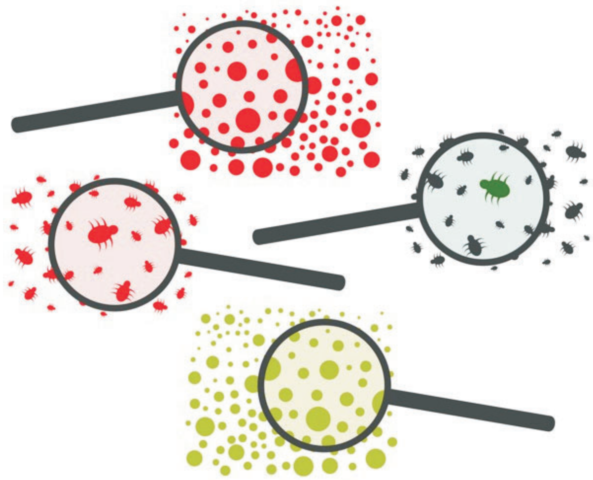
Detecting Microbes

IN 2006, a seventh grader did a science project, testing for *Escherichia coli* bacteria in fast-food restaurant ice and toilets. The ice had more bacteria, and her results made the national news. Hopefully, improved procedures have cleaned up the ice dispensers. It was not difficult for her to detect the bacteria, and most of us are glad she did. What else should be tested and how?



Objective:

Describe how and why microbes are detected in the water, food, and the environment.

Key Terms:

antibody
antigens
ATP
culture
ELISA
fluoresce
Limulus
lyse
media
microbes
pasteurization
wastewater

Understanding Microbe Detection

It is essential to find new methods of detecting dangerous and potentially fatal bacteria and other hazards in our environment.

TESTING FOR MICROBES

Microbes (microscopic living things that you often call "germs") are all around you. They can be collected from your water, your food, and virtually any surface around you. Most of them are harmless, but some can cause diseases if they enter the human body.

Why Test Water

Having a safe water supply is one of the greatest contributors to public health. Prior to processing water before drinking, there were outbreaks of serious diseases (e.g., cholera, typhoid fever, dysentery, and polio) across the United States.

Drinking Water

FIGURE 1. Public water supplies are tested for contamination before reaching households.

Public water supplies are cleaned prior to consumption. In addition, the water you use and throw away, **wastewater**, is cleaned before it is released into the environment. When the water comes from a surface source (e.g., a lake or a reservoir), it is more susceptible to contamination. As a result, it must go through a filtering process and be tested for harmful microbes. Well water for public supplies comes from deep wells and is usually free from microbes. However, it is still routinely tested. Private wells are shallower and more susceptible to contamination. They must be tested when installed and periodically after that—especially after floods.

Industrial Processes

Industrial processes use a great deal of water, which must be cleaned and tested to prevent interferences in the process. Removing harmful microbes is also necessary to protect the health of the workers who come in contact with the water.

Recreational Uses

Many public beaches are tested in the summer to ensure that the water is safe for swimming. Public pools rely on water treatment to keep them clean, but tests are still conducted as a precautionary measure.

Wastewater

Wastewater contains many potentially harmful substances that must be broken down. This breakdown is primarily accomplished by the action of some beneficial microbes. In wastewater treatment plants, the process is monitored by testing to be sure enough good microbes are present to do the job. Before it can be released, the wastewater is tested to be sure the process has destroyed the harmful microbes that might be present.

ON THE JOB…

CAREER CONNECTION: Water Treatment Operator

Do you want to protect the environment and public safety? Do you want a job where you work indoors and outdoors? Do you have good mechanical abilities and problem-solving skills? You are an excellent candidate for the position of water treatment operator.

The Bureau of Labor Statistics estimates that job prospects for water treatment operators are above average for the near future. Operators can be hired with a high school diploma and receive on-the-job training. Having a one-year certification or an associate degree will improve job prospects. Continued education to higher certifications improves earning potential.

Most operators work for local government units or private water companies. In small systems, one operator may run water treatment and wastewater treatment. In larger systems, there may be many levels of specialization. In large municipal systems, the supervisors may need a degree in engineering or a related science.

Operator earnings depend on the size of the system and the state in which it is located. Yearly earnings range from $22,000 to $55,000 with a median of $35,000.

More information can be found at:

Environmental Protection Agency—Office of Water
http://www.epa.gov/ow/

Water Environmental Federation—Professional Water Quality Workers
http://www.wef.org

U.S. Department of Labor—Bureau of Labor Statistics
http://www.bls.gov/oco/ocos229.htm

Why Test Food

Microbes in food can be beneficial or harmful. Food can be processed into desirable products such as yogurt, cheese, sauerkraut, or sausage by the actions of beneficial microbes. To control these processes the beneficial microbes must be monitored and potentially harmful ones detected and controlled.

FIGURE 2. Pasteurization has been used with dairy products for quite some time.

The Dairy Industry

The dairy industry has been using **pasteurization** (heating something hot enough to kill most harmful microbes) for many years. However, there are still microbes that have been transmitted in dairy products, so the need for constant testing in this industry is very important.

Harvesting Meat

You probably have heard more about the microbial contamination of meat. *Salmonella* in chicken and *E. coli* O157:H7 in ground beef have been in the news repeatedly. It is more difficult to sanitize every step in the meat production process than in the dairy industry.

Produce

It is also difficult to control the exposure to microbes in the fresh fruit and vegetable industry. Fresh produce is vulnerable to contamination from the soil, irrigation water, the workers, the processing, shipping, and display. *E. coli* O157:H7 has recently contaminated strawberries, spinach, and bean sprouts.

Why Test in Medicine

Since pharmaceuticals are taken when you are already sick, it is important that they do not carry any harmful microbes. Pharmaceutical production facilities are scrupulously clean, and the products are tested to be sure they are safe. Blood, blood products, and organs for transplant can never be microbe free, but they must be tested to prevent the transmission of serious microbial infection. Many infections require very specific treatment, so it is important that the microbe causing an infection is identified. Since some microbes can cause deadly infections, there is the possibility someone could use them for bioterrorism. The need for detecting such an attack is obvious.

DETECTING MICROBES

The swab and culture method of microbial detection is the classic scientific way to find microbes. It replaced the "see, smell, and touch" method. (If it looks rotten, smells rotten, or feels slimy rotten, it is rotten.) You need to detect microbes before you can see, smell, or touch them. Many surfaces or liquids can be touched with a swab. The swab transfers microbes to a growth **media** (a specific mixture of water and nutrients for the growth of microorganisms). The microbes on this media form a **culture** (growth of microbes under controlled conditions).

This method is used extensively, but it has some problems. Many harmful microbes require very specific media and a general media may miss them. Others are almost impossible to grow in the laboratory. If the microbes are present in very small numbers, it is easy to miss them with the swab. A very significant problem is that the test takes time, usually several days. However, some processes cannot wait for the results. Examples include food processes, medical tests, and beach contamination.

Enzyme-Linked Immunosorbant Assay

A new technology that is used extensively is Enzyme-linked Immunosorbant Assay (ELISA). **ELISA** is a technique used to detect the presence of an antigen or antibody. **Antigens** are protein specific to a microbe. The **antibody** is a product of the immune system of an animal that is specific to the antigen to be tested. The antibody is linked to an enzyme. In an ELISA test, the test materials are placed into micro wells and are fixed or stuck to the plastic. Known antibodies are placed into the wells. Various antibodies can be placed in different wells to test for a variety of antigens or microbes. The wells are rinsed, and the antibodies stay in wells containing compatible antigens. The wells are then exposed to a signal compound. It may cause a color change or it may **fluoresce** (glow) under specific light. The intensity of the color or fluorescence can be used to determine the concentration of the antigen.

Since the ELISA test is very specific, it is used for any microbe that has had an antibody produced for it. The test can be done very quickly. A quick strep test at your doctor will take minutes rather than the old swab and culture tests that required days of waiting for results. In situations with many possible microbes, several tests can be run simultaneously. It is even possible to automate the process, thereby saving more time and personnel resources. The main limitation is that even though you can test for many microbes, you will only be testing for the antigen placed in a well. Any microbe you do not specifically test for will not show up in any way.

FIGURE 3. A micropipette with multiple tips allows for more rapid ELISA tests. (Courtesy, CDC)

Polymerase Chain Reaction

Newer technologies allow us to test for microbes by looking for specific DNA sequences. It is not as fast as ELISA. DNA tests take hours to days (unlike on TV). DNA can tell us that there are sequences recognizable as being from a bacteria or a specific group of bacteria. Sometimes we know which bacteria are present, and sometimes we know that there are hundreds of bacteria present. But we only recognize a few as ones that have been cultured and identified. When samples are small, it is possible to enhance or multiply the DNA with a process called Polymerase Chain Reaction (PCR). These technologies can also test for RNA that is specific to a particular microbe.

LAL Test

The *Limulus* amebocyte lysate (LAL test) is a way of detecting Gram-negative bacteria. **Limulus** is the genus name for the horseshoe crab, which are ocean animals that are actually more closely related to spiders. These animals are captured when they come ashore to breed, and some blood is collected before they are released. The amebocytes (blood cells) are placed in pure water, which causes them to **lyse** (break open). The lysate is then freeze-dried and used for the testing. When the lysate comes in contact with the Gram-negative microbe's cell membrane chemicals or their endotoxins, it gels. The assay is primarily used to test pharmaceuticals that are to be injected into the human body.

Luminescent ATP Test

Another test is used to detect living cells. It is not specific to any microbe, but it will indicate living cells are present where you do not want them to be. A sample is taken from your test material and treated to cause any live cells to lyse. All living cells contain adenosine triphosphate (**ATP**), which is the energy compound of life. The lysate is mixed in a small well with an enzyme (luciferase) and a protein (luciferin). When the energy of ATP is present, the luciferase stimulates a reaction in the luciferin. This causes it to luminesce (give off light). The luciferin and luciferase were originally found in fireflies and other bioluminescent organisms, but they are now obtained for testing from genetically engineered bacteria. If there were no live cells in the sample, there will be no ATP and no light.

New Technologies

New technologies are being developed, from nanotechnology that uses very small amounts to strobe lights that cause fungal toxins on corn to give off specific sound vibrations. An interesting machine in development is called the e-nose. It is a modern update on the "see, smell, and touch" method that detects *E. coli*.

Summary:

Microbes (microscopic living things you often call "germs") are all around you. Having a safe water supply is one of the greatest contributors to public health. Wastewater contains many potentially harmful substances that must be broken down before it is released into the environment. Microbes in food can be beneficial or harmful. Since pharmaceuticals are taken when you are already sick, it is important that they do not carry any harmful microbes.

The need to detect microbes in the environment is important. There are a number of microbe detection methods. The swab and culture method of microbial detection is the classic scientific way to find microbes. The ELISA test is faster and very specific. DNA tests, endotoxin LAL tests, and luminescent ATP tests are also used in specific instances.

Checking Your Knowledge:

1. Why is it important to test wastewater for microbes before it is discharged?
2. Why is it difficult to keep fresh produce free of microbes?
3. Pharmaceuticals are produced under strictly clean conditions, so why do we test for microbes?

4. What are the problems with the swab and culture method of microbe detection?
5. What are the benefits of the ELISA test?

Expanding Your Knowledge:

Contact your county or state health department and collect any available data on food-borne or water-borne disease outbreaks over the past five years. Are there seasonal or other patterns to the outbreaks? Do you have any suggestions to minimize the outbreaks?

Web Links:

Water Quality
http://www.epa.gov/epahome/learn.htm#water

Food Safety
http://www.foodsafety.gov

Water and Food-Borne Diseases
http://www.cdc.gov

Details on ELISA Test
http://en.wikipedia.org/wiki/ELISA

Agricultural Career Profiles
http://www.mycaert.com/career-profiles