Teacher's Notes

New Technologies in Vaccines

Overview

North Carolina is an innovative leader in vaccine research, development and manufacturing. Although some myths make vaccinations controversial among parents, vaccines actually are one of the safest and most cost-effective tools in medicine. They have prevented millions of deaths and disabilities since the smallpox vaccination was introduced in Europe in the 1700s. Most U.S. children are vaccinated against 14 or more diseases. Vaccine researchers are making exciting progress in developing new and improved vaccines, manufacturing processes and delivery methods. The goal of this chapter is to help students understand the science concepts involved in immune response and in new technologies for manufacturing vaccines, as well as the exciting career opportunities available in this field.

This chapter also is intended to provide a snapshot of some of the leading vaccine research and manufacturing efforts in North Carolina. However, it is not intended to be a comprehensive summary. For a broader overview of vaccine development efforts throughout the state, please refer to http://www.ncbiotech.org/sites/default/files/vaccineassetsbrochure.pdf, a brief booklet published by the North Carolina Vaccine Consortium. The consortium consists of industry, academic and education partners, including Duke University, the North Carolina Biosciences Organization, the North Carolina Biotechnology Center, North Carolina State University, the University of North Carolina at Chapel Hill and Wake Forest University. These partners align their research, product development, manufacturing and workforce training efforts for common goals.

Alignment with Standards

Biology Objectives from the Essential Standards

Bio.1.1: Understand the relationship between the structures and functions of cells and their organelles.

- **Bio.1.1.3:** Explain how instructions in DNA lead to cell differentiation and result in cells specialized to perform specific functions in multicellular organisms.

Bio.2.1: Analyze the interdependence of living organisms within their environments.

- **Bio.2.1.2:** Analyze the survival and reproductive success of organisms in terms of behavioral, structural and reproductive adaptations.

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Bio.3.3: Understand the application of DNA technology.

- **Bio.3.3.2:** Summarize how transgenic organisms are engineered to benefit society.
- **Bio.3.3.3:** Evaluate some of the ethical issues surrounding the use of DNA technology (including cloning, genetically modified organisms, stem cell research and Human Genome Project).

Bio.3.4: Explain the theory of evolution by natural selection as a mechanism for how species change over time.

- **Bio.3.4.3:** Explain how various disease agents (bacteria, viruses, chemicals) can influence natural selection.

Benchmarks from *Project 2061 Benchmarks for Science Literacy*

American Association for the Advancement of Science — Project 2061, 1993

6E/M7: Vaccines induce the body to build immunity to a disease without actually causing the disease itself.

6C/H1: The immune system functions to protect against microscopic organisms and foreign substances that enter from outside the body and against some cancer cells that arise within.

8F/H4: Inoculations use weakened germs (or parts of them) to stimulate the body's immune system to react. This reaction prepares the body to fight subsequent invasions by actual germs of that type. Some inoculations last for life.

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AP Biology Themes and Topics

Themes	Topics
Relationship of Structure to Function	Cells • Membranes
Science as a Process	Molecular Genetics • Viral structure and replication
Science, Technology and Society	Molecular Genetics • Nucleic acid technology and applications
Evolution	Structure and Function of Plants and Animals • Structural, physiological and behavioral adaptations

Biomedical Technology Objectives from 2004 Course Blueprint

BT02.00: Analyze biomedical ethics and legal principles.
- **BT02.02:** Analyze the ethical principles of biomedical technology.

BT06.00: Analyze issues of public health, infectious diseases and bioterrorism.
- **BT06.01:** Discuss the infectious disease process.
- **BT06.02:** Analyze the role of public health in the prevention of infectious diseases.
- **BT06.04:** Analyze emerging and re-emerging infectious diseases as a public health issue.
- **BT06.05:** Examine the containment of bioterrorism agents.

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BT09.00: Evaluate careers and techniques that use biomedical technology.
- **BT09.01:** Investigate laboratory careers.
- **BT09.04:** Outline biotechnology careers and genetics.
- **BT09.05:** Evaluate the importance of biomedical technology in a chosen health career.

BT10.00: Analyze biomedical research.
- **BT10.01:** Discuss biomedical research.
- **BT10.02:** Outline biomedical research methods.
- **BT10.03:** Analyze the benefits of biomedical research.

BT11.00: Analyze challenges to biomedical research.
- **BT11.01:** Interpret personal beliefs about biomedical research.
- **BT11.04:** Debate pros and cons of animal research and animal rights.

BT12.00: Analyze current issues in biomedical technology.

Key Vocabulary

- **Vaccination** is the introduction into the body of a weakened, killed or piece of a disease-causing agent to prevent disease.
- **Immunization** is the process by which the body becomes immune to a disease. A person can become immune by getting the disease or from a vaccination.
- An **epidemic** is when more people in a particular population get a disease than typically expected.
- A **pandemic** is when a disease outbreak is global or over large areas of the world.
- A **pathogen** is a disease-causing agent. It usually refers to a virus, bacteria, fungi or protozoan parasite.
- A **virus** is a small, infectious agent that only can replicate inside the cells of a living organism. It has a core of DNA or RNA surrounded by a protein coat and is not itself a living cell.

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- **Bacteria** are a large group of microorganisms. They are less complex than eukaryotes, they usually are unicellular, their DNA is not contained in a nucleus and they do not have membrane-bound organelles, such as mitochondria and chloroplasts. While some bacteria cause disease, the majority are harmless, and many are essential to life.

- **Antigens** are substances, usually proteins or polysaccharides, that cause the body to produce antibodies.

- **Antibodies** are special proteins produced by the body to attack foreign invaders such as pathogens.

New Technologies in Vaccines

Can you imagine eating a banana instead of getting a shot? Or using flu vaccine antigens produced by tobacco plants? Ever heard of vaccine biodefense or nano-printing new vaccines? Exciting research and development in each of these areas is taking place in North Carolina. This research promises to improve the health of people around the world and provide many employment opportunities right here in our state. This chapter and the accompanying activities are designed to help you understand some of the biggest challenges and latest advances in vaccine technology.

Responding to Pandemics

We usually think of the flu in terms of the inconvenience of a flu shot or of missing work or school for a week or two. But in 1918–19, a severe outbreak of a virulent strain of influenza (flu) virus killed between 50 and 100 million people worldwide. More than 500,000 people (of a total population of 103 million) died in the United States from this flu. Also known as the "Spanish Flu," the 1918 strain of influenza was unusual because it was more lethal and killed a greater percentage of young, healthy adults than more typical flu outbreaks. In 2009, a new strain of influenza emerged that had some characteristics similar to the 1918 flu strain. Health officials were worried. Fortunately, the 2009 strain proved to be less lethal than the 1918 strain, and global health systems were better prepared. Could a new strain of flu or some other disease cause a repeat of the 1918 scenario today? As you read through this chapter, consider the ways in which the world has changed since 1918. Do these changes make a deadly disease scenario more or less likely?

When an infectious disease breaks out worldwide, it is called a pandemic. Other infectious diseases besides influenza — such as smallpox, bubonic plague and HIV — have caused deadly pandemics as well. But because flu viruses include many rapidly changing strains and circulate among animals as well as people, they are particularly likely to cause pandemics. In fact, there have been several flu pandemics in the last 100 years, and the interconnected nature of our current global society only makes these more likely in the future. Therefore, global public health experts are working hard to prepare for future pandemics — particularly the next influenza pandemic. One of their key weapons against this disease is vaccination.

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The main goal of vaccination is to prevent disease. Scientists have developed vaccinations against many of the most threatening diseases of the past, including smallpox, polio, cholera, typhoid, yellow fever and influenza. The state of North Carolina requires vaccination against 10 different diseases by the time children start school, and the federal Centers for Diseases Control and Prevention recommends vaccination against five more. Vaccination not only protects the vaccinated person but also protects the community as a whole. By lowering the incidence of the disease in the population, vaccination protects even those who are too young to be vaccinated, those who are allergic to vaccine ingredients and those who have weakened immune systems. However, some diseases present difficult challenges to vaccine researchers. Researchers are working to solve these challenges and develop new vaccines for many devastating diseases, including HIV, malaria and chlamydia.

How Vaccinations Work: Overview of Immune Response and Components of Vaccines

Your immune system protects you from diseases caused by a wide variety of pathogenic organisms, including viruses, bacteria, protozoans and worms. It does this with a layered defense system that has an array of defenses from mechanical barriers (such as skin and mucus) to specific attacks on invading organisms. The purpose of a vaccination is to jump-start a specific immune response by introducing the person (or animal) being vaccinated to enough of a specific, disease-causing agent to cause an immune response but not enough to cause illness.

The human immune system is able to recognize and distinguish between protein (and some other) molecules that belong to its owner's body (self) and those that come from outside attackers (foreign).

For more…

Visit the official website of the Nobel Prize (nobelprize.org/educational/medicine/immunity) for additional readings about the immune system and to play *The Immune System*, an interactive game.

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The foreign proteins are called antigens. Several different types of white blood cells work together to eliminate these antigens. White blood cells recognize surface proteins on disease agents as antigens because they are different from their own body's proteins. Some types of white blood cells, such as macrophages, surround and engulf the invaders. The macrophages break the invaders down and display the invaders' surface proteins. This sets off a chain of immune system reactions, activating some cells that attack infected cells (T cells) and others (B cells) that make antibodies, or proteins shaped to attach to the antigens. The antibodies bond with the antigens, which marks them for destruction by macrophages and T cells. Once the immune system has learned to make antibodies against a particular disease, it makes both T and B memory cells. These memory cells remain in the body for many years, ready to launch an attack if that specific disease invades again. Vaccinations work by triggering this response, so the body creates memory cells ready to quickly recognize and destroy a particular pathogen.

There are several different types of vaccines, each requiring different approaches to design and manufacture. These include:

- Live but weakened disease agents
- Inactivated or killed disease agents
- Subunit vaccines
- Toxoid vaccines
- Conjugate vaccines
- DNA vaccines
- Recombinant vector vaccines

Many vaccines are made of live, attenuated (weakened) disease agents. (This category includes live virus vaccines. Even though viruses are not considered living organisms, functional virus particles are called "live.") Others vaccines are made of inactivated or "killed" disease agents. Still others contain only a subunit of the disease organism. Toxoid vaccines help the body develop antibodies to toxins released by bacteria rather than antibodies to the bacteria themselves. Some bacteria have polysaccharide coatings that disguise them from immature immune systems. Conjugate vaccines help the immune systems of infants, and young children recognize these bacteria by linking toxoids their

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systems recognize to the polysaccharide coatings. Two other types of vaccines — DNA vaccines and recombinant vector vaccines — are in experimental stages. Each of these types of vaccines provides a different mix of advantages and disadvantages.

The use of live but weakened or attenuated disease agents to vaccinate has a long history. In the 1700s, Edward Jenner was interested in the belief of farmers that cowpox infection prevented smallpox. Jenner deliberately infected people with cowpox, a mild disease related to smallpox. Following their recovery from cowpox, he deliberately tried to infect these people with smallpox and found them to be immune. Vaccination for smallpox using cowpox quickly spread around the world. Jenner's research techniques would be considered highly unethical today, but his work saved countless lives. While Jenner used a naturally occurring live virus to vaccinate against smallpox, subsequent researchers developed methods for weakening pathogens in the laboratory. This led eventually to the complete eradication of the smallpox disease.

Today, researchers weaken or attenuate pathogens by growing them in a series of non-human cell cultures and selecting for those with lowered capability to reproduce in humans. Since Jenner's time, live, attenuated vaccines have been developed for many other diseases, especially those caused by viruses. In the United States, common live, attenuated vaccines including MMR, chickenpox and the nasal spray form of flu vaccine. The advantage of live, attenuated vaccines is that they are very effective in inducing full protection against their diseases. However, there are several problems with this type of vaccine:

- The viruses are still "live" and can mutate to a more dangerous form
- Some people, especially those with weak immune systems (such as cancer or HIV patients), get sick even from the weakened form of the disease
- To remain effective, a live vaccine usually needs constant refrigeration all the way from the manufacturing stage until it is injected into the patient. Vaccines that require refrigeration can be difficult to distribute, particularly in poorer countries.

Inactivated or killed disease agents are made by destroying the disease agent's DNA with chemicals, heat or radiation to prevent it from reproducing, while

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keeping some of the disease organism's proteins intact. These proteins then are purified and packaged to make the vaccine. The proteins in the vaccine induce the specific immune response, allowing the body to recognize and attack the disease organism in future attacks. The flu shot uses inactivated flu virus. The form of the polio vaccine developed by Jonas Salk in the 1950s used inactivated viruses to induce immunity. The advantages of these vaccines are that they can't cause the actual disease and that the purified forms can be stored without refrigeration, which makes them cheaper and easier to distribute globally. The disadvantage is that the immune response they cause is weaker than the immune response caused by live, attenuated viruses. The weaker response means that more booster shots are required to maintain immunity.

Vaccine Fears: Fact vs. Fiction

Smallpox vaccination eradicated smallpox; the last wild (non-laboratory) case was recorded in 1977. Polio may be eradicated next. Many diseases that routinely caused deaths around the world in the past now have greatly reduced incidence in the U.S. However, some diseases are coming back. Both measles and whooping cough (pertussis) have infected an increasing number of people in the U.S. in the last several years. For a quick view of the problem, examine the Council on Foreign Relations' interactive map, at http://www.cfr.org/interactives/GH_Vaccine_Map. This resurgence in vaccine-preventable diseases is due to the fears many people have about vaccination.

Vaccination does have risks, but these risks are far outweighed by the risks of the diseases they prevent. For example, the risk of death or serious complications from a measles vaccination is less than 1 in 1,000,000 (one in a million), while 1 of 1,000 people who catch measles die (and many others suffer serious complications, such as pneumonia and ear infection). For example, 40% of the 216 people who caught measles in a 2008–09 outbreak in Hamburg, Germany, required hospitalization due to serious complications. In addition, researchers and manufacturers are working to develop safer vaccines.

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One common myth about vaccination is that it leads to autism in young children. This myth arose from a fraudulent study published in a British medical journal claiming that the MMR (measles/mumps/rubella) vaccine caused autism. After publication of this study, vaccination rates fell, many more children got sick and some of these children died. Two large studies since have shown there is no connection between vaccination and autism spectrum disorders. An investigation into the original study found that the researchers in this study changed the data to fit their conclusion and that they had received money from lawyers and parents who wanted to sue vaccine manufacturers. The journal retracted the study and the researcher involved lost his job and his license to practice medicine. Unfortunately, some people still believe this myth, and vaccination rates continue to be lower than optimal for protecting the whole population.

Another set of controversies developed around Gardasil and Cervarix, vaccines against HPV (human papilloma virus). HPV is a sexually transmitted disease that causes genital warts and cervical cancer. It also has been linked to other cancers, such as vulvar and vaginal cancers in women, penile cancers in men and anal and throat cancers in men and women. HPV is the most common sexually transmitted disease in the U.S., but most women fight the infection off without treatment. Each vaccination requires three doses and is relatively expensive, and it is not yet known how long the protection it provides will last. On the other hand, cervical cancer affects 11,000 women a year in the U.S. and kills almost 4,000. Worldwide, 275,000 women die each year from cervical cancer. Most of these cases would be prevented by vaccination, which protects against the most common cancer-causing strains of HPV. Vaccination also can prevent abnormal and precancerous cells in the cervix. These abnormal cells are found by Pap smears, during which cells taken from a woman's cervix are examined. An abnormal Pap smear result often leads to a biopsy. Therefore, the HPV vaccine also can reduce the number of biopsies required. Despite a study of more than 600,000 people vaccinated against HPV showing no increased risk of serious adverse events, some parents and politicians continue to claim it is a dangerous vaccine. The Food and Drug Administration and government agencies around the world continue to monitor the safety and effectiveness of all vaccines.

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There are also social controversies around HPV vaccination. The vaccination is most effective in young adolescents because they have a stronger immune response and because it is important to be vaccinated before exposure to the disease. Some states have proposed legislation requiring the vaccination for school attendance, and other states provide funding for education about vaccination or for vaccinations themselves. However, many people are uncomfortable with vaccinating children in this age group against a sexually transmitted disease. Some parents think vaccination may lead girls to feel it is safer to have sex. It is important for health care providers to discuss these issues with families as patients and their parents make decisions about vaccination. Health care providers need to explain clearly that this vaccination does not prevent pregnancy, other sexually transmitted diseases or even all forms of HPV. Whether it is appropriate for the government to require vaccination against a sexually transmitted disease remains controversial.

Vaccine Development Process

The Food and Drug Administration (FDA) must approve vaccines for use in the United States. To get that approval, each new vaccine goes through an extensive development and testing process that builds on lessons from previous vaccination development and includes *in vitro* testing, animal testing and several phases of clinical trials in humans. Researchers use cell and tissue culture to assess the cellular response to a new vaccine. Then, researchers use animals to assess safety and the level of immune response. Ferrets are used in flu vaccine research because the flu acts similarly in ferrets and people. Mice and monkeys are used in research for vaccines for other diseases. Researchers may test the vaccine by vaccinating animals and then trying to infect them. This is called a challenge study and, unlike in Edward Jenner's time, challenge studies rarely are performed in humans today.[1] After a new vaccine proves to be safe and effective in *in vitro* or animal tests, the researchers submit an application to the FDA to test it in humans. Each phase of human testing involves careful monitoring to be sure the vaccine is safe and to assess its level of efficacy. Even after a vaccine is approved for use in the public, the FDA continues to regulate

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its production. A national system for reporting problems with vaccinations is used to monitor the ongoing safety of vaccines.

Manufacturing Vaccines: Tools and Techniques

Different methods are used to manufacture different vaccines. The earliest forms of smallpox vaccination were actually inoculation — infecting a person with a mild form of the disease to prevent the more virulent form. This method consisted of putting material from a smallpox patient's skin lesions onto a scratch in the skin of the person being vaccinated. Catching smallpox through a skin lesion was slower than catching it through airborne transmission to the respiratory tract and allowed the person to mount an immune response before falling seriously ill. However, this method was imprecise and dangerous. Today's methods of vaccine production and delivery are much safer. Vaccine antigens are produced under highly regulated conditions. Manufacturers follow required good manufacturing practices, which ensure that the product is produced exactly the same way each time it is made and that each part of the manufacturing process is carefully documented, from raw materials through distribution to the patient. This ensures a pure product, exact doses and safe distribution. We will look at three important methods of vaccine manufacture in more detail: eggs, cell culture and plant culture.

Eggs

Since the 1940s, influenza vaccine has been made by injecting fertilized chicken eggs with live but weakened strains of influenza virus. The virus replicates in the egg for several days.[2] The virus then is separated from the egg and exposed to chemicals, which inactivates the virus DNA. Next, the outer proteins of the virus are purified and tested to measure the yield, concentration and sterility of these proteins. The vaccine then is packaged into vials and another round of testing follows to ensure each batch has the correct dose and is safe. This process uses hundreds of millions of specially produced eggs every year. It takes five to six months from the time the new strains of influenza are identified

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each year until the vaccine is available for use. Because contamination is a serious threat, every aspect of the process must be carefully controlled. Other problems include (a) the threat of egg shortage due to diseases in chickens and (b) the fact that some strains of virus grow more slowly in eggs or have much lower yield of antigen than other strains, which means it takes longer to produce an adequate supply of vaccine. If a new or deadly strain of pandemic flu emerges, many people might become ill before the vaccine could be ready. For these reasons, researchers are working hard to develop new manufacturing methods.

Cell Culture

One of these new methods of vaccine production is cell culture, where antigen is grown in large vats of cells. The cells are grown in vats called bioreactors, which are completely closed from any contact with the outside environment to ensure no contamination occurs. After the cell population has grown to fill the vats, they are infected with the virus. The virus replicates in the cells, producing lots of antigen and killing the cells. Next, the antigen is harvested and purified and then, as in egg culture, it is tested, packaged and tested again. As in any drug manufacturing process, there is careful quality control and documentation every step of the way, from the acquisition of raw materials through the distribution of the product to the end user. This process is significantly faster than the egg process.

Pharming

Another exciting development is the production of pharmaceuticals in plants. Medicago has a new, 97,000-square-foot facility in Research Triangle Park, N.C., dedicated to the production of plant-based vaccines. (A regulation football field is only 57,600 square feet.) This vaccine manufacturing process is different from the egg and cell culture methods because live vaccine is not used. Similarly to the egg and cell culture processes, the new strain of influenza first must be isolated and characterized. However, after a new strain of virus is isolated, the gene for its main surface protein is sequenced. Next, the sequence is inserted into a plasmid. Plasmids are small pieces of DNA, which can replicate independently inside bacterial cells. The plasmids then are transferred

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into *Agrobacterium tumefaciens*, a soil bacterium that normally causes plant disease by inserting its plasmids into the plant's cells. Next, tobacco plants, grown in a carefully controlled greenhouse, are put in a vacuum tank. The vacuum pump sucks air out of the spaces inside the leaf. When the vacuum pressure is released, the agrobacterium solution is pulled into the leaf, where it infects the plant cells. The plasmid with the virus surface protein is now inside the leaf cells, where it causes them to make the virus protein. After several days the leaves are harvested and the virus surface protein is extracted and purified. The proteins form virus-like particles (VLPs), which activate an immune response — but without the DNA. Finally, they are packaged for delivery as a vaccine. Clinical trials indicate they can cause a strong immune response. Medicago recently was able to produce 10 million doses of flu vaccine in one month, which is significantly faster than traditional methods of flu vaccine production. Because they lack DNA, the VLPs are safer, non-infectious and more stable for distribution.

Challenges in Vaccine Research

Many challenges remain in vaccine research. Some disease organisms pose particularly complex problems to vaccine developers. We need rapid and economical manufacturing techniques to produce large amounts of vaccine in order to be prepared for the possibility of a rapidly emerging pandemic disease. Vaccine products must be pure and must be stable for distribution. Vaccines that don't require refrigeration are much easier to distribute, particularly in poorer countries. Many researchers are working on new methods of vaccinating people. Some day you might be able to feed your children a special banana rather than taking them to the doctor for a shot.

In some cases, such as flu and HIV, the disease-causing virus mutates frequently. The mutations cause changes in the antigen proteins found on the surface of the virus. Even if a person is vaccinated, changes in these proteins mean that the immune system doesn't recognize the changed virus immediately, which in turn means the immune system can't fight the changed

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virus effectively. This is why people need a new influenza vaccination each year. In the case of the common cold, the number of different viruses that cause the disease make vaccination development impractical. More than 200 strains of viruses can cause the common cold, including rhinoviruses, coronaviruses and adenoviruses. It has been impossible so far to design a vaccination to prevent all of these. Most vaccine-preventable diseases are caused by viruses or bacteria. However, malaria, a major killer worldwide, is caused by a protozoan carried by mosquitoes. Protozoans are much larger than viruses or bacteria and present multiple possible surface proteins that the immune system could attack. Unfortunately, these protozoans also have a complex life cycle. Different stages of the parasite grow within different cells and tissues in the human host, and the population of protozoans is quite diverse. Even a single bite from an infected mosquito may expose a person to many genetically diverse protozoans with slightly different antigens. Researchers have not yet been able to find an antigen consistently displayed by the malaria protozoans.

Once an effective antigen for inducing immunity has been isolated, the next step is to find the best way to produce large amounts of the required protein quickly and economically.

Careers in Vaccine Manufacturing

Manufacturing vaccines is an exacting business. Like any manufacturing process, it requires bringing in the correct raw materials, production, packaging and distribution. Because of the sensitivity of the vaccine product, it is essential that every step of production and any deviation in the procedure be documented completely. Each process requires employees with special knowledge and skills.

Winnell Newman: Manager of Student Programs, BTEC
Winnell Newman is the manager of student programs at the Golden LEAF Biomanufacturing Training and Education Center (BTEC) at North Carolina State University. The BTEC provides education and training to students at all

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levels and to current employees to prepare them for work in biomanufacturing. It also provides analytical services to help industry clients improve processes. The BTEC has industry-standard equipment, which gives students hands-on practice in the latest techniques. Winnell's job is to make sure students know about the BTEC's wide range of courses and programs and to advise students who are interested in biomanufacturing careers. She is passionate about helping students understand and take advantage of the rich environment offered at N.C. State and the BTEC.

In addition to her work at the BTEC, Ms. Newman is a co-founder of Trana Discovery, a startup company spun off from research done at N.C. State. Winnell is part of the research team that developed a drug discovery technology based on tRNA. This technology can be used to screen chemicals for the capacity to inhibit tRNA and may lead to a new class of antibiotics. Trana Discovery will license its technology to partner companies that specialize in drug development. Winnell says forming a biotechnology company has been a learning process every step of the way, stretching her beyond science to learn about entrepreneurship, business and law.

Ms. Newman began her college career with an interest in medicine but later, when she had the opportunity to participate in research, became fascinated by the discovery process. She majored in biology, went on to get a master's degree in botanical studies at the University of North Carolina at Greensboro and then went to work at Ciba-Geigy (now known as Syngenta) in the Metabolism and Residues Studies department.

"I did many things wrong when I first went to college, but with hard work and wonderful mentors I was able to recover," Winnell says. "Now I am in the perfect job for me. I can empower students with the knowledge needed to be successful in this exciting field."

She advises college students in any field to pick a major for which they have a passion, to seek counseling, to find a mentor and to begin networking early. She also advises students to make coursework their top priority. She says GPA does matter!

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Jennifer Ruiz: Assistant Director of Bioprocess Services, BTEC
Dr. Jennifer Ruiz is the Assistant Director of Bioprocess Services at the Golden LEAF Biomanufacturing Training and Education Center (BTEC) at North Carolina State University and the project coordinator for the BTEC's international influenza vaccine manufacturing program. In addition to its many education initiatives, the BTEC provides industry with services, such as the manufacture of potential pharmaceuticals for preclinical testing and research to optimize production processes. Dr. Ruiz finds this work interesting because she deals with many different companies that take different approaches to similar problems. She is especially suited to this work because she has prior experience with both upstream production of therapeutics and downstream processing, in which the product is purified and prepared for delivery to patients.

In addition to working with companies to optimize their processes, Dr. Ruiz manages and teaches in a professional development program for scientists and engineers from influenza vaccine manufacturing companies worldwide. The program is funded by the Biomedical Advanced Research and Development Authority (BARDA) of the U.S. Department of Health and Human Services and helps ensure a rapid global response to influenza. In case of a pandemic, it is important for many countries in all regions of the world to be able to produce a vaccine. Each company has different equipment and challenges — but all are focused on the goal of quickly producing safe, effective vaccines. The courses at the BTEC teach the visiting scientists and engineers about the latest techniques for both egg and cell culture and about new technologies, such as the plant-based technology used by Medicago.

Dr. Ruiz originally is from Costa Rica. When she started college there, studying journalism appealed to her extroverted, people-oriented personality. As she continued her studies, however, she missed the math and science courses she enjoyed in high school. She loved chemistry and decided to go into chemical engineering because she felt it would be more likely to lead to employment in industry. When Dr. Ruiz completed her undergraduate studies, she applied to graduate schools in the United States and was awarded a full scholarship to Texas A&M University. At Texas A&M she sought out professors in biochemical and medical research and found an adviser and mentor in Dr.

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Theresa Good, who was researching the development of targeted drug therapies for various diseases. After completing her doctorate, Dr. Ruiz went on to develop vaccines at AlphaVax before coming to the BTEC. Her advice to students is to channel your abilities to where you can make the most of them.

References

1. See http://www.dovepress.com/the-utility-of-human-challenge-studies-in-vaccine-development-lessons--peer-reviewed-article-VDT for a downloadable article on human challenge studies in cholera vaccine development and http://www.ligocyte.com/downloads/Noro.pdf for discussion of the development of a norovirus vaccine that includes a human challenge study.
2. See http://www.virology.ws/2009/12/10/influenza-virus-growth-in-eggs for a picture of how different viruses are injected into eggs and a detailed description of the process.

Resources

Vaccines

http://www.historyofvaccines.org/content/timelines/all

The College of Physicians of Philadelphia has a very detailed, illustrated timeline of vaccine development and animations that shows how vaccines work.

Developing a Malaria Vaccine

http://www.scidev.net/en/policy-briefs/malaria-vaccines-research-problems-and-priorities.html

This article explains the challenges in developing a malaria vaccine, what is being tried and recent progress.

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Medicago

http://www.medicago.com

Medicago's website includes a step-by-step overview of the company's plant-based vaccine production process and reference information for scientific publications of related research.

Novartis Vaccines

http://www.novartisvaccines.com

This site includes an influenza vaccines information center and news releases about recent research and achievements.

Vaccine-Preventable Outbreaks

http://www.cfr.org/interactives/GH_Vaccine_Map

This interactive map by the Council on Foreign Relations shows outbreaks of diseases that could have been prevented by vaccines worldwide since 2008. You can click on each outbreak to get more information and links to news stories about the outbreak.

Egg-Based Vaccine Production Process

http://www.virology.ws/2009/12/10/influenza-virus-growth-in-eggs

This site has a picture of how different viruses are injected into eggs and a detailed description of the process.

MEDMYST

http://webadventures.rice.edu/stu/Games/MedMyst

This website has online games and class activities for middle school students in which students investigate infectious disease outbreaks. It was developed by the Rice University Center for Technology in Teaching and Learning.

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Council on Foreign Relations' Interactive Map of Vaccine Preventable Disease Outbreaks

http://www.cfr.org/interactives/GH_Vaccine_Map

This map plots outbreaks of vaccine-preventable diseases globally since 2008. Students can zoom in on areas of interest and click on a point to learn more about each outbreak.

Why Vaccines?

Learning Outcomes
- Students will investigate parts of the immune system.
- Students will explore how vaccines can program the immune system to fight specific disease.

Key Vocabulary
- Vaccine
- Immune system
- Macrophages
- Antigen
- Lymph nodes
- Lymphocytes
- T cells
- B cells
- Antibodies
- Memory cells

Time Required
- Approximately 10 minutes of prep time
- Approximately 60 minutes of class time for activity and discussion

Materials
- Game card (1 copy for each group of students)
- Game instructions (1 copy for each group of students)
- Game board (1 copy for each group of students)
- Computer

Background Information
The human immune system is able to recognize and distinguish between protein molecules that belong to its owner's body (self) and those that come from outside attackers (foreign). The foreign proteins are called antigens. Several different types of white blood cells work together to eliminate these antigens.

Why Vaccines? (continued)

Macrophages surround and engulf the invaders. Then the macrophages break the invaders down and display the invaders' surface proteins. This sets off a chain of immune system reactions, activating some cells that attack infected cells (T cells) and others that make antibodies (B cells). Antibodies are proteins shaped to attach to the antigens. The antibodies bond with the antigens; this marks them for destruction by macrophages and T cells. Once the immune system has learned to make antibodies against a particular disease, it makes both T and B memory cells. These memory cells remain in the body for many years, ready to launch an attack if that specific disease invades again. Vaccinations work by triggering this response so the body creates memory cells ready to recognize and destroy a particular pathogen quickly.

Teaching Notes

This activity allows the students to simulate the process of fighting infections. First, you will tell a story to get the students thinking about the challenges the immune system faces. Then you will spend a few minutes having students role-play key components of the immune response. The students will repeat the process by playing a game and seeing how much more quickly and efficiently the body is able to react once the body has been vaccinated.

Before class begins, create the game packets. In a ziplock bag, place instructions for the game and cards. You may want to laminate the cards, instructions and game board. Print extra cards in case some are lost. To expand the game, you may want to create a set of medical histories, which would be given to each player. The medical histories may state that the player is allergic to the vaccine ingredients in a specific vaccine. Another history may state that the player has a compromised immune system, with death occurring if exposed to a given disease.

Procedure

Create a story to introduce students to the idea of invaders in a secure land. For example: "You are the emperor ruling over a kingdom. People enter and leave the kingdom, visiting friends and family and participating in commerce. Suddenly, you notice several people have been killed near the main gate — but it isn't obvious who killed them or how they were killed. An invader apparently has entered the fortress. What do you do?" Allow the students to think for one minute. Next, provide two minutes for the students to discuss with a partner how they would handle the situation. Finally, allow the students to share their ideas with the whole class.

Why Vaccines? (continued)

Explain to the students that these types of questions — how to identify the invader, how they would attack the invader, how to prevent an attack from similar invaders — are the same questions the body must consider when encountering a germ or microbe that causes an infection.

Below is suggested wording for an explanation to give as the students act out the immune system's response. You should adjust the story to meet the needs of your classroom. You may be the narrator or assign a student to that role while you give directions. Give the role-playing students signs with the roles and props as suggested in the chart on the next page.

Number of Students	Role	Prop
1	Microbe	Paper microbe antigens taped to the role sign
1	Macrophage	Plastic forks and spoons
1	Lymph Nodes	Immune system headquarters sign
1	T Cells	Cell phone
1	B Cells	Paper antibody guns

Activity Script
Explain that the room is the body. Have the Microbe wait outside the classroom. Tell the Macrophage to walk quietly around the class. Place the Lymph Nodes at the head of the class sitting behind a desk. Have the B Cell and T Cell hide behind the desk. Instruct each character to announce "I am…" as he or she enters the scene.

Narrator: Today we are in a healthy body and all the cells are happily working together until a Microbe enters the body.

Microbe student enters the room.

Narrator: Notice the surface antigens on the Microbe. Each microbe has its own unique antigens. The body recognizes any foreign antigens as enemy invaders. At first the body does not notice the Microbe.

Why Vaccines? (continued)

Macrophage walks around the room freely.

Narrator: The job of the Macrophage is to move around the body looking for antigens that do not belong. The Macrophage notices the Microbe's antigen and begins to attack ... or digest the Microbe.

The Macrophage uses the plastic fork/spoon to pretend to eat the Microbe. The Macrophage removes the antigens from the Microbe. The Microbe sits down and then quietly moves back to the classroom door.

Narrator: The Macrophage takes the Microbe's antigens to the Lymph Nodes. The Lymph Nodes are the headquarters of the immune system. B Cells are produced in the bone marrow and T Cells mature in the thymus.

The B Cell and T Cell stand up like superheroes. B Cells walk around the class with the paper antibody guns, looking for Microbes.

Narrator: B cells make antibodies. Antibodies fight specific Microbes by bonding with the exactly matching antigens. This marks the Microbe for destruction by T Cells and Macrophages. If the body never has encountered a particular type of Microbe before, the B Cells will not have antibodies ready to attack.

T Cells walk around the room looking as if they are talking on the cell phone and are ready to "bounce" someone out of the class.

Narrator: T Cells are the next soldiers in the war against infections. Just as different soldiers have different jobs, so do different T Cells. Some attack the invaders, others help B Cells mature, others secrete important chemicals and still others regulate the immune response and prevent autoimmune disorders. Some T Cells communicate with other items. If a body has not been exposed to a particular type of Microbe before, it will not know how to fight that type of Microbe. However, once the B Cells and T Cells have been programmed to attack the virus, Memory B Cells and Memory T Cells will exist in the body, ready mount a quick response if the same type of Microbe appears again. A vaccine introduces a small amount of weakened or dead Microbe into the body. In response, the body will make antibodies and create Memory Cells to fight. If the body later is exposed to a large, strong Microbe population, the body will immediately begin destroying the Microbes with the Memory Cells.

Why Vaccines? (continued)

Activity Game
Now that students have seen the immune system's response to a germ and heard how vaccines work, they will play a game involving the cards at the end of this lesson plan.

Put students in groups of 4 to 6. Give each player 3 B cell cards, 3 T cell cards and 6 immune point cards.

Each turn, a player may choose to whether to buy a vaccine. Vaccines cost 1 immune point each. The player must decide to buy Vaccine 1, 2, 3 or 4. After buying the vaccine, the player may change the 1 B cell and 1 T cell into memory cells for the purchased vaccine. Then the player must draw a germ card. If his or her immune system has a quick response because the correct vaccine was given, the player will be given 3 immune points. If the player does not have the correct memory cells to match the germ, he or she must pay 2 immune points.

This process of drawing germs will continue until all germs have been exposed or someone runs out of immune points. The player with the most immune points wins.

After playing one or two rounds of the game, ask the students to identify the two discoveries or conclusions they can make from playing the game. To focus the discussion, ask the students, "What happened when a person got a vaccine? Did the vaccine always match the germ they got? What happens if you did not buy a vaccine? What would happen if you were allergic to the ingredients in Vaccine 1?"

Discussion Questions

- What immediately fights microbes that enter the body?
- How can the body's immune system be strengthened to fight infections?
- How does a vaccine help a body fight infections?
- Why are memory cells important?

Assessment

Students work independently to create a flowchart showing how vaccines "teach" the immune system to attack to a germ using the following terms: vaccine, macrophages, antigen, lymph nodes, T cells, B cells, antibodies, memory cells.

Why Vaccines? (continued)

Extension

Students may review the immune system's response to infections and vaccines by visiting *How Vaccines Work*, at http://www.historyofvaccines.org/content/how-vaccines-work. Students also may research how specific germs affect the human body.

Other Resources

How a Vaccine Works
http://www.youtube.com/watch?v=7MaiT5w5NWQ

How Vaccines Work
http://www.youtube.com/watch?v=k3CWxekGt7Y

Why Vaccines? (continued)

Game Cards (for 1 group of 4 students)

B Cell	**B Cell**	**B Cell**
B Cell	**B Cell**	**B Cell**
B Cell	**B Cell**	**B Cell**
B Cell	**B Cell**	**B Cell**

Why Vaccines? (continued)

T Cell	T Cell	T Cell
T Cell	T Cell	T Cell
T Cell	T Cell	T Cell
T Cell	T Cell	T Cell

Why Vaccines? (continued)

1 Immune Point	**1 Immune Point**	**1 Immune Point**
1 Immune Point	**1 Immune Point**	**1 Immune Point**
1 Immune Point	**1 Immune Point**	**1 Immune Point**
1 Immune Point	**1 Immune Point**	**1 Immune Point**

Why Vaccines? (continued)

Vaccine 1	**Vaccine 1**	**Vaccine 1**
Vaccine 2	**Vaccine 2**	**Vaccine 2**
Vaccine 3	**Vaccine 3**	**Vaccine 3**
Vaccine 4	**Vaccine 4**	**Vaccine 4**

Why Vaccines? (continued)

Memory B Cell Germ 1	**Memory T Cell Germ 1**	**Memory B Cell Germ 2**
Memory B Cell Germ 1	**Memory T Cell Germ 1**	**Memory B Cell Germ 2**
Memory B Cell Germ 1	**Memory T Cell Germ 1**	**Memory B Cell Germ 2**
Memory B Cell Germ 1	**Memory T Cell Germ 1**	**Memory B Cell Germ 2**

Why Vaccines? (continued)

Memory T Cell Germ 2	**Memory B Cell Germ 3**	**Memory T Cell Germ 3**
Memory T Cell Germ 2	**Memory B Cell Germ 3**	**Memory T Cell Germ 3**
Memory T Cell Germ 2	**Memory B Cell Germ 3**	**Memory T Cell Germ 3**
Memory T Cell Germ 2	**Memory B Cell Germ 3**	**Memory T Cell Germ 3**

Why Vaccines? (continued)

Memory B Cell Germ 4	**Memory T Cell Germ 4**	**Germ 1**
Memory B Cell Germ 4	**Memory T Cell Germ 4**	**Germ 1**
Memory B Cell Germ 4	**Memory T Cell Germ 4**	**Germ 1**
Memory B Cell Germ 4	**Memory T Cell Germ 4**	**Germ 1**

Why Vaccines? (continued)

Germ 2	**Germ 3**	**Germ 4**
Germ 2	**Germ 3**	**Germ 4**
Germ 2	**Germ 3**	**Germ 4**
Germ 2	**Germ 3**	**Germ 4**

Why Vaccines? (continued)

Game Board

B Cell Discard	**T Cell** Discard
Germ Discard	**Vaccine** Discard

Why Vaccines? (continued)

Gameplay Instructions

How to Play the *Why Vaccines?* Board Game

Before the Game:

- Give each player 3 B Cell cards, 3 T Cell cards and 6 Immune Point cards.
- Vaccine cards should be sorted and placed right-side up.
- Germ cards should be shuffled and placed upside down.
- Immune cards should be stacked right-side up.
- Sort and stack each of the other types of cards face-up.

On a Player's Turn:

- The player may choose to whether to buy a Vaccine. Vaccines cost 1 immune point each.
- If the player decides to buy a Vaccine, he or she then must decide whether to buy Vaccine 1, 2, 3 or 4. After buying the Vaccine, the player changes the 1 B Cell and 1 T Cell into 1 Memory B Cell and 1 Memory T Cell for the purchased Vaccine.
- Next, the player draws a Germ card. If his or her immune system has a quick response because the correct Vaccine was given, he or she will be given 3 Immune Points. If the player does not have the correct Memory Cells to match the Germ, he or she must pay 2 Immune Points.

How the Game Ends:

- Players take turns until all Germs have been exposed or until a player runs out of Immune Points.
- Once the game ends, the player with the most Immune Points is the winner.

Population Changes

Learning Outcomes
- Students will compare and contrast the spread of infectious diseases in vaccinated and unvaccinated populations.

Key Vocabulary
- Infectious disease
- Epidemic
- Pandemic

Time Required
- Approximately 10 minutes of teacher prep time
- Approximately 30 minutes of class time for epidemic solution activity
- Approximately 10 minutes of class time for computer simulation and discussion

Materials (per student)
- Small paper cup or test tube
- Small pipette
- NaOH solution (such as 0.1M)
- Phenolphthalein or red cabbage indicator
- Distilled water
- Computer model

Background Information
Throughout history, infectious diseases have crippled towns and even countries. Thousands of people have died from infectious diseases. The first time a person is exposed to a disease, his or her body learns to make antibodies specific to that disease. In subsequent exposures, the body is able to quickly recognize the microbe and fight it with the proper antibodies and memory cells. Vaccination primes the body to prepare the specific antibodies and memory cells by exposure to weakened or killed forms of the disease organism, so the body is prepared without getting sick. If a large enough portion of the population has been vaccinated, a degree of protection exists for people who can't be vaccinated for some reason (e.g. too young or immunocompromised.) This is called herd immunity.

Population Changes (continued)

Teaching Notes

This initial activity allows students to simulate how infectious diseases are spread in populations with varying levels of protection from vaccination. The NaOH solution is made by placing 1g of NaOH in a container and adding distilled water to make 250mL of solution. If you have 30 students, fill 29 cups (or test tubes) with distilled water. One cup should be filled with NaOH solution. This is the index case. Number all the cups, including the one with NaOH solution, randomly, making sure to keep track of which cup has the NaOH solution. The person with this cup will be the initially infected person.

The computer model requires a computer that is connected to the Internet and is able to run Adobe Flash. The computer simulation may be completed as a whole-class discussion or a small group activity.

Safety

Make sure students do not ingest the liquid in the cups. If the water is spilled, rinse the area and dry the surface carefully.

Procedure

Use a primary resource on the influenza epidemic of 1918, such as *A Letter from Camp Devins, MA*, from *American Experience* by PBS, in which a doctor shares what is happening at a military base during the influenza epidemic of 1918. This resource can be found at http://www.pbs.org/wgbh/americanexperience/features/primary-resources/influenza-letter.

Other resources:

- *The Influenza Epidemic and How We Tried to Control It*
  http://library.buffalo.edu/libraries/units/hsl/resources/guides/flu45.pdf
- *Influenza Vignettes*
  http://library.buffalo.edu/libraries/units/hsl/resources/guides/flu129.pdf
- *A Retrospect of the Influenza Epidemic*
  http://library.buffalo.edu/libraries/units/hsl/resources/guides/flu949.pdf
- Video interviews
  http://www.pbs.org/wgbh/americanexperience/features/primary-resourcesinfluenza-letter

Population Changes (continued)

Ask students questions to initiate their thinking about epidemics, such as:
- How would you feel if someone in your family were affected by this infectious disease?
- What would you do?
- What would you want to learn before coming into contact with the disease?
- What would you want to learn after coming into contact with the disease?

Students will now learn what it is like to be a part of a population, Class City, in which an infectious disease is introduced. Provide each student with a cup (or test tube) of liquid and a disposable pipette. Explain that they now may exchange fluid over the next 5 minutes with at least 3 different people in the class. (Encourage the students to use the pipette to mix the fluid after each exchange.) Students should keep track of their exchanges in the *Population Changes* handout.

Discussion questions for the students after the swap:
- Who came into contact with the most people?
- Who came into contact with the fewest number of people?
- Who would you expect to come down with the disease?

To discover who is infected, students should visit the Clinic. (The Clinic will be an area of the classroom in which the phenolphthalein is located.) At the Clinic, the teacher adds 1 drop of test solution (phenolphthalein) to each cup. If the student's solution turns pink, the student is infected. If the student's solution is clear, he or she is not infected. Have each student indicate on the whiteboard whether he or she is infected.

Break the students into small groups. Each group will serve as a CDC Epidemiology Team. The task is to analyze the patterns of transmission and determine the index case (the first infected person). The groups may create a list of question to ask other groups to clarify their data.

Allow each group to share its analysis with the whole class. Tell the students which number cup was the index case. Ask the students to name some ways we can prevent the spread of the disease. Ask the students what would have happened if half of the class had been vaccinated for this disease. Help the students see that when a large proportion of people are vaccinated against a disease, the disease will not spread as rapidly through the population because the number of suitable hosts is very low.

The *Disease Lab* from the Habitable Planet and the Annenburg Foundation provides an online simulation to provide a visual example of the difference in infectious diseases in unvaccinated and vaccinated populations. The simulation,

Population Changes (continued)

available at http://www.learner.org/courses/envsci/interactives/disease/disease.html, also will allow you to change how the population interacts with each other and how virulent the disease is. You can work through as a class or assign students to try various scenarios in small groups. Please note this simulation uses fictional diseases, such as impfluenza, which sound like common diseases, such as influenza.

Scenario 1
Open the simulator and choose impfluenza as the disease. Choose a population density of "high" and a population mixing of "high."

Ask students to predict how the contagious group will change if the population mixing changes to "medium" and "low." Run a simulation with population mixing of "medium." Run a third simulation with a population mixing of "low."

Scenario 2
Open the simulator and choose impfluenza as the disease. Choose a population density of "low" and a population mixing of "medium."

Ask students to predict how the at-risk group will change if the population density changes to "medium" and "high." Run a simulation with population density of "medium." Run a third simulation with a population density of "high."

Scenario 3
Open the simulator and choose impfluenza as the disease. Choose a population density of "high" and a population mixing of "medium." Choose "Vaccine" and a vaccinated percentage of none.

Ask students to predict how the at-risk group will change if the vaccinated percentage changes increase to 10%, 25% and finally 50%. Run a simulation at each vaccination percentage.

Assessment

Students should complete the *Population Changes* handout. Students should work together to discover the initial cause of the infectious disease. Students also may write a letter to a friend in another class explaining what it is like to be part of a population in which an infectious disease in introduced.

Population Changes (continued)

Extension

The activity may be extended by learning how medical advances have assisted in the prevention of disease spread through the online simulation, *Illsville*, from the History of Vaccines website or how the body reacts to disease in the game *The Immune System*, from the Nobel Prize website. Students also may read and discuss *Vaccines for Pandemic Threats*. Both can be found at http://www.historyofvaccines.org/content/articles/vaccines-pandemic-threats.

Population Changes

A mysterious disease has entered the population of Class City. Today you will be interacting with other classmates. Record the classmates with whom you have contact (swap liquid) throughout the activity in the table below.

Contact	Student Name/ Cup Number	Infected? (Yes or No)	Analysis of Contact
1			
2			
3			
4			
5			
6			
7			
8			
9			

Visit the clinic and record if you are infected on the whiteboard. Use the class data to record if the classmates with whom you were in contact are infected.

Your small group will serve as the CDC Epidemiology Team. You must analyze the contact to determine who you believe was the index case (the initially infected individual). If your group needs to ask another group a question, one member may travel to other groups to ask a question. Each small group may ask other groups a total of three questions.

Population Changes (continued)

Observations

Explain who your group has concluded to be the index case (initially infected individual), and why.

Disease Lab **Scenario 1**	
Population Density	**Population Mixing**
Observations	
Population Density	**Population Mixing**
Observations	
Population Density	**Population Mixing**
Observations	

Population Changes (continued)

Disease Lab **Scenario 2**	
Population Density	**Population Mixing**
Observations	
Population Density	**Population Mixing**
Observations	
Population Density	**Population Mixing**
Observations	

Population Changes (continued)

Disease Lab **Scenario 3**		
Population Density	**Population Mixing**	**Vaccine**
Observations		
Population Density	**Population Mixing**	**Vaccine**
Observations		
Population Density	**Population Mixing**	**Vaccine**
Observations		

Population Changes (continued)

Discussion Questions

1. What is an infectious disease?

2. What is an epidemic?

3. How can vaccinations prevent the spread of infectious diseases?

Culturing Cells

Procedure adapted from Mapping Your Future: Careers in Biomanufacturing *by the North Carolina Association for Biomedical Research.*

Learning Outcomes

- Students will determine the advantages of cell culture-based vaccine manufacturing over traditional, egg-based vaccine manufacturing.
- Students will learn the engineering challenges in supporting a culture of cell.

Key Vocabulary

- Vaccine
- Cell
- Culture

Time Required

- Approximately 10 minutes of prep time
- Approximately 90 minutes of class time for the activity

Materials

Per Class:

- Measuring spoons (teaspoon and tablespoon)
- Measuring cup (1 cup)
- Microwave/hot plate
- Thermometer
- Timer
- 1 jar of baker's yeast
- 1 bag of sugar
- Water
- Tape

Per Group:

- Clear bottle
- 1 yard of string
- Ruler

Culturing Cells (continued)

Background Information

Several hundred years ago, infectious diseases including plague and smallpox ran rampant and often killed substantial portions of the population. But in the early 1700s, the idea of inoculation (the deliberate introduction of disease organisms) to protect against smallpox emerged in Western Europe. Smallpox inoculation, which had been practiced in India, China and the Middle East, was tried in England and North America. People were infected deliberately with dried pus from a sick person placed on a scratch in their skin. This provided protection to the survivors — but it often led to dangerous cases of smallpox. However, Edward Jenner, the father of immunology, researched the effect of inoculation with cowpox, a milder form of the disease. This provided protection without the danger. Since then, much safer ways of exposing people to disease antigens have been developed. These vaccines now are available for many different diseases. The methods for producing vaccines also have changed as science has progressed.

Until now, most influenza vaccines have been produced using eggs, but recent advances are providing for vaccine production using mammalian cell culture. This process will allow faster and more efficient production of vaccines.

Teaching Notes

In this activity, students will be encouraging fermentation by the yeast cells in a bioreactor. The successful fermentation will be determined by the amount of carbon dioxide produced. Yeast may be placed in an empty water bottle with warm water and sugar. Then, place a balloon over the bottle and secure it with tape. The carbon dioxide will cause the balloon to expand. The volume of the balloon can be measured to determine the amount of carbon dioxide produced. Teacher may choose to create a more engineered project by allowing groups to determine how much sugar is added to the bioreactor.

Before doing this experiment, use a thermometer to ensure the water is warmed to between 100° and 110° F. If the water is warmer, the yeast may be killed. If the water is colder, the yeast may not react. Provide students with measuring spoons or balances to measure the yeast and sugar. Provide tape to secure the balloons to the water bottles.

Safety

Make sure students do not ingest the material. The balloon can pop or fly off the bottle if it is not properly secured.

Culturing Cells (continued)

Procedure

Ask your students to list what they would need to create a production facility for tomato plants. Point out that they would need to provide optimal conditions for the plants to grow. Vaccines also are grown in production facilities — typically eggs. It takes approximately four to five months to produce an influenza vaccine. In the past 10 years, the process of producing vaccines in mammalian cell cultures was developed.

Divide the students into groups of four to complete an annotated reading activity. Provide each student with a copy of *Cell Culture-Based Vaccine Production: Technological Options,* by Rino Rappuoli. It is available at http://www.nae.edu/File.aspx?id=7399 and is from *The Bridge*, an engineering and society journal published by the National Academy of Engineering.

Students should read silently for 5 minutes, circling unknown words, highlighting definitions of terms and starring important ideas. After 5 minutes of silent reading, allow the students 10 minutes to share in their groups what they marked. Finally, allow each group to share a key point with the entire class.

Students will mimic this process by producing products with yeast cells in a simple bioreactor. Strictly speaking, a bioreactor is a chamber where product formation occurs. The large-scale bioreactor is the heart of the vaccine production process, and it is a sophisticated piece of equipment that creates a highly controlled and sterile environment for the cells to grow and produce the carbon dioxide. The largest bioreactors can be several stories tall, but the cells that grow in those large reactors begin production in small containers like the bioreactor that will be used today.

Divide the students into groups and allow each group to follow the instructions from the *Culturing Cells* handout. Make sure the students are following the instructions and using proper safety techniques.

Assessment

Students should complete the *Culturing Cells* handout. Students should work together to complete the activity and form a clear conclusion.

Extension

After reviewing the group's revised procedure, allow students to test their idea for increasing the production of carbon dioxide. In addition, the activity may

Culturing Cells (continued)

be extended by viewing *Cell Culture* on YouTube, at http://www.youtube.com/watch?v=0iQqOuINeZI. Students then may discuss how this process compares to and contrasts from the process in this activity.

The students also may repeat the experiment with minor modifications to test for the presence of carbon dioxide. In this experiment, yeast, sugar and warm water is placed in a flask, with tubing placed in an inverted graduated cylinder. The graduated cylinder then is placed in a water-filled beaker.

The carbon dioxide will replace the water and begin to fill the graduated cylinder. You can prove to the students that the gas produced is carbon dioxide by placing a burning splint in the gas container. The flaming splint will be extinguished in the presence of carbon dioxide.

Culturing Cells

Materials

- 1 teaspoon of active, dry yeast (not rapid-rise)
- 1 cup of warm water (between 100° F and 110° F)
- 1 tablespoon of sugar (sucrose)
- 1 round rubber balloon
- 1 small (0.5L to 1L) clear, empty bottle, such as a water bottle
- String
- Ruler
- Tape

Instructions

1. Collect materials and supplies from the instructor.
2. Stretch out the balloon repeatedly so it will be easy to blow up later.
3. Pour the yeast and sugar into the clear water bottle. Then add the warm water.
4. Gently rotate the bottle to mix the materials. Avoid coating the sides of the bottle with yeast.
5. Attach the balloon to the mouth of the bottle. Add tape to secure the balloon to the bottle.
6. Write the time the bioreactor begins. Within a few minutes, you will see bubbling. The balloon then will begin to inflate.
7. Take measurements of the balloon's circumference with the string every 3 minutes. Record the time and the circumference.

Time	Circumference	Observations

Culturing Cells (continued)

Discussion Questions

1. What demonstrates that the bioreactor is producing carbon dioxide?

2. What would you consider changing to increase production of the carbon dioxide?

3. Design an experiment to test your idea for increasing production of carbon dioxide. Make sure you explain how the increased production will be determined.

Plant-Based Vaccines: Coming to a Disease Near You

Learning Outcomes
- Students will explore how plants are used in the production of vaccines.
- Students will identify a disease that scientist are trying to treat through plant-based vaccines.
- Students will explore the current research on how plant-based vaccines are used to treat a specific disease.

Key Vocabulary
- Plant-based vaccines

Time Required
- Approximately 15 minutes of prep time
- Approximately 60 minutes for research of plant-based vaccines
- Approximately 30 minutes for presentations

Materials (per group)
- Examples from the Internet of research involving plant-based vaccines

Background Information
Vaccines are used to prevent — and, in some cases, eradicate — diseases. How quickly and efficiently vaccines can be produced is very important when an epidemic occurs. Plants have been found to be a great media for producing the antigens for disease. Once the antigen protein for a specific disease has been isolated, the protein gene is transferred to bacteria. The plant then is inoculated with the bacteria and begins to produce the protein needed for the vaccinations. This process takes weeks instead of the months needed for traditional vaccine production.

Plant-Based Vaccines: Coming to a Disease Near You (continued)

Teaching Notes

Consider showing a news clip or reading a news story about plant-based vaccines to explain the importance of this research. With your class, create a list of plants that are being used in current vaccine research. See the Resources section at the end of this lesson plan for resources that your students can use to create the list of plants used in vaccine research. You may choose to create a webpage with bookmarks to sources for plant-based vaccines to ensure your students use reputable sources.

Safety

No safety concerns.

Procedure

Display a map of current disease outbreaks for which a vaccine is available. Use the *Global Health Observatory Map Gallery* (http://gamapserver.who.int/mapLibrary) from the World Health Organization to display maps from around the world. The search engine allows the user to pinpoint outbreaks in specific areas of the world and look at specific diseases. If this lesson is taught in influenza season, consider using the weekly influenza summary map of the United States by the Centers for Disease Control and Prevention (http://www.cdc.gov/flu/weekly/usmap.htm).

Ask the students to discuss with a partner possibilities for why the outbreak is located where it is and what the barriers are for getting the vaccine to the area in need. After this discussion, the student teams should record their ideas and share them with the entire class.

The following is a short list of topics that student teams should discuss. Many others topics may be included as well.

- Location
- Characteristics of the people affected (e.g. poverty, beliefs, travel)
- Vaccine factors
  - » Difficulty in production
  - » Cost of production

Plant-Based Vaccines: Coming to a Disease Near You (continued)

- » Storage requirements
- » Delivery of vaccine
- » Timeline for production
- » Match with disease

Explain to students that some of these barriers may be overcome by using plant-based vaccines. To provide information about why plant-based vaccines are important, assign students to read articles, such as:

- *Darpa's Flu Fighters Ramp Up Veggie-Based Vaccines*
  http://www.wired.com/dangerroom/2012/07/vaccines

- *Plant-Based Vaccine Manufacturing*
  http://www.historyofvaccines.org/content/blog/plant-based-vaccine-manufacturing

- *Transgenic Plant Based Edible Vaccines: A Technology Landscape*
  http://www.geiper.com/Reports/Transgenicplant.pdf

Students should record the information they are gathering from these sources in the student data sheet in the *Plant-Based Vaccines: Coming to a Disease Near You* handout. Provide an opportunity for students to share their findings with the class.

Students now will compete to show which plant is the best vaccine base. Divide the class into groups and allow each group to choose a plant to research. Each group should determine which disease(s) are being studied in its chosen plant and why its chosen plant is a good media for growth. Monitor student research to make sure reputable sources are being used. Remind students to document the resources used to find the information.

Next, groups will create an advertisement for the best plant vaccine base. The advertisement may be digital or paper/pencil. The advertisement must identify the plant and show vaccines for which diseases are being researched in conjunction with this plant. The advertisement also should show the benefits of using the specific plant.

Once the students complete the advertisement, provide an opportunity for each group to share the plant with the class and why it think the plant is the best vaccine base.

Plant-Based Vaccines: Coming to a Disease Near You (continued)

Assessment

Students may complete the *Plant-Based Vaccines: Coming to a Disease Near You* handout. Students may present their advertisements to the class.

Extension

Students may research the FDA process and timeline for new vaccines to move from research to public distribution.

Other Resources

BioBytes: Biotechnology and Plant Made Pharmaceuticals
http://www.youtube.com/watch?v=iiuAC0U9VxY

Bioreactor engineering for recombinant protein production in plant cell suspension cultures
http://download.bioon.com.cn/upload/month_0912/20091206_b515bf297f4d29c30cffuH7qrX6KIjMF.attach.pdf

Could Lettuce Cure Diabetes?
http://www.youtube.com/watch?v=GffVIeXC6wQ

Creating plant-based vaccines
http://www.youtube.com/watch?v=2OI3uXeVz9E

Curing Diabetes with Lettuce
http://www.youtube.com/watch?v=me5bJ3Y3uPQ

Medicago English Video
http://www.youtube.com/watch?v=JTeCtiFiDEs

NC RISING | Medicago | UNC-TV
http://www.youtube.com/watch?v=rMCaKTjex3Y

Plant-Based Vaccines: Coming to a Disease Near You (continued)

Plant-made vaccines in support of the Millennium Development Goals
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3075396

Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives
http://www.ufv.br/dbv/pgfvg/BVE684/htms/pdfs_revisao/trangenicos_transformacao/Vaccine%20antigen%20production%20in%20transgenic%20plants.pdf

Plant-Based Vaccines: Coming to a Disease Near You

Plant-based vaccines are revolutionizing the production of vaccines. This activity will provide the opportunity to study plant-based vaccines and the plants that serve as hosts.

Procedure and Observations

1. Research why plants are a good base for vaccines. Use the sources provided by the teacher. Record your reasons below.

2. Now that you know a little about plant-based vaccines, choose a plant to research:

   _____

   Before continuing, have your teacher approve your plant: _____

Plant-Based Vaccines: Coming to a Disease Near You (continued)

3. Now it is time to research why the plant is the best base for vaccination production.

   You have a list of questions to answer. As you research, you also should add a couple questions of your own to the list. Keep track of your notes and sources in the chart below. (A list of suggested sources will be provided.)

	Notes	**Website/Last Update**
What disease is being researched in connection with the plant you have selected?	1.	
	2.	
	3.	
	4.	
What are the advantages of using this plant as a vaccine base?	1.	
	2.	
	3.	
	4.	

Plant-Based Vaccines: Coming to a Disease Near You (continued)

4. After you have completed the research, create an advertisement for the plant, illustrating why it is the best vaccine base. The advertisement may be hand-drawn or digitally-created. The advertisement must identify the plant and must show which diseases are being researched with this plant. The advertisement also should show the benefits of using this specific plant. At the end of this process, you will have the opportunity to share your advertisement with the class.