and off the translation, and that can affect how quickly the body will break down the mRNA.
- **Poly (A) tail** – a long stretch of adenine nucleotides that enhances the stability of the mRNA and encourages its translation.

Once we designed our mRNA vaccine, we will clone the sequence into a plasmid and transform it into *E. coli*. This will allow us to make millions upon millions of copies of the mRNA that can be combined with the lipid mixture and used to immunize the population.

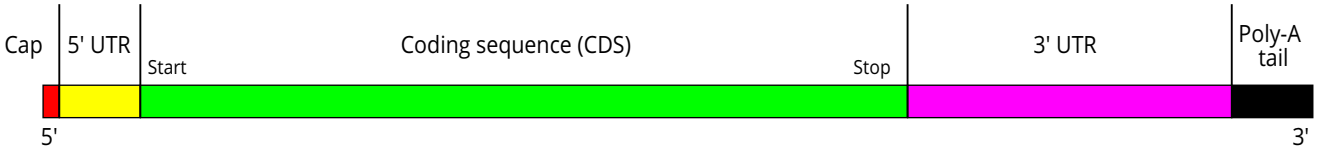


Figure 9: The structure of a typical human protein coding mRNA including the untranslated regions (UTRs).

FIGURE 9 IMAGE CREDIT: Daylite, Public domain, via Wikimedia Commons.

Module II: Building an mRNA Vaccine

In this exercise, you have the sequence for multiple elements of mRNA, including protein coding regions and regulatory sequences. You will choose the ones necessary to build the most effective mRNA vaccine. We will then test the efficacy of the vaccine in modules III and IV.

1. **REVIEW** the available mRNA parts (see APPENDIX B) and **SELECT** which ones will make the best vaccine.
2. Using scissors, **CUT OUT** the selected mRNA parts.
3. Using tape or a glue stick, **CONNECT** the mRNA pieces. For easy assembly, remove extra tabs when necessary.
4. **SHOW** your mRNA vaccine to your classmates and explain why you chose specific parts. Together, **CHOOSE** the best mRNA vaccine for testing.

Module III: Calculating Vaccine Efficacy

We have enrolled 3000 people in this Phase III clinical trial, and we are monitoring them regularly for the novel disease using the Enzyme Linked ImmunoSorbent Assay, or ELISA. The ELISA is a highly sensitive test that uses antibodies to detect the presence of specific molecules within a complex sample. It is often used as a preliminary screening test because it is simple and fast to perform. This ELISA specifically looks for antigens in the blood which denote that a patient has been infected by our pathogen of interest.

The basic ELISA follows a few simple steps:

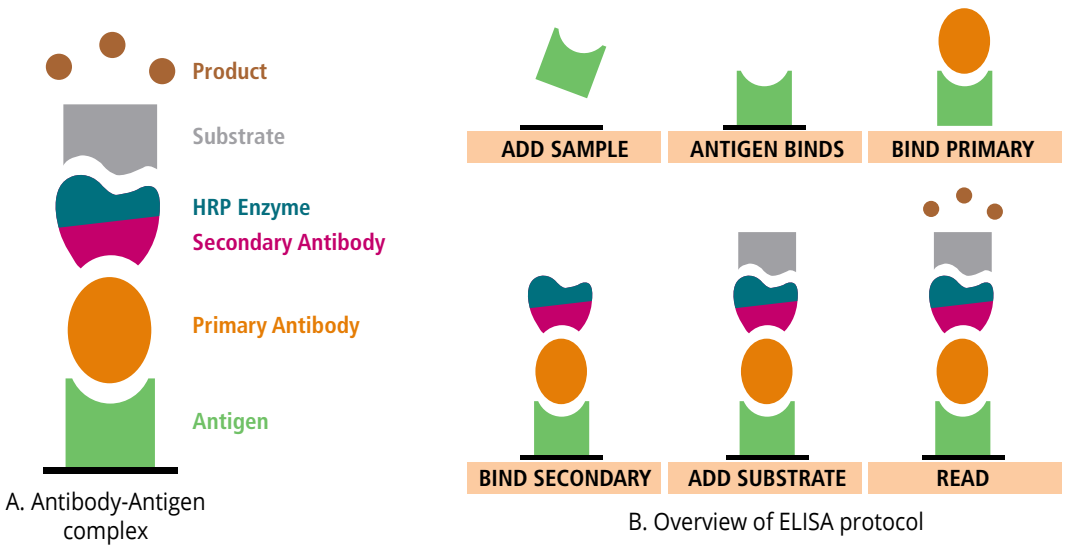
1. The **sample** is added to the wells of the microtiter plate, where it adheres to the plastic through hydrophobic and electrostatic interactions. If the sample includes antigens, they will adhere to the plate.

2. After washing away any excess sample, the wells are "**blocked**" with a protein-containing buffer to prevent non-specific interactions.

3. The **primary antibody** is added to the wells, where it recognizes the antigen and binds through electrostatic interactions. This forms the antibody-antigen complex. Excess antibody is washed out of the wells.

4. The **secondary antibody**, which recognizes the primary antibody, is added to the wells. If the antibody-antigen complex has formed in the well, the secondary antibody remains in the well after washing. Before performing the experiment, the secondary antibody is covalently linked to an enzyme that allows us to detect the presence of the antibody-antigen complex.

5. The **substrate** is added to all the wells where it reacts with the enzyme. It either produces color (chromogenic detection) or light (fluorogenic detection) in wells where there is antigen-antibody complex. Since each enzyme can quickly break down many substrate molecules into product, we can get results in a few minutes.



A. Antibody-Antigen complex

B. Overview of ELISA protocol

Figure 10: Optimized ELISA workflow.

Module III: Calculating Vaccine Efficacy, continued

In this module, we are working to determine the safety and efficacy of our candidate vaccine. As with other vaccine clinical trials, our trial is double blind, meaning that neither the researchers nor the patients know who gets the vaccine or the placebo. Since the clinician does not know which treatment the patient has received, they cannot accidentally reveal which medication is being administered. Thus, we can identify which side effects result from the vaccine, and which do not.

We split our group of volunteers into two cohorts of 1500, half of whom have received the vaccine and half who received a placebo. Over time, we have used the ELISA to test them for the disease. This data allows us to calculate the vaccine efficacy, or VE. This formula describes the percent reduction of a disease in the vaccinated population when compared to the unvaccinated population. In simpler terms, the vaccine efficacy compares how often vaccinated people get sick when compared to unvaccinated.

A large group of enrolled patients remains to be tested for our disease. In this module, you will test the final samples for disease using the ELISA. After the patients are unblinded, you will contribute the classroom data set to the larger data set and use the results to calculate the VE.

The vaccine efficacy (or VE) describes the percent reduction of a disease in the vaccinated population when compared to the unvaccinated population. In simpler terms, the vaccine efficacy compares how often vaccinated people get sick when compared to unvaccinated.

We can calculate the VE using a simple formula, below:

$$VE = \frac{\text{Risk of disease in unvaccinated group} - \text{risk of disease in vaccinated group}}{\text{Risk of disease in unvaccinated group}}$$

The risks are calculated using the experimentally collected data, where

$$\text{Risk} = \frac{\text{infected people in group}}{\text{Total people in group}}$$

So, in a situation where there is a hypothetical outbreak of a vaccine-preventable disease in a school, this is how researchers calculate the risks and the VE.

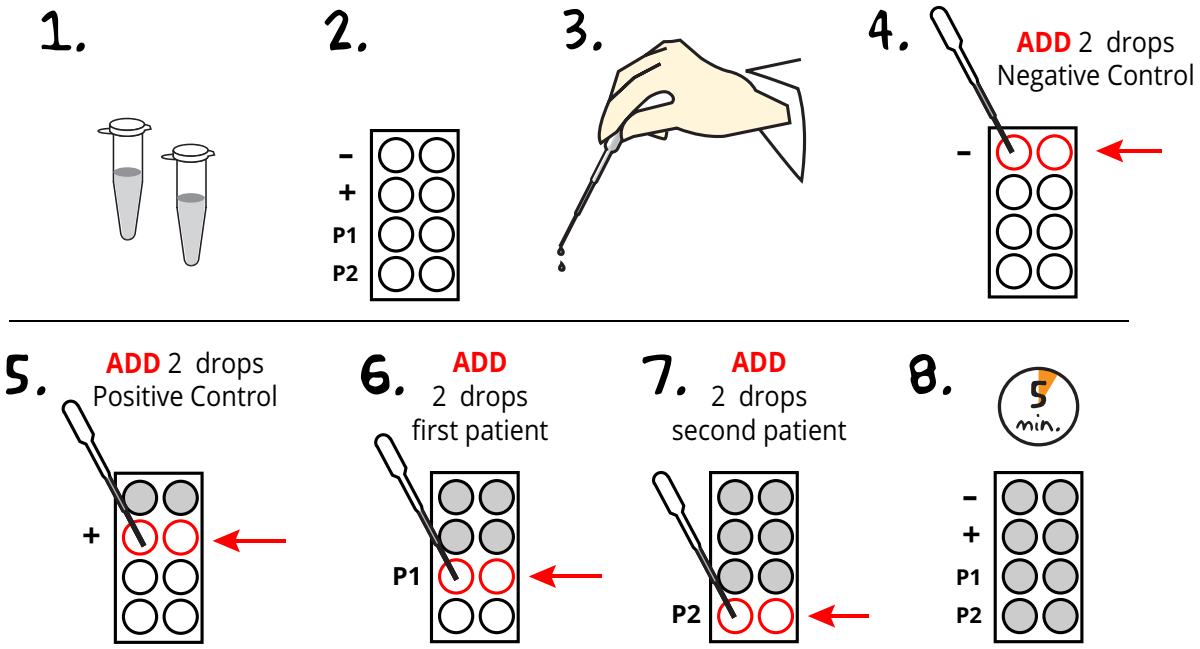
	disease positive	disease negative	Total
Vaccinated	20	129	149
Unvaccinated	5	6	11

The risk of disease for the vaccinated is 20/149 = 0.134
The risk of disease for the unvaccinated is 5/11 = 0.454

The VE = (0.454 – 0.134)/0.454 = 0.705.

As a percentage, the VE is 70.5%, which means there is a 70.5% reduction in the occurrence of the specific disease in the population because of vaccination.

Module III: Calculating Vaccine Efficacy, continued



1. **SELECT** two anonymous patient samples at random for testing. **RECORD** the numbers in your lab notebook. These samples will be your first and second patient samples. You will not know whether they received the vaccine or the placebo until the study is unblinded at the end. **OBTAIN** all additional reagents from your teacher.

2. **LABEL** the bottom of wells according to the chart.

3. **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.

4. **ADD** two (2) drops of Negative Control (D - Neg CTRL) into each of the two negative control wells (Row 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.

5. **ADD** two (2) drops of Positive Control (E - Pos CTRL) into each of the two positive control wells (Row 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.

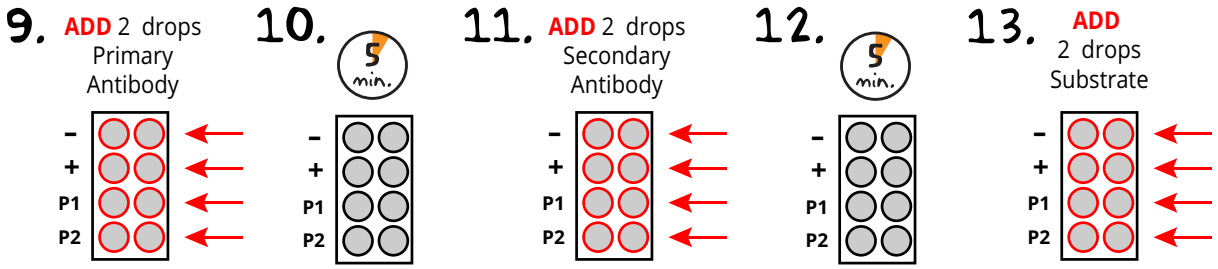
6. **ADD** two (2) drops of the first patient sample into each of the two Patient 1 wells (Row 3). **RETURN** unused sample to the tube. **RINSE** the pipet several times in distilled or DI water. **DISCARD** and **REPLACE** the water used for washing the pipet.

7. **ADD** two (2) drops of the second patient sample into each of the two Patient 2 wells (Row 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.

8. **INCUBATE** the plate for five (5) minutes at room temperature. ***(NOTE: This is a simplified version of an ELISA. Normally, incubation steps are followed by a step to wash of any unbound reagents.)***

continued

Module III: Calculating Vaccine Efficacy, continued



9. Carefully **ADD** two (2) drops of primary antibody (H-1°AB) 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.

10. **INCUBATE** the plate for five (5) minutes at room temperature.

11. Using a new pipet, **ADD** two (2) drops of secondary antibody (I-2°AB) 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.

12. **INCUBATE** the plate for five (5) minutes at room temperature.

13. **ADD** two (2) drops of substrate (J-Sub) into all wells.

14. **OBSERVE** and **RECORD** results in your laboratory notebook.

To calculate the vaccine efficacy:

1. The teacher will unblind the data, meaning that you will know whether your patient has received the vaccine or the placebo. Your patient will fall into one of these four categories:
   - Vaccinated, confirmed sick
   - Vaccinated, not sick
   - Unvaccinated, confirmed sick
   - Unvaccinated, not sick

2. Using this information and the equations on page 15, **CALCULATE** the risk for the vaccinated and unvaccinated populations and the VE. Do you think the vaccine is effective?

TABLE B

	Confirmed Sick	Not Sick	TOTAL
Vaccinated	147 + _____	1323 + _____	1470 + _____
Unvaccinated	947 + _____	523 + _____	1470 + _____

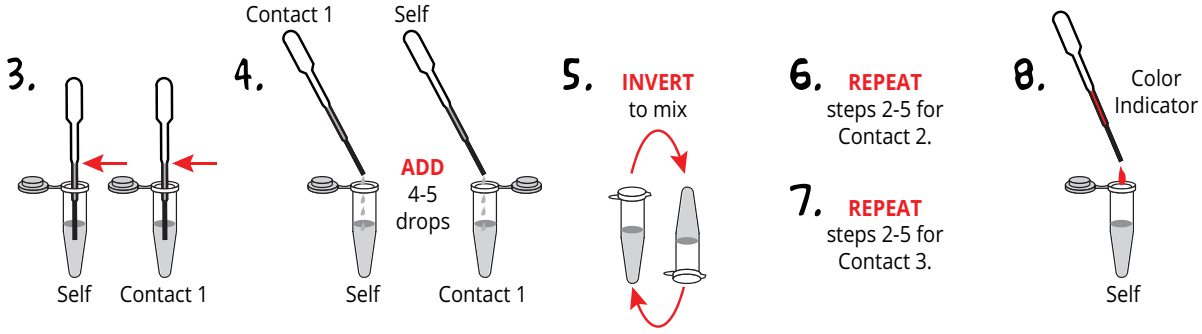
Module IV: Do Vaccines Stop Diseases from Spreading?

We have developed our vaccine and tested it in clinical trials, where it was shown to be highly effective. Now, we will study how our novel disease travels through a community after 50% of the population has been immunized.

1. **OBTAIN** the necessary reagents for the experiment:
   a. plastic transfer pipet
   b. numbered conical tube containing 4 mL of a clear solution.
   c. a printout of Table C.1 (See Appendix C)

   The tube represents your simulated biological "self". The liquid represents saliva, blood, or any other body fluid that can be responsible for disease transmission. **LABEL** the tube with your name and **RECORD** your code in the first row of Column A. Half of the samples have been immunized against the virus. At this point you are not aware if you are a carrier of the infection or if you have been immunized.

2. Randomly **SELECT** a classmate for your first contact. **RECORD** this person's code as "Contact 1" in Table C.1.



3. Using the transfer pipet, **REMOVE** enough solution from your tube to reach the second line on the pipet. Your contact should do the same.

4. **ADD** 4-5 large drops of the solution in your dropper to your partner's tube. They will **ADD** 4-5 large drops of their solution to your tube. **RETURN** any solution remaining in your pipet to your tube.

5. **CAP** the tube tightly and **INVERT** 3-4 times to mix.

6. **REPEAT** for a second time (steps 2 through 5) by randomly selecting a second student and exchanging fluids.

7. **REPEAT** for a third time (steps 2 through 5) by randomly selecting a third student and exchanging fluids. At this point, you should have close to the same volume that you started with. However, the composition will be different – it will be a mixture of your "self" and the fluids added by your three contacts.

8. **ADD** one drop of color indicator to your tube. If your solution remains clear, then you have not been infected. If your solution turns pink, you have been infected. Note this result in Table C.2.

Module IV: Do Vaccines Stop Diseases from Spreading?, continued

9. With your class, **COMPLETE** and **ANALYZE** Table C.2. (Table C.2 can be found in Appendix C)

    a. How many students have a pink sample?

    b. What percentage of the students does that cover?

    c. Using the data, can you create a flowchart to determine the suspected carriers?

    d. At this point, your teacher will let you know which individuals are vaccinated. Did the vaccine stop the spread of the disease in the community? What would happen if more or less of the community has been vaccinated?

Study Questions

1. What are the two sets of defenses used by the human body to prevent disease? Define each in one sentence.

2. How are antibodies and antigen receptors the same? How are they different?

3. What is a vaccine and how does it work?

4. Pertussis (or whooping cough) is a vaccine preventable childhood disease characterized by cold symptoms and characteristic fits of coughing. It can be fatal, especially in infants, which is why routine vaccination is important.

   The DTaP vaccine is given in several doses, meaning that a child can be unvaccinated, under vaccinated, or fully vaccinated. Older children can be fully vaccinated, plus receive a booster at an older age. To determine the efficacy of the vaccine in the real world, researchers followed 469,982 children for an extended period. Table D below shows the results of the study. With this data and the formulas from Module III (page 15), calculate the relative risks and the vaccine efficacy for each group.

TABLE D

	Whooping Cough	No illness	Total
Unvaccinated	99	4571	4670
Undervaccinated	36	14064	14100
Fully vaccinated	515	328472	328987
Booster	88	122137	122225
Totals	738	469244	469982

Instructor's Guide

ADVANCE PREPARATION:

PREPARATION FOR:	WHAT TO DO:	WHEN?	TIME REQUIRED:
Module I	Prepare and aliquot solutions, label tubes.	Up to one month before performing the experiment.	45 min.
Module II	Print out pieces, acquire scissors and tape or glue.	Any time before performing the experiment.	15 min.
Module III	Prepare and aliquot solutions, label tubes.	Up to one month before performing the experiment.	45 min.
Module IV	Prepare and aliquot solutions, label tubes.	Up to one month before performing the experiment.	45 min.

Pre-Lab Preparations: Module I

MODULE I - TEACHER PREP																														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Initial Disease status (+/-)																														
Final Disease status (+/-)																														

This module can accommodate between 10-30 students. For best results, we recommend 20 or more students take part in the exercise. The exercise requires an even number of students. If there are not an even number of students, the instructor should participate in the exercise.

1. Print out the following:
   - One copy of the teacher table (above)
   - Enough student tables (Appendix A) so that every participant has one.

2. Count out the number of 5 mL snap top tubes necessary for the module (1 tube per student) and put the rest aside.

3. Prepare a NaOH stock solution by completely dissolving the NaOH pellets (component A) in 10 mL distilled or deionized water. (Note, this solution must be used within one month of preparation. This stock will be used again in Module IV, so plan to perform Module IV within one month of Module I.)

4. Prepare the "infected" or "carrier" solution by adding 2 ml of the NaOH stock solution to 18 ml distilled or deionized water. Dispense 4 ml of this solution to unlabeled tubes based on the number of students in the class, as indicated below. Mark each sample with a random number and record these numbers in your teacher table. (Need help, try **https://www.random.org/integers/**)
   a. For 10 students, prepare one 4 mL infected sample
   b. For 11-19 students, prepare two 4 mL infected samples
   c. For 20-30 students, prepare three 4 mL infected samples

5. Prepare the "uninfected" or "non-carrier" samples by dispensing 4 mL of distilled water into the remaining tubes. Number and note the number in the teacher's table.

6. Save all remaining solutions for Module IV. They can be stored at room temperature

7. Instruct students to use caution when working with these chemicals. Students should wear gloves, safety goggles, and protective clothing.

FOR MODULE I
Each student group should receive:

- One numbered tube of "self" solution
- One large transfer pipet
- Lab marker
- Printed table

Pre-Lab Preparations: Module II

Students can work individually or in groups. Print out enough of the component mRNA parts (Appendix B) for the class. The pages should be printed single sided at 100% scale. Each student or group will also need a pair of scissors and adhesive tape or glue.

At the end of the exercise, students will explain why they chose the pieces that they did. The class should come to a consensus as to which parts are "best" when building the mRNA vaccine and that it will be "tested" in the next two modules.

FOR MODULE II
Each student or group should receive:

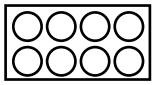
- Copy of Appendix B
- Scissors
- Adhesive tape or glue

Pre-Lab Preparations: Module III

MODULE III - TEACHER PREP																														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Vaccine status (+/-)																														
Final Disease status (+/-)																														
Sample #	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Vaccine status (+/-)																														
Final Disease status (+/-)																														

In this module, students will perform simulated medical diagnostic ELISAs. The reagents are shelf stable, do not require dilution or resuspension, and can be aliquoted in advance for distribution to students.

1. Cut the microtiter plates into a 2x4 grid as shown. Each student will receive one 2x4 well piece.



2. Label and dispense solutions:

   a. Label thirty 1.5 mL snap-top tubes with the D–Neg CTRL sticker. Label thirty 1.5 mL snap-top tubes with the E–Pos CTRL sticker. Dispense 200 µL of Component D (Negative Control) and Component E (Positive Control) into the appropriate tubes.

   b. In this step, we will be creating our "blinded" patient samples for testing. A small number of vaccinated samples will test positive, and a small number of unvaccinated samples will test negative. This data will allow us to calculate the vaccine efficiency. Each student will receive two randomized patient samples.
      i. Print the Module III Teacher Table.
      ii. Using a marker, label sixty 1.5 mL snap-top tubes with numbers from 1-60.
      iii. Choose thirty of the labeled tubes at random. These will be your "vaccinated" patient samples. Record the numbers in the teacher table as positive (+) for the vaccine. Select three tubes at random and dispense 200 µL of the Positive Patient Sample (F) into the tubes and record them as having a positive (+) test. The remaining 27 tubes should be recorded as negative (-) for the disease. Dispense 200 µL of the Negative Patient Sample (G) into the remaining tubes.
      iv. The remaining thirty labeled tubes represent your "unvaccinated" (placebo) patient samples. Record the numbers in the teacher table as negative (-) for the vaccine. Select 20 tubes at random and dispense 200 uL of the Positive Patient Sample (F) into the tubes and record them as having a positive (+) test. The remaining 10 tubes should be recorded as negative (-) for the disease. Dispense 200 uL of the Negative Patient Sample (G) into the remaining tubes.

   c. Label 30 tubes with the “H - 1°AB” stickers. Dispense 400 µL of solution H into each tube.

   d. Label 30 tubes with the “I – 2°Ab” stickers. Dispense 400 µL of solution I into each tube.

   e. Label 30 tubes with the “J–Sub” stickers. Dispense 400 µL of solution J into each tube.

FOR MODULE III
Each student should receive:

- One 2 x 4 well microtiter grid
- 3 Small transfer pipets
- 1 Tube of each sample (7 tubes total)
- 2 Patient samples, chosen by student at random

Pre-Lab Preparations: Module IV

MODULE IV - TEACHER PREP																														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Disease status (+/ - / V)																														
Final Disease status (+/-)																														

This module can accommodate between 10-30 students. For best results, we recommend 20 or more students take part in the exercise. The exercise requires an even number of students. If there are not an even number of students, the instructor should participate in the exercise. Students do not need to be assigned to the same number or infection status as the first exercise.

1. Count out the number of 5 mL snap top tubes necessary for the module and put the rest aside. Split the tubes into two equal pools, one which represents the vaccinated population, and one which will be the carrier and infected population.

2. Prepare the "Vaccine" solution by diluting the component C (Vaccine Buffer) in 85 mL water. This solution is resistant to color change when "infected" with NaOH solution. Dispense 4 mL of the solution into the vaccinated population tubes. Randomly number the tubes and record them with a "V" in your teacher table.

3. You will need up to 12 mL of the "infected" solution, depending on how large your class is. Remaining "infected" solution from Module I can be used. Prepare additional "infected" solution by adding 2 mL of the NaOH stock solution to 18 mL distilled or deionized water. The remaining solution from Module I can be mixed the new solution. Dispense 4 mL of this solution to the correct number of unlabeled tubes (see below). Mark each sample with a random, unassigned number and record these numbers in your teacher table.

   a. For 10 students, prepare one 4 mL infected sample.
   b. For 11-19 students, prepare two 4 mL infected samples.
   c. For 20-30 students, prepare three 4 mL infected samples.

4. Prepare the "unvaccinated" samples using distilled water as in Module I. Dispense 4 mL into the remaining unlabeled tubes and assign each remaining tube a number.

5. Instruct students to use caution when working with these chemicals. Students should wear gloves, safety goggles, and protective clothing.

FOR MODULE IV
Each student group should receive:

- 1 Tube of "carrier" or "non-carrier" solution
- 1 Large transfer pipet
- Lab marker
- Printed table

Experiment Results and Analysis

MODULE I

Below is a simulation performed with 20 samples.
The three initial infection samples are marked in red.
The subsequent infections are marked in orange.

TABLE A.2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Rnd 1	8	20	16	9	10	14	11	1	4	5	7	17	15	6	13	3	12	19	18	2
Rnd 2	19	8	4	3	16	13	18	2	10	9	15	20	6	17	11	5	14	7	1	12
Rnd 3	3	9	1	12	19	15	10	13	2	7	14	4	8	11	6	17	16	20	5	18
Results																				

In this simulation individuals 6, 12, and 16 were initially infected. During round 1 they infected individuals 3, 14, and 17. By the 3rd round most of the community (16/20) was infected. That represents 80% of the population.

After three rounds of transfer, your results may look like this. Students should work backwards from the results to try to identify the initial sources of infection. However, it may be difficult to come to a clear conclusion. The lesson will help you start a discussion about the challenges facing public health officials when contact tracing to identify the source of an infection. For example, if too many people in an area are infected, it's hard to determine where the source of the infection was in the community, even with testing.

MODULE II

The consensus mRNA vaccine should include the following pieces for the following reasons:

- **5' Cap** – this helps make an mRNA stable and it encourages its translation.
- **5' UTR "high translation"** – this sequence contains elements that encourage translation of the mRNA.
- **Viral Surface Protein coding region** – since this protein is on the outside of the virus, any virus particles that get into the body will be quickly identified by the immune system and targeted for destruction.
- **3' UTR "Stable mRNA"** – this sequence will ensure that the mRNA lasts long enough to create a lot of protein, creating an immune response.
- **Poly (A) tail** – this element enhances the stability of the mRNA and encourages its translation.

5' CAP	5' UTR High Translation	Coding Sequence Viral Surface Protein	3' UTR Stable mRNA	Poly (A) Tail

Experiment Results and Analysis

MODULE III

CLASSROOM DATA – 60 patients, each student tests two samples. For classrooms with fewer than 30 students, be sure to add the missing data points from your teacher data sheet (or allow students to test more than two patient samples). Positive results will be bright pink and negative results will be clear. All students should have the same controls, but the patient sample results will vary. This is to be expected based on the random selection of patient samples.

Total (including student data)

TABLE B			
	Confirmed Sick	Not Sick	TOTAL
Vaccinated	150	1350	1500
Unvaccinated	967	533	1500

Using this complete data set, students will calculate the risk for unvaccinated, the risk for vaccinated, and the VE.

Risk for vaccinated = 150/1500 = 0.10
Risk for unvaccinated = 967/1500=0.645

VE = (0.645-0.10)/0.645 = 0.845 or 84.5%. Yes, the vaccine is effective.

Experiment Results and Analysis, continued

MODULE IV

Below is a simulation performed with 20 samples.
The three initial infection samples are marked in red.
The vaccinated samples are in green.
The subsequent infections are marked in orange.

TABLE C.2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Rnd 1	13	15	20	7	10	14	4	16	17	5	18	19	1	6	2	8	9	11	12	3
Rnd 2	17	9	5	13	3	12	18	19	2	20	15	6	4	16	11	14	1	7	8	10
Rnd 3	16	18	8	6	15	4	12	3	19	11	10	7	14	13	5	1	20	2	9	17
Results																				

In this simulation individuals 5, 11, and 19 were initially infected. Because of vaccination in the population, they were unable to infect as many people as in Module I. By the third round, only four additional people had been infected (7/20 total). The rest avoided infection. That represents 35% of the population.

After three rounds of transfer, your results may look like this. Students should work backwards from the results to try to identify the initial sources of infection. Again, it may be difficult to come to a clear conclusion as to the source of the infection, as vaccinated samples should not become infected. If they do, this would be considered a "breakthrough infection," which is infection by a pathogen after vaccination. This experiment has been optimized to prevent breakthrough infection, but there is a possibility if the students transfer volumes greater than 1 mL at each transfer.

Students should compare the number of positive cases at the end of this module vs the first module to see how vaccination reduces the numbers of infection in a community. This is an example of herd immunity, where there are fewer susceptible people in a population, which reduces the spread of disease.

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

TABLE A.1				
Recipient Code (Self)	Contact 1	Contact 2	Contact 3	Final Color

TABLE A.2																													
Round 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Round 2																														
Round 3																														
RESULTS																														

Printable mRNA Vaccine Components

Print one copy, single sided, for student or student group.

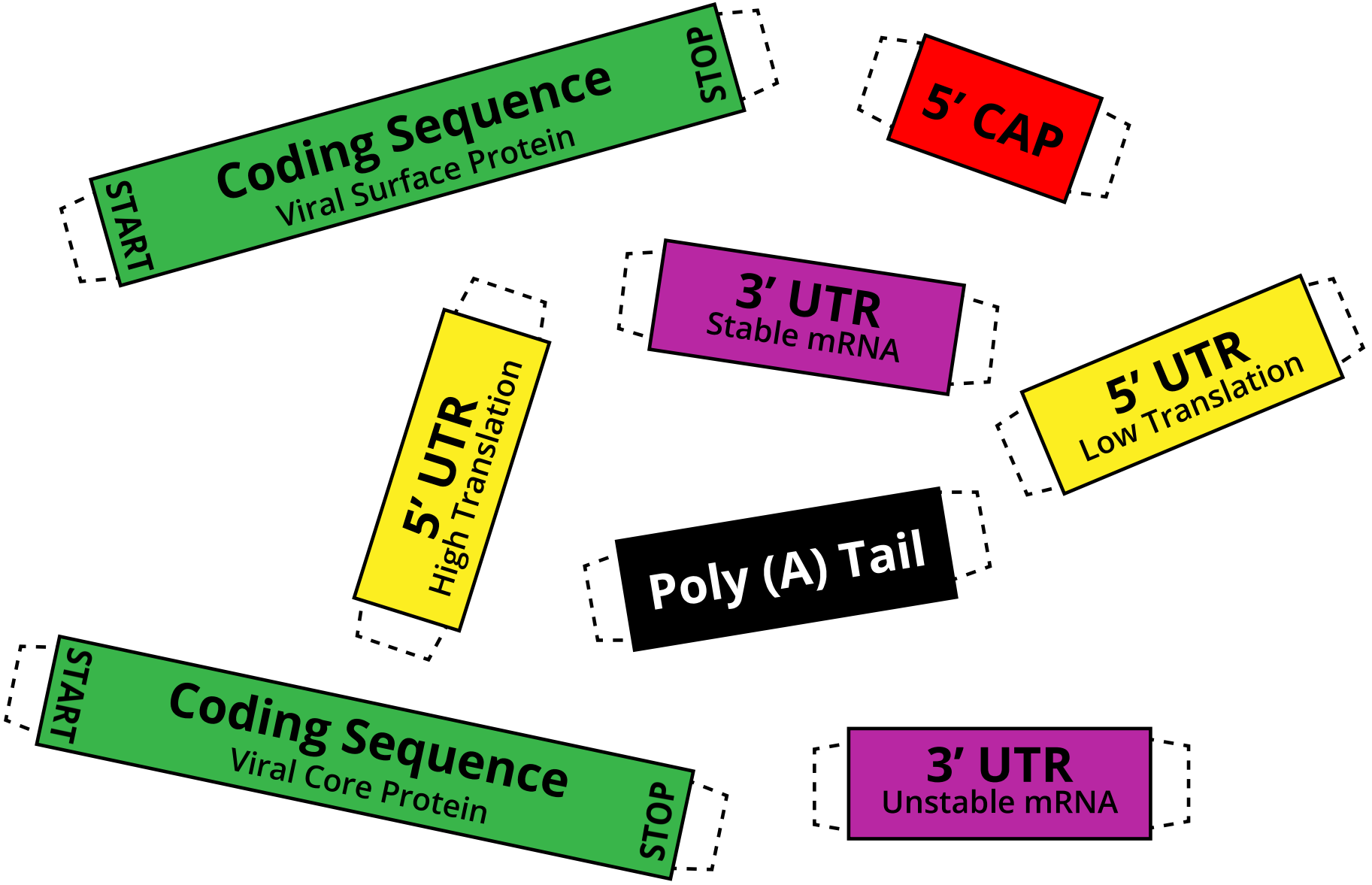


TABLE C.1				
Recipient Code (Self)	Contact 1	Contact 2	Contact 3	Final Color

TABLE C.2																													
Round 1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Round 2																														
Round 3																														
RESULTS																														