Making Vaccines

**Background:** A vaccine against a disease works by generating an immune response in the body against some kind of pathogen—a virus, bacterium or some other agent that causes the disease. Normally when a pathogen invades the body, the immune system works to get rid of the pathogen. Often, though, the immune system gets a slow start, which gives the pathogen time to multiply and cause trouble. What a vaccine does is expose the immune system to a less-threatening version of a pathogen and, in effect, prime it to recognize and quickly eliminate the innocuous pathogen’s harmful counterpart, should it ever invade the body.

**Objective:** Explore the process of creating six vaccines in your own virtual laboratory. Understand the possible technique options to produce each vaccine.

**Directions:**
2. Click on the link, “Making Vaccines”
3. Read the directions and follow the prompts given.
4. Fill out the chart below.

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Disease For Which Vaccine Protects</th>
<th>How the Vaccine is Made</th>
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<td>Similar Pathogen</td>
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<td>Toxoid Vaccine</td>
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<td>Naked DNA Vaccine</td>
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<td>Attenuated Vaccine</td>
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**Questions:** Please answer the following questions.

1. Why might you get a mild case of the flu after getting a flu vaccine?

2. Why do you have to get an annual flu shot but you are only vaccinated against chicken pox one time?

3. Why is it important to vaccinate against rare diseases?

4. Considering what you learned about vaccinations, why do you think most are given during the first year of life?

5. Do you think there will be new vaccinations required during your lifetime? Explain your answer.
Making Vaccines – On Line Lab (if not able to get online)

Can’t pull the website up? Not able to get internet? Below is the information you need to complete the worksheet.

Live vaccines contain living pathogens. These pathogens invade cells within the body and use those cells to produce many copies of themselves, just as their more harmful counterparts would. The "similar pathogen" and "attenuated" vaccines discussed in this feature are examples of live vaccines. Although these vaccines trigger a full immune response, there is a small risk of the viruses within evolving into more-virulent strains. Non-live vaccines contain agents that do not reproduce in the body. "Killed," "subunit," and "toxoid" are examples of non-live vaccines. These vaccines trigger a partial immune response. Genetic vaccines are non-live vaccines that trigger a full immune response.

The procedures outlined in this feature have been greatly simplified. Also, some steps are meant to show what is done but not how. For example, a gene cannot be plucked out of DNA using tweezers, and there's no box-like device called a purifier that can extract toxins from bacteria as well as viruses from pus.

Making Vaccines
Similar-pathogen vaccine: smallpox virus

Step 1
Use the sterile petri dish to collect fluid from pustules on the cow’s udder.

To create a vaccine that will protect you against a pathogen, you usually begin with that pathogen and alter it in some way. Not so with smallpox. To create this vaccine, you begin with another virus that is similar to the smallpox virus, yet different enough not to bring on the smallpox disease once it enters your body. This similar virus is cowpox.

The cow to the left has been intentionally infected with cowpox virus. The fluid that you collect from virus-caused pustules on the cow's udder contains many copies of the virus.

Step 2
Use the purifier to isolate the viruses

Smallpox vaccines contains cowpox viruses but not the bacteria and other impurities found in the fluid collected from such pustules.

To make the vaccine, therefore, you'll need to separate the cowpox viruses from the rest of the fluid.

Step 3
Fill the syringe with the purified cowpox viruses.

The smallpox vaccine is a live vaccine; the cowpox viruses it contains will invade cells in your body, multiply, and spread to other cells in your body, just as the smallpox viruses would. And as with smallpox, the body's immune system will mount an attack against the cowpox and subsequently always "remember" what it looks like. Then, if cowpox or the similar smallpox ever enters the body, the immune system will quickly get rid of the invaders.
The smallpox vaccine is complete.

Congratulations. You have just created a vaccine for smallpox.

At one time, cows were used to create the smallpox vaccine. In fact, the decades-old stockpile in the U.S. today was made using live calves through a process similar to the one outlined here. Advancements in biotechnology, however, have led to more efficient procedures that make use of bioreactors.

Making Vaccines
Attenuated vaccine: measles virus

You are about to create a live-attenuated vaccine, which means that you need to alter a pathogen—in this case a measles virus—so that it will still invade cells in the body and use those cells to make many copies of itself, just as would any other live virus. The altered virus must be similar enough to the original measles virus to stimulate an immune response, but not so similar that it brings on the disease itself.

To create a new strain of the virus, you'll need to let it grow in a tissue culture.

Step 1
Use the tissue culture to grow new viruses.

The tissue culture is an artificial growth medium for the virus. You will intentionally make the environment of the culture different than that of the natural human environment. For this vaccine, you'll keep the culture at a lower temperature.

Over time, the virus will evolve into strains that grow better in the lower temperature. Strains that grow especially well in this cooler environment are selected and allowed to evolve into new strains. These strains are more likely to have a difficult time growing in the warmer environment of the human body. After many generations, a strain is selected that grows slow enough in humans to allow the immune system to eliminate it before it spreads.

Congratulations. You have just produced a live-attenuated measles vaccine.

Like the smallpox vaccine, the virus within the vaccine will invade body cells, multiply within the cells, then spread to other body cells. The virus used in the measles vaccine today took almost ten years to create. The starting stock for the virus originated from a virus living in a child in 1954.

Live-attenuated vaccines are also used to protect the body against mumps, rubella, polio, and yellow fever.

Done
The measles vaccine is complete.

Select another pathogen.
Making Vaccines
Killed vaccine: polio virus

The goal in creating a killed vaccine is to disable a pathogen's replicating ability (its ability to enter cells and multiply) while keeping intact its shape and other characteristics that will generate an immune response against the actual pathogen. When the body is exposed to the killed polio vaccine, its immune system will set up a defense that will attack any live polio viruses that it may encounter later.

To produce this vaccine, you first need many copies of the polio virus. You can grow these in a tissue culture.

Step 1
Use the tissue culture to grow new viruses.

The polio virus uses the cells within the tissue culture to produce many copies of itself.

These copies of the virus need to be separated from the tissue culture.

Step 2
Use the purifier to isolate the polio viruses.

There are several ways to inactivate a virus or bacteria for use in a vaccine. One way is to expose the pathogen to heat. This is how the bacteria in the typhoid vaccine is inactivated. Another way is to use radiation.

For the polio vaccine developed by Jonas Salk in 1954, formaldehyde was used. You'll use formaldehyde in creating your polio vaccine, too.

Step 3
Use formaldehyde to kill the viruses.

The dead viruses in your polio vaccine will not produce a full immune response when injected in a body. This is true for all vaccines that are not live. For this reason, these vaccines usually require booster shots.

Step 4
Fill the syringe with the killed polio virus.
Congratulations. You have produced a killed polio vaccine.

There are two polio vaccines widely used today. One is Salk's killed vaccine; the other is a live-attenuated vaccine first developed by Albert Sabin.

In addition to polio and typhus, killed vaccines are used to prevent influenza, typhoid, and rabies.

**Making Vaccines**

**Toxoid vaccine: tetanus**

With a toxoid vaccine, the goal is to condition the immune system to combat not an invading virus or bacteria but rather a toxin produced by that invading virus or bacteria. The tetanus shot is such a vaccine. Tetanus is a disease caused by toxins created by the bacteria *Clostridium tetani*. The vaccine conditions the body's immune system to eliminate these toxins.

To produce the vaccine, you first need to grow many copies of the *Clostridium tetani* bacteria.

**Step 1**
Use the growth medium to grow new copies of the *Clostridium tetani* bacteria.

While in the growth medium, the bacterial cells produce the toxin, which are toxic molecules that are often released by the cells.

To produce the vaccine, you'll need to separate these molecules from the bacteria and the growth medium.

**Step 2**
Isolate the toxins with the purifier.

In this state, the toxin would be harmful to the human body. To make the vaccine, it needs to be neutralized.

Sometimes formaldehyde is used to neutralize toxins. For your vaccine, you'll use aluminum salts to decrease its harmful effects.

**Step 3**
Add aluminum salts to the purified toxins.
The toxin would work as a vaccine now, but it wouldn't stimulate a strong immune response. To increase the response, an "adjuvant" is added to the vaccine. For the tetanus vaccine, another vaccine acts as the adjuvant. This other vaccine inoculates against pertussis. The vaccine for diphtheria -- also a toxoid vaccine -- is also often added to the tetanus/pertussis combo, making for the DPT vaccine.

Step 4
Fill the syringe with the treated toxins.

Done
The tetanus vaccine is complete.

Select another pathogen.

Making Vaccines
Subunit vaccine: hepatitis B

A subunit vaccine makes use of just a small portion of a pathogen. For a virus, the vaccine can contain just a piece of the protein coat that surrounds the virus's DNA (or RNA). Even small portion of a virus is sometimes enough to stimulate an immune response in the body.

There are several ways to produce a vaccine for hepatitis B vaccine. For your vaccine, you'll use genetic engineering techniques.

Step 1
Use the tweezers to pull out a segment of DNA from the hepatitis B virus.

A segment of the virus's DNA is responsible for the production of the virus's protein coat. You will add this segment to the DNA within a yeast cell.

The yeast cell, as it grows, will "read" the viral DNA incorporated in its own DNA and produce the protein that makes up the protein coat of hepatitis B.

Step 2
Add the segment of DNA to the DNA of a yeast cell (which is in the yeast culture).
Step 3
Use the purifier to isolate the hepatitis B antigen produced by the yeast cells.

The vaccine, once administered, will stimulate the immune system to attack the antigen (i.e., the protein coat). Then, if the inoculated person is later exposed to the virus, the immune system will quickly respond to the invader and eliminate it before it has a chance to spread widely.

To finish making the vaccine, you need to separate the proteins from the yeast cells.

Step 4
Fill the syringe with the purified hepatitis B antigen.

The isolated hepatitis B protein, produced by the yeast cells, contains none of the viral DNA that makes hepatitis B harmful. Therefore, there is no possibility of it causing the disease.

Congratulations. You have produced a subunit vaccine for hepatitis B.

Another example in the subunit category is the anthrax vaccine approved in the U.S. (The countries of the former Soviet Union have an attenuated version of the vaccine.) The U.S. vaccine is currently administered to military personnel.

Done
The hepatitis B vaccine is complete.

Select another pathogen.

Making Vaccines
Naked-DNA vaccine: HIV

Step 1
Use the growth medium, which includes PCR primers, to make billions of copies of a single gene.

Genetic vaccines, sometimes called naked-DNA vaccines, are currently being developed to fight diseases such as AIDS. The goal of these vaccines is to use a gene from a pathogen to generate an immune response. A gene contains the instructions to create a protein. With a genetic vaccine, small loops of DNA in the vaccine invade body cells and incorporate themselves into the cells' nuclei. Once there, the cells read the instructions and produce the gene's protein.

Using a technique called PCR, which stands for polymerase chain reaction, you'll make many copies of a specific gene. The work of finding the gene and copying sequences of its DNA is done by "primers."
Step 2
Combine the virus genes with vectors.

To make your genetic vaccine, you'll use vectors. Vectors are agents that are able to enter and instruct cells to create proteins based on the vector's DNA code. In this case, the vectors are loops of double-stranded DNA. You can exploit the vector's ability to create proteins by splicing a gene from the virus into a vector. The cell that the vector later invades will then produce proteins created by the virus.

The vectors and copied genes have been treated with restriction enzymes, which are agents that cut DNA sequences at known locations. The enzymes have cut open the round vectors and trimmed the ends of the copied genes.

Step 3
Add bacteria to the vectors to allow the altered vectors to replicate.

The ends of the vectors have again come together, but now with a gene spliced into the loop. You'll need many copies of the vector/gene loop for your genetic vaccine. These copies can be produced with the help of bacteria.

Vectors are capable of self-replicating when within a bacterial host, as long as that host is in an environment conducive to growing. After you combine the vectors and bacteria, the vectors will be shocked into the bacteria.

Step 4
Use the purifier to separate the altered vectors from the bacteria.

The final vaccine should include only the vectors, so you'll need to separate them from the bacteria after enough copies have been produced. This can be done with a detergent, which ruptures the cell walls of the bacteria and frees the DNA within.

The relatively large bacterial DNA can then be separated from the smaller DNA loop that makes up the vector.

Step 5
Fill the syringe with the altered vectors.

Upon inoculation, billions of copies of the altered vector will enter the body. Of these, only 1 percent will work their way into the nuclei of body cells. But that's enough.

The body's immune system responds to these proteins once they leave the cell. But more importantly, it also reacts to proteins that are incorporated into the cells' walls. So in addition to mounting an attack against the free-floating proteins, the immune system attacks and eliminates cells that have been colonized by a pathogen. The vaccine, then, works like a live vaccine, but without the risk. (With a live vaccine, the pathogen can continue to replicate and destroy cells as it does so.)
Congratulations. You have just produced a naked-DNA HIV vaccine.

Trials for a genetic vaccine that may protect against AIDS began in 1995. These vaccines, which contained HIV genes, were given to patients who already were infected with HIV. A year later, the trials were expanded to test people without HIV. These trials are still being conducted and have not yet produced conclusive results.

Human trials for genetic vaccines against herpes, influenza, malaria, and hepatitis B are also underway.

Note: Although the genetic material of HIV is RNA, the procedure for making the vaccine is similar.

Once you have completed your lab paper. Turn this back in to your teacher along with your lab sheet.