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SECTION 1
DIAGNOSTIC SERVICES IN HEALTH CARE
Imagine you have been feeling fatigued for several weeks and you visit a doctor to find out what’s wrong. Because fatigue is a sign of many diseases, the doctor must figure out its cause in order to treat you properly. The cause might be anemia. It might be a low-grade infection. Maybe it’s allergies or diabetes. The doctor will ask you some questions and do a physical examination to narrow the possibilities. He or she might also order laboratory tests, such as a blood test or urinalysis.

A wide range of diagnostic services are available to help medical professionals diagnose the cause of various symptoms, choose a treatment plan, monitor the progression of diseases and determine the effectiveness of treatment. Many of these tests also are used in forensic medicine (applying medical knowledge to legal issues). In order for a diagnostic test to be accurate, correct procedures must be followed. It also is important to understand the possibility of both false negative and false positive tests. New technologies are helping clinicians diagnose conditions more quickly and helping researchers better understand the origins of disease conditions.

QUALITY PROCEDURES
Diagnostic tests may be done in a doctor’s office, hospital or clinic laboratory. Some
Pathology & Forensic Science: Diagnostic Services in Health Care

Tests require a patient specimen (sample) of blood, urine or other body tissue, while other tests involve imaging technology. Patient specimens may be sent to a private, commercial facility, often called a reference laboratory. Careful adherence to standard laboratory procedures for either type of test — from collecting the patient specimen or image to testing and reporting results — is essential to patient safety.

Hospitals and large clinics often use barcode labels to automate recordkeeping. Each specimen or image also is labeled with at least two patient identifiers, such as name and date of birth. Identifiers that aren’t necessarily specific to each patient, such as hospital room number, no longer are used. Labeling should be done in the presence of the patient to prevent misidentification of records. If you give blood or have a lab test done, you should make sure the person performing the test checks and rechecks your identifying information. This is part of a national effort to improve patient safety by accurately identifying patient specimens and images.

Lab tests are becoming more and more automated, and the diagnostic machines used for these tests only produce accurate results when the patient specimens are collected and packaged correctly, are stored in conditions that avoid sunlight and extreme temperatures, and are transported quickly. To protect workers and the public from possibly infectious samples, the samples must be packaged carefully and treated as biological hazards for transport. After testing is complete, the specimens usually must be kept for a period specified by regulations and then disposed in accordance with federal and state regulations. In addition to the biohazards presented by patient tissue, potential hazards are posed by the chemical reagents used in the tests.

Diagnostic machines also must be well maintained and calibrated correctly. Congress passed the Clinical Laboratory Improvement Amendments in 1988 to ensure the accuracy of diagnostic testing. Under this law, the federal government regulates many aspects of laboratory operation, including the training of employees and the types and quality of instruments used to analyze samples. Laboratory personnel follow specific protocols for each test. These are known as standard operating procedures, or SOPs. An SOP ensures a test is done the same way each time, which helps ensure the accuracy of the results. Laboratory procedures are documented extensively in diagnostic testing, just as they are during the manufacture of vaccines, therapeutic drugs and medical devices. Records must be kept of the calibration and maintenance of instrumentation, the reagents used and any problems that occur. To check if the tests are accurate, laboratories regularly test known samples. They also are required to participate in proficiency testing, in which an outside agency sends unannounced samples to be tested.
In this case, the outside agency knows the correct results, while the laboratory does not. Laboratories are inspected regularly by their accrediting organizations as well. During these inspections, procedures and documentation are checked.

**REFERENCE RANGE**

What is “normal?” When your doctor receives a result from a diagnostic test, he or she must interpret the results. A result that is normal for one person could indicate a problem for another. Results of many diagnostic tests vary according to the patient’s age, gender, recently eaten foods, whether he/she is exercising or resting and other factors. Therefore, the “normal” range is not the same for all patients. Results also can vary depending on the procedures followed and the equipment used, so it is important to interpret test results in the context of the lab where the test was performed. A reference range is determined for a particular population (such as pregnant women or healthy men between ages 65 and 70) by averaging the test results for a large number of people and adding/subtracting two standard deviations to/from the average. Good practice is for the laboratory to provide a reference range of “normal” results for the particular test and laboratory and for the doctor to interpret the results for the particular patient.

**BLOOD**

Blood has many useful components for monitoring health, disease and physiological state, and for establishing identity. Some common blood tests include complete blood count, blood smear, glucose tolerance, basic metabolic profile and lipid profile. Blood or other body fluids also may be tested for the presence of specific antibodies or antigens. Blood usually is collected by a doctor, nurse or specially trained phlebotomist. Depending on the type of blood tests needed, blood first must be processed in various ways. An anti-coagulant often is added to prevent clotting. Centrifugation is used to separate blood cells from the serum. Automation allows for fast, accurate testing of large numbers of samples. Advances in technology mean that smaller volumes of blood can be used.
to provide accurate results. And while a variety of laboratory machines do the different blood tests, humans still are needed to ensure the machines function properly and the results are interpreted correctly. Finally, blood always should be treated as a potential biological hazard.

**URINALYSIS**

Urinalysis refers to a group of tests done on urine to detect urinary tract infections, metabolic disorders (such as diabetes) and kidney disorders. A urinalysis includes up to three steps. First, the urine is inspected visually. It should be pale yellow and clear. Cloudy urine might indicate an infection, while dark urine might indicate blood. Second, a series of chemical tests are performed, usually using chemical test strips. These indicate the concentration of the urine, the pH of the urine and the presence of protein, glucose, ketones and several other substances that shouldn’t be in the urine. Third, if abnormal results are found, a microscopic examination of the urine is done to look for cells, microorganisms and crystals. Other urine tests also may be performed depending on the results of these tests and other conditions the patient may have. Doctors can do the basic urinalysis tests in their offices, but more advanced tests and cultures are sent out to a laboratory.

**CULTURES**

Cultures are tests in which a laboratory attempts to grow microorganisms that could be making a patient sick in order to identify the microorganism and determine what type of antibiotics will provide effective treatment. The patient specimen might be a throat swab, a stool sample, urine, blood, sputum from a cough or pus from a wound. The patient specimen is placed on a nutrient media and incubated at a specific temperature for 18 to 24 hours. A lab technician examines the plate and performs further tests to identify any bacteria or fungi growing on it. Healthy people have many normal bacteria in their digestive tracts and on their skin that do not cause harm. In fact, some are helpful and necessary for good health. Therefore, it is important to distinguish nonharmful organisms from pathogenic ones. If the organism is pathogenic, the lab also can test to see which antibiotics are effective.

**BIOPSY**

In a biopsy, a sample of tissue is removed from the body to determine the presence and progression of a disease or disorder. Some biopsies require surgery, while others use a needle to remove the sample while a doctor palpitates the lump or uses X-ray or ultrasound imaging to guide the needle to the right spot. Depending on the tissue being biopsied, the procedure is not too invasive and usually can be done on an outpatient basis. The sample then is examined under a microscope by a pathologist. Biopsies frequently are used to detect whether a suspicious lump or mole is cancerous. They also are used to detect
other conditions, such as celiac disease.

**RAPID DIAGNOSTIC TEST TECHNOLOGIES**

Rapid diagnostic tests, such as rapid strep or rapid influenza tests, depend on detecting and signaling the presence of particular molecules. These molecules vary with the test. Some tests are designed to detect a particular molecule found in the disease organism, while others detect antibodies made by the patient in response to the disease. Still others detect the level of hormones or other metabolic chemicals found in the body. The tests are built with detector molecules that bind specifically to the molecules to be detected. The bound molecules then clump or are captured on a specific line or spot. In each case this reaction creates a visual signal. Some rapid diagnostic tests are very accurate, but others can’t easily distinguish between a patient with a current acute infection and a patient with antibodies to an infection from which he or she already has recovered. Rapid diagnostic tests are especially useful when a quick diagnosis is important for screening or treatment and in places where laboratory testing is unavailable.

**IMAGING**

X-rays, ultrasound, magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT scan) and computed axial tomography (CAT scan) are examples of diagnostic imaging techniques in common use today. They are important because they allow early, noninvasive detection of many potentially fatal conditions that can be treated successfully. They also provide pregnancy monitoring and diagnosis and treatment of common problems, such as dental decay and broken bones. Specialized technicians are required to operate and maintain the machines and carry out the tests for each type of diagnostic imaging. The images themselves usually are interpreted by a doctor who specializes in radiology.

**HOME MEDICAL TEST KITS**

From HIV status to cholesterol level, many diagnostic tests are available at the pharmacy for home use. Home-use diagnostic medical tests are regulated by the U.S. Food and Drug Administration, or FDA, which has approved many more medical tests for people to use at home in recent years. Some of these tests can be processed and interpreted at home immediately. Others
are no more than home collection kits that require the consumer to send a specimen to a laboratory, which performs the actual test.

The tests work in a variety of ways. For example, people taking certain medications must monitor their blood regularly to be sure the medication level is not causing complications. People with diabetes monitor their blood glucose at home. People who take COUMADIN, a blood thinner for preventing blood clots and strokes, must monitor their blood clotting time carefully to be sure they are taking the correct amount of medication. They do this by pricking their finger, squeezing out a drop of blood and inserting it into a meter device, which measures the clotting time. Just as with machines in a laboratory, the meter must be tested and calibrated with sample solutions. Many home tests, such as pregnancy tests and cholesterol tests, use the rapid diagnostic test technologies described previously.

Home test kits offer patients privacy, allow for frequent monitoring and often reduce costs. But depending on the circumstances, the use of home test kits also can cause problems. Some tests are more difficult to administer at home or are more difficult to interpret. Sometimes fake or expired tests that could produce incorrect results are sold over the Internet. Inaccurate results can have negative consequences for public health, especially when the test is for an infectious disease. Another concern is that instead of seeing a doctor, patients may decide to treat themselves — often inadequately. On the other hand, if a doctor orders a test, he or she would discuss treatment options when providing the patient with the test results. Because of these concerns, some people have fought against approval of some kinds of home-use diagnostic tests.

**GENETIC TESTS**

Genetic testing sounds futuristic, but many genetic tests already are in use today. As scientists gain understanding of the role of genetics in disease, genetic testing is becoming increasingly important. For example, every baby born in North Carolina is tested for more than 25 genetic conditions at birth even if he or she shows no immediate symptoms of a problem. Conditions such as sickle cell disease and PKU are included in newborn screening programs because early diagnosis and treatment can make a critical difference to a patient’s long-term health. Other tests
help people make decisions about whether to have children and how to plan financially based on whether they have inherited a disorder that won’t show up until middle age (such as Huntington’s disease). Some people are interested in whether they have inherited certain disease-specific susceptibility genes. Genes also can determine a person’s response to certain medications. The study of these responses, known as pharmacogenomics, is an exciting, relatively new and rapidly growing area of research. Genetic testing can involve direct tests of patient DNA or indirect methods, such as determining the level or conformation of a protein in the blood.

**ADVANCES IN DIAGNOSTIC TESTING**

Imagine going to the doctor’s office and breathing into a small tube at check-in. When the doctor sees you a few minutes later, he or she tells you the breath sample you provided already has been screened for diabetes, five common types of cancer and several infectious diseases, and that you show no signs of any of these diseases. No costly blood tests, no need to urinate into a cup, no uncomfortable mammogram and no invasive biopsy needed! This scenario is still in the future — but it’s coming closer with recent advances in diagnostic breath analysis.

Researchers are working on noninvasive breath tests for many diseases, including lung, throat and breast cancer, multiple sclerosis and tuberculosis. Exhaled breath has more than 1,000 volatile organic compounds (VOCs). Most of these compounds are found in very low concentrations in exhaled breath — they are measured in parts per million or even billion — so instruments must be very sensitive in order to detect and quantify them. Different diseases cause different characteristic patterns of VOCs in a patient’s exhaled breath. Breath analysis devices collect VOCs in the patient’s breath, detect them with gas chromatography-mass spectrometry and analyze the patterns. Other researchers are working on similar breath tests for important chemicals used in monitoring conditions such as blood glucose in diabetics and nitrous oxides in asthma patients. This kind of testing could allow for rapid, less invasive testing for many conditions and eventually could lower costs.

**CAREERS IN DIAGNOSTIC MEDICINE**

**Pathologists**

Pathologists are medical doctors who specialize in the diagnosis and study of diseases and disease processes. Pathologists may specialize in clinical laboratory testing, anatomic pathology (examination of cells taken from a patient in a biopsy), forensic pathology or the development of new diagnostic tests and instruments. Both hospital and reference laboratories generally are directed by a pathologist or Ph.D.-level scientist with a degree in clinical laboratory medicine. A large hospital
or commercial laboratory might have different departments (such as hematology, toxicology, microbiology and surgical pathology) headed by specialists.

**Medical Laboratory Technologists & Technicians**

More than 300,000 people work as medical technologists and medical laboratory technicians in U.S. hospitals, laboratories and physician offices. This is a growing occupation due to (a) a national population that is both increasing and aging and (b) the development of new diagnostic tests. Medical technologists generally have a bachelor’s degree in science and specific training in the skills needed in a clinical laboratory setting. Technicians usually have at least an associate degree and additional, specific training in clinical laboratory sciences. Technologists perform diagnostic tests, maintain and calibrate laboratory instruments, and document all their work. Just like other medical professionals, medical technologists and technicians must follow laws and ethical guidelines to protect patients.

**Nuclear Medicine Technologists**

Nuclear medicine technologists operate advanced imaging machines (MRI, PET, CT) and use radiopharmaceuticals to help diagnose and treat diseases. Radiopharmaceuticals use small amounts of radioactive molecules that often are attached to pharmacologically active molecules. New radiopharmaceuticals are being developed that will lead to improved treatment for some diseases. Nuclear medicine technologist is a small but rapidly growing occupation. It requires an associate degree in nuclear medicine technology and the passing of certification exams.

**Phlebotomists**

Phlebotomists draw blood specimens from patients and work with clinics and laboratories to ensure patient safety by managing and documenting specimens. Phlebotomy requires only a one-semester certificate program but does not pay as well as a laboratory technician job.

**RESOURCES**

**Lab Tests Online**

Lab Tests Online is produced by the American Association for Clinical Chemistry and has clear, peer-reviewed, explanatory materials for many common laboratory tests. For an excellent article on reference ranges, visit labtestsonline.org/understanding/features/ref-ranges.

**KidsHealth**

The KidsHealth website is produced by the nonprofit Nemours Center for Children’s Health Media. It includes material for many common lab tests and educational material on a variety of topics for parents, teens, kids and educators. Audio and many articles are available in both English and Spanish. Videos are available for some topics. For lab test explanations, visit
kidshealth.org/parent/system and click on Medical Tests and Exams.

**North Carolina Community Colleges**
North Carolina Community Colleges have more than 50 different Health Care programs, including certificates and associate degrees in laboratory technology and diagnostic medicine. Each college’s website provides career information, such as typical tasks, training required and salary ranges for graduates of its programs.

For career information about medical laboratory technology, phlebotomy and radiography in Wake Tech’s Health Sciences Division, visit health.waketech.edu.

For information about cardiac and vascular sonography, computer tomography, magnetic resonance imaging, nuclear medicine technology and radiography at Johnston Community College, visit johnstoncc.edu/healthsciencesprograms.aspx.

**ExploreHEALTHCareers**
ExploreHEALTHCareers is a website with information on a wide variety of health care-related careers, including diagnostic specialties. Visit explorehealthcareers.org.

**UNC Chapel Hill**
The University of North Carolina at Chapel Hill has both bachelor’s and master’s degree programs in clinical laboratory science. For the Division of Clinical Laboratory Science, visit med.unc.edu/ahs/clinical.

For stories of a day in the life of five clinical laboratory scientists in different settings, each featuring a patient emergency, visit med.unc.edu/ahs/clinical/files/clsatwork2001.pdf.

**American Medical Technologists**
American Medical Technologists is a non-profit association for allied health professionals. For extensive career information for people interested in working in diagnostic medicine and clinical laboratories, visit americanmedtech.org.

**American College of Pathologists**
To view a sample accreditation checklist for a clinical laboratory that shows how laboratories are inspected and demonstrate quality procedures, visit www.cap.org/apps/docs/laboratory_accreditation/sample_checklist.pdf.

**Labcompare**
Labcompare is a commercial site with good explanations and pictures of the equipment used in diagnostic laboratories. Students could research equipment needed for various tests on this site. Visit labcompare.com.

**Mensanna Research**
Mensanna Research explains the history of breath analysis in medicine and its work in developing breath analysis tests for
cancers, flu and tuberculosis. Its website also has a list of downloadable research publications. Visit menssanaresearch.com.

**Nanowerk**
For an article about nanotechnology-based sensors that are used to diagnose disease using a patient’s exhaled breath sensors, visit nanowerk.com/spotlight/spotid=28303.php.

**Rapid Diagnostic Testing**
For information on how various rapid diagnostic tests work, visit rapid-diagnostics.org/technologies and rapid-diagnostics.org/tech-lateral-howitworks.

**BIO Ventures for Global Health**
For more information on how various rapid diagnostic tests work, visit bvgh.org/Biopharmaceutical-Solutions/Global-Health-Primer/Targets/cid/ViewDetails/ItemID/16.aspx.

**U.S. National Library of Medicine**
For an article that provides a clear explanation of pharmacogenomics and links to additional information, visit ghr.nlm.nih.gov/handbook/genomicresearch/pharmacogenomics.
WHAT ARE MY TEST RESULTS?

TEACHER ACTIVITY OVERVIEW

LEARNING OUTCOMES
• Students will trace the steps traveled by a lab sample from when the sample is taken until the results are returned.
• Students will create a simple set of standard operating procedures (SOPs) for other students to follow.
• Students will complete simple lab tests on simulated blood/urine.
• Students will create a report of lab results.

KEY VOCABULARY
• Quality control
• Lab technician
• Urine specimen
• Tracking system

TIME REQUIRED
• Approximately 30 minutes of teacher prep time
• Approximately 90 minutes of class time for lab and discussion
MATERIALS REQUIRED

For Class Discussion

- Pitcher
- Measuring spoon
- Measuring cup
- Sugar (4- or 5-pound bag)
- Kool-Aid (3 to 5 packets)
- Water (2 to 3 gallons)
- Cups

For 15 Lab Sets

- 45 test tubes
- 15 test tube rack
- Healthy simulated urine sample (“Sample #1”)
  - Make 5 cups of chicken broth by combining 5 cups of water and 5 cubes of chicken bouillon.
- Unknown simulated urine sample (“Sample #2”)
  - Add a few drops of chicken blood to 1 cup of chicken broth.
- Unknown simulated urine sample (“Sample #3”)
  - Add 1 teaspoon of milk to 1 cup of chicken broth.
- Unknown simulated urine sample (“Sample #4”)
  - Add 1 teaspoon of acetone to 1 cup of chicken broth.
- Unknown simulated urine sample (“Sample #5”)
  - Add 1 teaspoon of Mountain Dew to 1 cup of chicken broth.
- 45 urinalysis reagent test strips
  - May be purchased from Carolina Biological (carolina.com) or online drugstores.
- Paper towels for each station

BACKGROUND INFORMATION

Have you ever felt sick? What happens? Sometimes people who are ill go to the doctor for an examination. The doctor may order some blood or urine tests. This seems like a basic procedure, but there are multiple steps that must be observed for accurate testing results. The steps are written in a standard operating procedure, or SOP. The introductory class discussion will help demonstrate to the students the importance of specific SOPs.

The sample should be collected properly. For urinalysis, the patient must follow the instructions for a clean urine catch. In a blood test, the phlebotomist must follow proper
safety procedures to draw the blood from the patient. The sample should be labeled with the date, person’s name and test. The sample is logged in the system from the time the sample is collected until the results are reported. The sample then is sent to the lab and analyzed. At the lab, technicians must follow SOPs to complete the lab test. The test results then are sent to the requesting physician. In this activity, students will learn about the process involved in sample collection, analysis and results. Students also will be able to formulate an SOP for a urinalysis.

TEACHING NOTES
Real urine samples should not be used in classrooms. Simulated urine samples should be used instead. Simulated urine can be purchased from Carolina Biological, online drugstores or made by the teacher. There are several recipes for simulated urine designed to simulate different disorders. Items such as Mountain Dew and chicken bouillon can be used to create simulated urine.

Healthy urine can be simulated by adding a cube of chicken bouillon to a cup of water. A few drops of chicken blood can be added to the chicken broth to test positive for blood. A small amount of milk can be added to the urine sample to make the sample cloudy. A few drops of acetone can be added to the urine sample to test positive for ketones. The smell of the acetone also may be noticed in this sample as a marker for ketones. Flat Mountain Dew will test positive for glucose, or glucose may be added to the base urine sample.

This activity is designed so the teacher may provide each group with a sample of the “healthy” urine. The teacher then may make each of the “sick” urine samples and fill 7 or 8 labeled test tubes with each sample. Each lab group then may choose 2 of the “sick” urine samples to analyze. This way groups will be able to analyze different patients and/or collections of patients.

SAFETY
Students should use proper laboratory techniques. Even though the materials are common household items, the materials used in this lab should not be ingested. Students should wear safety goggles. Proper care should be taken when disposing the reagent strips.

PROCEDURE
Begin the class by allowing students to work with a partner. Give them the task of writing the instructions for making Kool-Aid when provided a pitcher, measuring cup, measuring spoon, sugar and Kool-Aid packet. Provide them with 5 to 7 minutes to write the instructions.
Two groups may come to the front of the class to exchange instructions. One group will read the instructions out loud and follow the instructions exactly to create the drink. If proper sanitary measures were taken, provide cups for those who would like to try the “drink.” After a few groups have tried to create the drink, lead the class in a discussion. Examine what changes should be made to the instructions to create a quality drink. Explain that this set of instructions is known as a standard operating procedure, or SOP. Industry and health care organizations have quality control checklists to make sure everyone performing specific tasks or tests follows the same steps for quality assurance.

Explain to the students that today they will be lab technicians at a local medical laboratory. They will choose 3 different samples of simulated urine to test and share the data with the attending physician. Students will follow the instruction on the student worksheet (next page).

After completing the worksheet, students should write their own quality control checklist for the reagent test strip.

**ASSESSMENT**
Students should complete the student worksheet. After completing the lab activity, students should work in groups to create a quality control checklist for the reagent test strip.

**EXTENSION**
This activity may be extended by researching career options on selected websites, such as Diagnostic Detectives: The Medical Laboratory Professions (medlabcareers.msu.edu/resources.html) and Who’s Who in the Lab: A Look at Laboratory Professionals (labtestsonline.org/lab/who/start/1).
Doctors often order medical tests to help diagnose a person’s illness. The three main examinations that may be conducted for a urinalysis are physical examination, chemical examination and microscopic examination. In this activity, you will perform physical and chemical examinations of several different urine samples.

**PROCEDURE**
Record all observations in the chart on the next page.

**Physical Examination**
1. Waft to detect if the sample has an odor.
2. Observe the color of the sample.
3. Observe the clarity of the sample.

**Chemical Examination**
4. Follow the instructions on the reagent strip bottle to test for common indicators.
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DISCUSSION QUESTIONS

1. Which sample is normal? Why do the results indicate the identified sample is correct?

2. What do you think the other samples indicate?
3. Today you used the instructions for the reagent strip to test the urine samples. Please translate these instructions into common language and write a quality control checklist for the chemical reagent test strips.

4. Once the tests have been completed, it is very important for the results to be relayed to the doctor and patient. Please write the test results in an organized and concise fashion. Remember that the lab technicians relay the results but do not diagnose the illness.
Extension
Now that the results have been relayed to the doctor, pretend to be a physician. Use the medical dictionary or online sources to identify what illnesses are indicated by the test results.
SECTION 2

AUTOPSY
An autopsy is a specialized examination of a corpse to determine how and why someone died and to investigate any disease or injury. There are two classifications for autopsies: forensic and clinical.

A forensic autopsy is required in cases where criminal activity is suspected, cases where the body has not been identified, cases where the cause of death is not clear and many cases involving accidental death. A clinical autopsy is done in an attempt to better understand disease progression and effects of treatments and to learn how to prevent deaths.

Autopsies are done by medical doctors who specialize in diagnosing disease, or pathologists. They may be assisted by an autopsy technician (sometimes known as a “diener”). Forensic pathologists have special training in autopsies and evidence collection. An autopsy usually involves an examination of the exterior of the body and a surgical examination of internal systems and organs. Additional examinations and analyses are done to reveal cellular changes and the presence of various chemicals in the body.

A forensic autopsy team may include forensic anthropologists and forensic toxicologists. A forensic anthropologist is called on to apply archeological and physical anthropology techniques, particularly when the remains are significantly decayed or skeletalized. A forensic toxicologist analyzes samples from the body to detect the presence of drugs and poisons. New advances in imaging techniques allow digital autopsies, magnetic resonance imaging (MRI) and multislice computed tomography (MSCT) to supplement or, in some cases, replace physical autopsies.

In North Carolina, a medical examiner is responsible for determining the cause of death in all accidents, unexpected deaths, unwitnessed deaths, drug overdoses and suspected homicides and suicides. If a
person doesn’t die of a known disease at a hospital, the case is usually the responsibility of a medical examiner, who must view the body and determine the cause of death. The medical examiner decides if an autopsy is needed and orders one, if necessary. The time and cause of death have important legal implications for insurance, wills and criminal cases. If the medical examiner requires an autopsy, permission from the family is not required.

**AUTOPSY PROCEDURES**

Autopsy procedures begin when a body is received, typically by a hospital morgue or a medical examiner’s office. Particularly in the case of forensic autopsies, specific procedures are followed to preserve evidence and avoid contamination. For example, the corpse’s hands are bagged to preserve evidence, such as blood under the fingernails and gunshot residue. The body is covered and transported in a new body bag or evidence sheet to reduce the possibility of evidence contamination during transport. Otherwise, a stray hair from someone legitimately at the scene could confuse the investigation.

At the morgue, the pathologist and any assistants or observers wear gowns, gloves and face shields to protect themselves from potential infection and to avoid contaminating evidence. Notes and photographs are taken at each step. An X-ray is taken of the body, which preserves evidence of fractures and the location of any bullets. The pathologist examines and photographs the body in the clothes in which it arrived. Evidence, such as gunshot residue, hair samples and fibers, is collected and preserved. Once the evidence is collected, the body is undressed and examined carefully for wounds and external abnormalities. For example, tiny dots of blood under the eyelids are a sign of petechial hemorrhage, which indicates the person may have suffocated while trying to breathe against resistance. Once the examination is complete, the body is cleaned, weighed and measured, and all identifying features, such as sex, race, eye color, hair type/color, apparent age, scars, tattoos and birthmarks, are noted. At this point, blood, oral fluid and tissue samples may be taken for toxicological analysis.

After the external examination, the pathologist opens the body to examine the internal organs. This begins with an incision that exposes the rib cage and abdomen so the pathologist can remove and weigh the internal organs and check them for injuries and abnormalities. As the pathologist examines internal organs, he or she may collect tissue samples for microscopic study, culturing disease organisms or toxicology analysis. If the brain must be examined in detail, it is removed and placed in formalin fluid for several weeks so it can be sliced.

When internal examination is complete, the pathologist puts the body back
together, hiding signs of the autopsy so the family of the deceased person can view the body and release it to a mortuary to prepare for a funeral.

**TIME OF DEATH**

In forensic cases, determining the amount of time since death, or postmortem interval, may be important. The pathologist must take careful notes about conditions of the body that provide evidence of the time elapsed since death.

The body changes and eventually decomposes in predictable ways after death. For example, the body cools at a predictable rate after death, depending on the ambient temperature. Blood settles to the lowest parts of the body, which results in discoloration of tissue, or livor mortis. The extent of livor mortis in various tissues can help investigators determine the postmortem interval and position of the body at death.

Rigor mortis is the stiffening of body muscles that occurs after death due to chemical changes. When muscle cells don’t have oxygen, they can’t do cellular respiration. This means they don’t produce ATP, a molecule that transports chemical energy within cells for metabolism. Without ATP, muscles can’t transport calcium ions out of the cells, which in turn causes the muscle fibers to contract. These fibers can’t relax without ATP, so rigor mortis begins to set in between 2 and 6 hours after death. The muscles are affected in a predictable sequence and reach maximum stiffness approximately 12 hours after death. After 12 hours, the muscles relax gradually as decomposition begins.

Decomposition of the body proceeds in predictable stages and is caused by the growth of bacteria in various tissues. Environmental conditions, especially temperature and moisture, affect the speed of decomposition. Digestion also proceeds at a predictable rate, so the contents of the stomach and intestines can provide valuable clues. Certain insects typically lay eggs in dead bodies at predictable times after death. These eggs mature into larvae and pupate on a predictable timeline. A forensic entomologist may be called in to examine insects found on a decaying body and provide an estimate of time since death. For instance, evidence from forensic entomology was important in exonerating Alan Gell of the 1995 murder of Allen Jenkins in Bertie County, N.C., when it showed Gell already was in jail when the murder took place. (Visit [deathpenaltyinfo.org/node/1905](http://deathpenaltyinfo.org/node/1905) to read more.)

**VOCABULARY STUDY**

*Mortis* is the Latin word for “death.” Look for the root word *mort* in the following terms:
- Livor mortis
- Mortician
- Mortuary
- Postmortem
- Rigor mortis
AUTOPSY AS A RESEARCH TOOL
Autopsy is one of the oldest research tools in medicine and continues to be essential to understanding disease processes and developing new treatments. It is the best way to confirm certain disease diagnoses, which are essential to policy and public health decisions. Clinical autopsies also act as a quality control measure for doctors, hospitals and new treatments. For example, autopsy is an important tool for Alzheimer’s disease researchers. A 2007 study done by Dr. Julie Schneider and colleagues at the Rush Alzheimer’s Disease Center, in Chicago, compared clinical diagnoses with autopsy results. The study revealed that many people with clinical signs of dementia showed multiple disease processes in their brains, some of which could have been treated or prevented. These types of studies help researchers improve diagnostic methods.

Another important area of research involving autopsies is traumatic brain injury. Traumatic brain injury is a growing concern because of the number of veterans returning from war with brain injuries caused by explosives. While these injuries don’t always show up on an X-ray or MRI, they do cause a wide range of symptoms. Some of these symptoms appear days or weeks after the trauma and can last or even continue to worsen for years. Autopsies show that some of these symptoms are associated with changes in brain anatomy.

Research on athletes in contact sports, particularly boxing and football, is showing that these players are at risk of degenerative brain diseases due to repeated concussions. Traumatic brain injury, even early in life, is a known risk factor for Alzheimer’s disease and for chronic traumatic encephalopathy (CTE). Like Alzheimer’s patients, people with CTE show signs of brain degeneration, such as memory loss, confusion, impaired judgment, aggression, depression and progressive dementia. Onset often comes at a much younger age than in typical Alzheimer’s patients. Brain autopsy after death is the only sure way to diagnose CTE and differentiate it from other neurodegenerative diseases. Autopsies show reduced brain size and weight as well as distinctive tangles of tau protein in the brains of people who die from CTE. Recent autopsies of retired professional football players who died from suicide showed clear signs of CTE. Even autopsies of very young athletes with a history of repeated head trauma but who died from other causes have uncovered the beginnings of abnormal tau protein deposits. Researchers are working to find biomarkers to diagnose CTE in living patients and are investigating therapeutic drugs that might work after an injury to break down or prevent tau protein deposits.

Autopsy research can change understanding of how treatments are working and shape recommendations regarding
clinical practice. Recent research has revealed differences in the way healing progresses when different kinds of coronary stents are placed in the heart. Arteries can become blocked over time with atherosclerotic plaque, which is caused by inflammation of the arteries and deposits of cholesterol and lipids. Narrowing of the arteries reduces blood flow and increases blood pressure. The plaque can break off and cause blockage in other places. A blockage in the brain can cause a stroke, while a blockage in the coronary artery can cause a heart attack. A coronary stent is a tube in the coronary artery to cover the lesion and relieve the blockage.

Dr. Renu Virmani, of the CVPath Institute, is a pathologist who does autopsies on cardiac patients that focus on examining the coronary arteries and implanted stents to better understand the effects of coronary stent treatment. Her findings have resulted in changes to the recommended medications provided after stent insertion.

**BARRIERS TO AUTOPTSY**

The autopsy rate in the United States has declined sharply during the past several decades, from 19.3% of all deaths in 1972 to 8.5% in 2007. It now is particularly uncommon for an autopsy to be performed when a person dies in a hospital due to disease. This decline is partly due to the increasing cost of autopsy. Because insurance does not cover the cost of clinical autopsies, they generally are paid for by the patient’s family, the hospital or, in some cases, research funding. Clinicians sometimes fear that an autopsy would show a problem with their care. Other times, they think modern diagnostic tools have reduced the need for autopsy. This is problematic because autopsy research has shown that many times patients are misdiagnosed or have another contributing disease condition that might have been treated if it had been diagnosed. Autopsies also can show the need for changes in treatment protocols.

A medical examiner does not need the consent of the family to order a forensic autopsy. However, clinical autopsies generally are ordered by physicians and must be consented to by the family of the deceased person. The experiences of different cultural and ethnic groups have caused some of these groups to distrust medical researchers and thus object to clinical autopsy. Many members of minority groups know of cases in which researchers showed disregard for the well-being of research subjects and may have experienced poor treatment from the medical system themselves. Other people fear what an autopsy might reveal about the deceased person’s lifestyle. Still others fear the body won’t be treated with respect or will be disfigured.

Not surprisingly, different religions feel differently about autopsies. Most religions have specific beliefs and practices related to death and the treatment of bodies.
In some cases, they encourage immediate burial and forbid desecration of the body — both of which rule out an autopsy. However, most religions make an exception when an autopsy could save another person’s life.

Laws in at least 11 states allow a family to express a religious objection that must be taken into consideration by the medical examiner when deciding whether to order an autopsy. The medical examiner still may require an autopsy if there is a question of homicide or if public health issues are involved. Medical examiners sometimes work with religious leaders and community groups to help overcome objections to forensic autopsy. When the need for an autopsy is explained with sensitivity and people are assured the body will be treated with respect, a much higher percentage of families give consent.

**RESOURCES**

**U.S. National Library of Medicine**
For an exhibition titled *Visible Proofs: Forensic Views of the Body*, which includes the history and technology of forensic medicine, videos and a variety of case studies, visit [nlm.nih.gov/visibleproofs/education/medical](http://nlm.nih.gov/visibleproofs/education/medical).

For a list of excellent resources for teachers and students, visit [nlm.nih.gov/visibleproofs/resources/weblinks.html](http://nlm.nih.gov/visibleproofs/resources/weblinks.html).

**The Virtual Autopsy**
The Virtual Autopsy is a website created by British medical students that has numerous case studies with photos of tissues and organs. Students can click on the different parts of the body, review the photos and findings, and then choose a cause of death. Interactive feedback guides student understanding of the underlying medical condition and autopsy findings. Visit [www.le.ac.uk/pathology/teach/va](http://www.le.ac.uk/pathology/teach/va).

**HowStuffWorks**
The article *How Autopsies Work* provides a step-by-step explanation of the autopsy with pictures and links to other sites. Visit [science.howstuffworks.com/autopsy](http://science.howstuffworks.com/autopsy).

**Medscape**

**BrainLine.org**
This site is funded by the Defense and Veterans Brain Injury Center and has many resources for people with brain injuries and their families, such as links to summaries of recent research. Visit [brainline.org](http://brainline.org).

**Boston University**
Boston University’s Center for the Study of Traumatic Encephalopathy has many useful resources, including links to academic
research papers, case studies and images. Visit bu.edu/cste.

Post Mortem: Death Investigation in America
For a series of articles and programs on forensic investigations done by ProPublica and PBS Frontline, visit propublica.org/series/post-mortem and pbs.org/wgbh/pages/frontline/post-mortem.

For an article that includes discussion of Dr. Virmani’s work on stents, visit propublica.org/article/without-autopsies-hospitals-bury-their-mistakes.
LEARNING OUTCOMES
• Students will examine what happens to the body from the time of death until the autopsy.
• Students will be able to list when forensic autopsies are required in the state of North Carolina.
• Students will be able to describe what happens during both a traditional autopsy and a virtual autopsy.
• Students will discuss economic, religious and cultural concerns with autopsy.
• Students will perform a virtual autopsy and determine the cause of death from information provided.

KEY VOCABULARY
• Medical examiner
• Autopsy
• Virtual autopsy

TIME REQUIRED
• Approximately 10 minutes of teacher prep time
• Approximately 100 minutes of class time for lab and discussion
MATERIALS REQUIRED

- Cash register tape
- Sticky notes
- Handouts
- Internet access
- RealPlayer (computer program used to play certain audio files)

BACKGROUND INFORMATION

From *Perry Mason* to *Colombo* to *NCIS*, murder investigations have been a prominent part of TV programming for decades. Largely due to the popularity of these shows, the public often believes it “knows” what happens to a body once a murder has been discovered. However, many people would be shocked by the procedures and time required to gather, test and analyze samples from crime scenes. This activity is designed to introduce students to concepts related to when an autopsy is required, what happens during the autopsy and what can be learned from the autopsy.

The process of crime scene investigation varies from locality to locality, and autopsy rules vary from state to state. This activity focuses on the processes outlined by the U.S. Department of Justice and the North Carolina Office of the Chief Medical Examiner. If you are interested in other processes, many of the resources will provide alternative perspectives. The extension activity will help students see how problems with crime investigations perpetuate problems into the justice system.

Autopsies traditionally have required the cadaver to be examined both externally and internally. The internal examination typically requires cutting the cadaver open and examining internal organs and removing tissue samples. This disturbance of the body and delay of burial causes problem for some religious and cultural groups. With the advancement of MRIs and CT scans, virtual autopsies now are possible in many situations. Students will look at the special concerns of some groups and how virtual autopsy can assist pathologists and medical examiners.

TEACHING NOTES

Murder scene investigations are popular classroom activities. If you are interested in conducting an investigation in the classroom or virtually, here are some suggested resources:

- Incorporating Biotechnology into the Classroom: Crime Scene Investigation ([www.public.asu.edu/~langland/forensics.html](http://www.public.asu.edu/~langland/forensics.html))
- Autopsy of a Murder ([centredessciencesdemontreal.com/static/autopsy/flash.htm](http://centredessciencesdemontreal.com/static/autopsy/flash.htm))
Safety

Remind students to have mature conversations about this subject. It is important to remember that a student might have experienced the traumatic death of a loved one. It is essential for teachers to create a safe place for students to discuss this material. This is a great time to discuss the ethical behaviors that should be seen in medical and law enforcement personnel.

Procedure

Begin by showing a clip from a television crime investigation. Many shows have YouTube channels with clips, such as NCIS (youtube.com/show/ncis). Break the students into groups of 3 or 4 and ask them to create a timeline using the cash register tape. Assume the body was found at noon on Monday for all timelines. Provide 5 to 7 minutes for the groups to list the actions and include estimated times for everything that happens to the body from the time it is found until it is given to the family for burial. Post the timelines in front of the class, lining up start times. This will make it easy to discuss similarities and differences among the groups.

Provide each group with a student worksheet to complete. The student worksheets are in WebQuest format (webquest.org). The WebQuest and task may be placed on a website such as Edmodo (edmodo.com) for the students to access. Students may then use a computer, tablet or phone to access the information.

After completing the WebQuest, allow the groups to use sticky notes to modify their timelines. Check student understanding with discussion questions such as, “What are the reasons an autopsy may be required in North Carolina?” and “What happens during the internal portion of the autopsy?”

Assessment

Students will complete the student worksheet WebQuest. Groups may produce a brochure to be given to the local hospital to explain the autopsy process. A rubric for the brochure is attached.
EXTENSION

Encourage students to choose an autopsy case study, such as those provided by the University of Pittsburgh Medical School database (path.upmc.edu/cases.html). Students may then examine the visual and written data from the autopsy and try to formulate a simplistic diagnosis. Students may check their diagnoses by clicking on the final diagnosis.

Students also may watch Post Mortem: Death Investigation in America (pbs.org/wgbh/pages/frontline/post-mortem). This PBS show will introduce students to many of the challenges faced by police and crime scene investigators in the current legal system.
Once a body is discovered at a crime scene, it takes a fascinating journey before it is released for burial. Your task is to discover and report back to others what happens to the body along the way.

**PROCEDURE**

**Crime Scene Investigation**

1. Read *A Guide to Death Scene Investigation* ([nij.gov/nij/topics/law-enforcement/investigations/crime-scene/guides/death-investigation](http://nij.gov/nij/topics/law-enforcement/investigations/crime-scene/guides/death-investigation)). Click on the links at the bottom of the page to answer the following questions:

   a. What is the first thing a death scene investigator should do upon arriving at the scene?

      __________________________________________________________

      __________________________________________________________

      __________________________________________________________

   b. What is the procedure for chain of custody?

      __________________________________________________________

      __________________________________________________________

      __________________________________________________________

      __________________________________________________________
c. When evaluating the scene, what are at least 4 of the steps that should be done?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

d. What happens to the body when it is documented and evaluated?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

________________________________________________________________________

2. Listen to Science of Forensics on NPR’s Talk of the Nation (npr.org/templates/rundowns/rundown.php?prgId=5&prgDate=9-7-2001; click the RealPlayer icon next to the podcast title) and list 3 things forensic scientists must think of during an investigation:

a. Thing #1:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

b. Thing #2:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

c. Thing #3:
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
Autopsy

3. Read the Guidelines, Rules, and Statutes of the North Carolina Office of the Chief Medical Examiner (www.ocme.dhhs.nc.gov/rules/guidelines) and answer the following questions:

   a. When does the state of North Carolina require an autopsy to be done on a body?

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

   b. Who conducts a required autopsy?

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

4. Read How Autopsies Work (science.howstuffworks.com/autopsy4.htm), from the external to the internal investigations of an autopsy.

   a. What happens in an external investigation?

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

   b. Where is the first cut on the body usually made?

   __________________________________________________________
c. What happens to the internal organs?

Virtual Autopsy
5. Read/watch 2 of the following articles/videos:
   » Virtual Autopsy in Forensic Medicine
     (medical.siemens.com/siemens/it_IT/gg_ct_FBAs/files/CIP/Out_of_the_ordinary/
      Virtual_Autopsy_in_Forensic_Medicine.pdf)
   » Forensics Revolution: Virtual Autopsies Provide New Insights into Death
     (spiegel.de/international/europe/new-virtual-autopsy-procedure-is-changing-
      forensics-a-875657.html)
   » Virtual autopsy: does it spell the end of the scalpel?
     (guardian.co.uk/science/2013/feb/23/virtual-autopsy-virtopsy-forensic-science)
   » Die Zukunft heisst Virtopsy / The future is Virtopsy
     (www.virtopsy.com/movies; scroll down to find the video and choose one of the English
      versions of the activity)
a. What tests or scans may be conducted in a virtual autopsy?

b. What are the benefits of virtual autopsy?

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**Religious & Cultural Considerations**

6. Read 1 of the following articles:
   » *Religions and the Autopsy*  
     *(emedicine.medscape.com/article/1705993-overview#showall)*  
   » *Religious and Cultural Considerations for Autopsy*  
     *(ohsu.edu/xd/health/services/doernbecher/research-education/research/pape-family-pediatric-research-institute/upload/Religious-and-Cultural-Considerations-for-Autopsy.pdf)*

a. Which religions and cultures do not favor autopsies?

b. How can a medical professional help ease concerns people might have about autopsies?
Virtual Autopsy Activity
7. Visit The Virtual Autopsy (www.le.ac.uk/pathology/teach/va) and choose a case. Review the data from the autopsy and try to diagnose the cause of death. Check to see if your cause of death was correct.

a. Which case did you choose?

b. What is your initial cause of death? (If you do not know the specific medical term, explain which part of the body causes concern.)

c. Did you diagnose the case correctly?

d. Which piece of information helped (or would have helped) the diagnosis?
SECTION 3
DNA ANALYSIS
Every living organism — from the smallest bacteria to the tallest trees to the most complex animals — needs a set of instructions to live, grow, develop and reproduce. These instructions are encoded in long molecules of deoxyribonucleic acid, or DNA. Each DNA molecule has a twisted ladder shape, known as a double helix, and is formed from two long, complementary chains of nucleotides. There are four different nucleotides: adenine (A), thymine (T), cytosine (C) and guanine (G). Each nucleotide is made up of a sugar, a phosphate and a nitrogenous base. The sides of the DNA ladder in every organism are made of a chain in which the sugar and phosphate parts of the nucleotides are alternated. The rungs of the ladder are made of nitrogenous bases, and the order of these bases forms the code that provides the template for all the proteins made by an organism. Each base has a complementary base that fits like a chemical puzzle piece and forms the opposite half of the rung. A always pairs with T, and C always pairs with G. This pairing enables the cell to unzip DNA molecules and form two new strands, each of which is identical to the original strand. This method of replication allows each cell and organism to pass its information on to the next generation. Human DNA is arranged into 23 pairs of chromosomes in the nucleus of each cell. Each chromosome is a long, coiled DNA molecule packaged with protein. A full set of human chromosomes contains 6 billion pairs of nitrogenous bases. That is a lot of information!
Recent research has shown that these 6 billion base pairs code for only about 20,000 proteins — many fewer than previously expected. The bases that code for proteins (or for functional RNA units such as ribosomes and transfer RNA) are known as genes. The rest of the DNA is known as non-coding DNA. Non-coding DNA sometimes used to be called junk DNA, but scientists now are finding that parts of these non-coding regions play important regulatory roles.

Your traits include everything from your height and eye color to your personality and susceptibility to various medical conditions. Genes interact with the environment to produce these traits. Some traits, such as height and weight, are strongly influenced by the environment. Other traits are influenced very little or not at all by the environment.

**How Unique is Your DNA?**

You might have heard some of the following statements:

- Human DNA is more than 95% similar to chimpanzee DNA.
- Your DNA is 50% identical to your mother’s DNA and 50% identical to your father’s DNA.
- Unless you have an identical twin, nobody has DNA just like yours.

But what do these statements mean? What is actually being compared here? How unique is each individual person’s DNA?

Everybody has genes for proteins that provide the essential structures and functions of the body. For example, everybody
has genes for cytochrome c, a protein that plays an essential role in the electron transport chain. Everybody has genes for hemoglobin, a protein in blood that transports oxygen. Slight variations in these genes provide individuals with their unique characteristics, their personal combination of hair color, skin color, height, personality and biochemistry. If you compare the DNA sequence of two humans, approximately 999 out of every 1,000 pairs will be the same. This is why humans are said to be 99.9% alike. But in every 1,000 pairs, approximately 1 of the pairs will differ. It is these differences that make each person's DNA sequence unique (unless he or she is an identical twin).

Not only are humans similar to one another, but we also are similar to other organisms in many ways. This is because many gene sequences are substantially similar in widely different organisms. The more closely related and similar two species are in body structure and biochemistry, the more similar are the sequence of the bases that form their DNA code. Sequences that are very similar across different species are known as conserved sequences because they have been preserved from generation to generation over evolutionary time. For example, the gene that codes for the protein cytochrome c is very similar in plants and animals. Its critical function in energy cycling helps conserve its sequence. Any changes or mutations in the protein coding sequence would tend to be lethal or put the organism at a strong disadvantage. The non-coding sequences, on the other hand, may not experience the same level of evolutionary pressure to be similar. The non-coding region is where the most differences are seen between species and between individuals of the same species.

APPLICATIONS OF DNA ANALYSIS

Using DNA to Identify People

Because each person's DNA is unique, DNA is like a fingerprint. If you have a sample of body tissue that includes blood, semen, saliva, skin or hair, it can be tested to see if the DNA it contains matches that of a particular person. However, sequencing all 6 billion base pairs would be far more time consuming and expensive than is practical. Therefore, DNA fingerprinting or profiling for forensic purposes concentrates on a few highly variable regions of DNA and a kind of DNA known as a short tandem repeat, or STR. STRs are short, three- to four-nucleotide sequences of DNA that repeat over and over and are found in non-coding regions of DNA. In the United States, the Federal Bureau of Investigation, or FBI, maintains a database that contains DNA profiles of convicted criminals, some arrestees, missing persons, relatives of missing persons and unidentified remains. These DNA profiles record information from 13 different STR locations, or core loci, on the chromosomes.

The number of STR repeats at a particular location is known as an allele and varies
considerably from person to person. Each person has two alleles for each location — one from each parent. While it is common for two people to share the same alleles at one location, it would be unlikely for even closely related people to have the same alleles at all 13 locations. The probability of an exact DNA match for all 13 locations differs by ethnic group and depends on how common the alleles in a particular profile are, but it is always very small. If a person does not match a sample at all 13 locations, he or she can be excluded as a possible source of the sample.

As of 2013, there have been 307 post-conviction exonerations in the U.S. based on DNA evidence. In 149 of these cases the true source of the sample has been identified. (Visit innocenceproject.org/know for more information.) If a person does match a sample at all 13 locations, it is still important to understand how his or her DNA came to be in the sample. Careful police and laboratory procedures must be followed to establish a chain of custody for the sample and to prevent cross-contamination, considering that a small amount of DNA from a police officer or lab worker could confuse the profile with extra alleles.

DNA is used to identify close relatives as well. In addition to the FBI database used in missing person and unidentified remains cases, numerous laboratories perform tests to establish paternity and other family relationships in custody, inheritance, immigration and other legal cases. To establish paternity, labs usually compare results at 16 loci, including the 13 core loci used by the FBI. If the family relationship test is being done for legal reasons, it is necessary to establish a chain of custody for the sample — just as in a criminal case. DNA also is used to identify remains from wars, mass murders and disasters. While paternity and maternity testing can exclude someone positively or give a very high probability of inclusion, the results from siblings, cousins and other, more distant family relationships are more difficult to interpret.

**Beyond Identification**

Careful analysis of a person’s genes can provide a wealth of health information. For example, Angelina Jolie was able to discover through genetic testing that she shared her mother’s risk for breast and ovarian cancer due to inherited mutations in a tumor-suppressing BRCA gene. People can find out whether they have inherited genes that affect their risk for many other diseases and conditions. Because genes are such an important factor in health, the U.S. enacted the Genetic Information Nondiscrimination Act, or GINA, in 2008 to prevent employment and insurance discrimination based on genetic information. Still, it is important to realize that for most conditions a single gene is only part of the prognosis. Other genes may have a moderating effect. A person’s environment, including what he or she eats and drinks,
what microorganisms he or she is exposed to, exercise habits, exposure to sunlight and many other factors, also plays a role in how disease conditions are manifested.

Newborn screening programs test infants for genetic and other conditions that should be treated immediately but that might not be immediately obvious. For example, it is important for a baby with phenylketonuria to have a special diet from birth to avoid mental retardation, seizures and albinism. Babies with sickle cell disease need specialized care to prevent infections and organ damage and to treat pain. Newborn screening for genetic disorders involves testing a tiny amount of blood taken by pricking the baby’s heel.

Some laboratories now will perform genetic tests and provide a person with a variety of information on his or her ancestry, potential relatives and gene variations relative to a wide range of medical conditions with a known genetic component. These services help adoptees without known biological family members understand their risks for various medical conditions and, in some cases, find biological relatives. One such company, 23andMe, provides ancestry and medical risk information by looking at more than 1 million places where the human genome is known to have single nucleotide variations, or SNPs.

Genetic testing also is important for understanding the prognosis and treatment of certain types of cancer. Cancer cells usually have several mutations that make them different from a patient’s other cells. Because each of the trillions of cells that make up a person’s body is descended from the initial zygote formed at conception, it is not surprising that harmful copying mistakes sometimes occur when cells reproduce. Cancerous cells usually have inherited several harmful mutations that together allow the cell to evade the body’s usual defense mechanisms against unrestrained growth. In some cases, cancer researchers have been able to match specific treatments for specific mutations. For example, breast cancer patients whose cells test positive for receptors known as HER2 are helped by the drug Herceptin, which blocks these receptors. A drug called XALKORI helps the 3% to 5% of lung cancer patients whose cancers test positive for a mutation in the anaplastic lymphoma kinase gene. Researchers are developing a better understanding of cancer-causing mechanisms at the molecular level. As a result, research to develop tests for mutations active in particular cancers and to develop drugs that target these mutations is rapidly expanding.

**TECHNIQUES FOR ANALYZING DNA**

**Karyotyping**

Karyotyping is a technique for looking at an organism’s set of chromosomes. Some
genetic disorders are caused by extra, missing or rearranged chromosomes or pieces of chromosomes. Prenatal testing often is used to detect these disorders before a baby even is born. Chromosomes condense during cell division, which makes them — and any abnormalities they might contain — easier to see. A cell in metaphase is stained and photographed under a microscope. Digital images of the chromosomes then are cut out, paired and organized by size. Because each chromosome should be a specific size and have a specific banding pattern, abnormalities can be detected. Special techniques such as spectral karyotyping and fluorescence in situ hybridization, or FISH, use probes that stick to known places on the chromosomes in order to color, or “paint,” specific parts of the chromosomes. This makes it easier to spot certain types of rearrangements.

**Polymerase Chain Reaction**
Polymerase chain reaction, or PCR, is an extremely important technique in DNA testing. PCR is used to make enough copies of DNA segments so the DNA can be sequenced, profiled or tested for a particular genetic disorder. Millions of exact copies of a specific sequence can be made in just a few hours with simple, inexpensive tools. This is called amplification. PCR requires short pieces of DNA, known as primers, which must be complementary to the beginning and end of the desired DNA sequence. The desired primers can be ordered from commercial suppliers. The target DNA sequence alternately is heated and cooled with the primers and DNA polymerase, a special enzyme that replicates DNA by synthesizing the complementary strand. PCR allows DNA profiling to be performed from an extremely small sample of DNA.

**Gel Electrophoresis**
Gel electrophoresis is a technique for separating molecules by length and charge, including DNA, RNA and proteins. In DNA profiling, the DNA fragments of interest are pieces of DNA that contain the STR regions. PCR is used to make millions of copies of these specific fragments of DNA from
a sample. The fragments then are inserted into a special gel and electricity is applied to attract the molecules through the gel. The smaller fragments with fewer STR repeats move faster. Next, the fragments are stained so they will show up. The result is a banded pattern that is compared to a reference pattern to determine which STR alleles are in the sample.

**Microarrays**

Microarrays can detect the presence and activity of thousands of genes at the same time. A microarray is made by attaching or printing DNA probes on a glass slide, chip or membrane in an organized array. The probes are designed to attach to complementary DNA. The sample DNA is amplified using PCR and cut up using special enzymes that will divide the DNA at specific places. Next, the solution containing the sample DNA is incubated with the microarray. When the sample DNA matches the probe DNA, it will stick together, or hybridize, and will remain on the chip while the rest is washed off. The hybridized probes then can be triggered to glow, which is detected by a reader. This tells the researcher what DNA or RNA was found in the sample. The chip can be designed to detect single nucleotide polymorphisms, or tiny, one-base mutations that make one gene different from another. This technology is used to give people detailed information about their ancestry and genes for particular traits. Microarrays are used to study which genes are active during development and in cancer cells, as well as cell response to pathogens and various treatments.

**RESOURCES**

**Freakonomics**

The article *Are the F.B.I.’s Probabilities About DNA Matches Crazy?* from the Freakonomics website provides a good discussion about the accuracy of DNA profiling. Visit [freakonomics.com/2008/08/19/are-the-fbis-probabilities-about-dna-matches-crazy](freakonomics.com/2008/08/19/are-the-fbis-probabilities-about-dna-matches-crazy).

To help your students understand this issue, discuss the birthday problem before reading the Freakonomics article. This problem is explained at [mathforum.org/dr.math/faq/faq.birthdayprob.html](mathforum.org/dr.math/faq/faq.birthdayprob.html).

**University of Utah**

For a virtual gel electrophoresis lab from the Learn.Genetics website of the University of Utah’s Genetic Science Learning Center, visit [learn.genetics.utah.edu/content/labs/gel](learn.genetics.utah.edu/content/labs/gel).

For accompanying explanations of how this technique is used in forensics, visit [learn.genetics.utah.edu/content/labs/gel/forensics](learn.genetics.utah.edu/content/labs/gel/forensics).

**Baylor University**

The article *Discovery, development, and current applications of DNA identity testing*, from Baylor University Medical Center, provides a very readable, historical discussion.
of the development and current uses of DNA identity testing. Visit ncbi.nlm.nih.gov/pmc/articles/PMC1200713.

**Nature**
For an article that asks whether it is ethical to keep a database of convicted felons’ DNA profiles and whether we can rely on DNA fingerprints for conviction, visit nature.com/scitable/topicpage/forensics-dna-fingerprinting-and-codis-736.

**Cold Spring Harbor Laboratory**
For a step-by-step animation and explanation of polymerase chain reaction from the DNA Learning Center at Cold Spring Harbor Laboratory, visit dnalc.org/resources/animations/pcr.html.

**23andMe**
For an explanation of how 23andMe’s genotyping technology works and for example of the use of microarrays in DNA analysis, visit 23andme.com/more/genotyping.
LEARNING OUTCOMES
• Students will define DNA databanking.
• Students will discuss DNA databanking in the United States and around the world.
• Students will write a letter to state/federal officials about how DNA swabs should be collected or stored after an interaction with law officers.

KEY VOCABULARY
• DNA
• DNA databanking

TIME REQUIRED
• Approximately 10 minutes of teacher prep time
• Approximately 90 minutes of class time for research and discussion

MATERIALS REQUIRED
• Computer with projection device
• Copies of articles (electronic or printed)
• Addresses of political leaders, such as the president, the governor or senators

BACKGROUND INFORMATION
Deoxyribonucleic acid, or DNA, contains unique, hereditary information found in the nucleus of organisms. The information in DNA is unique for each individual and may be used
to identify an individual. One group that uses the uniqueness of DNA is law enforcement. Some states have created DNA databanks that store the DNA from criminal suspects and/or convicts to use in criminal investigations. This use of genetic material by forensic scientists is called DNA profiling. DNA databanks have been created in several states in the U.S. and countries around the world. Ethical questions remain as to how and when this genetic information should be used.

**TEACHING NOTES**

This activity is designed to provide students with an opportunity to explore ethical questions that arise from DNA databanking. It is important to remember that students might have a personal connection to someone in the criminal justice system or an unsolved criminal case. Therefore, some attention should be paid to creating a safe atmosphere for students to discuss this controversial topic. Before getting started, have the students develop discussion rules. Also encourage the students to look at the topic from different points of view. Ask the students to pretend to put on someone else’s “glasses” to see the issue from a different perspective. Ask how their views might be different if they were arrested or if they were the victim of a violent crime.

**PROCEDURE**

Begin with the question, “What is DNA?” Provide time for the students to explain how they define DNA. Review the chemical nature of DNA and the way in which it codes for proteins that in turn determine the characteristics of organisms. Ask students, “Why is DNA important?” Help students understand the uniqueness of DNA and why it may be used to solve problems. Watch one or more of the video clips listed below to explain how forensic scientists use DNA to help solve criminal cases.

- **Catching Killers: All Signs Point to Innocent**
  (youtube.com/watch?v=Fz6p8Eg1Z3w)
  » This video, from the Smithsonian Channel, explains how DNA was used in the first criminal case.

- **Catching Killers: Traces of a Serial Killer**
  (youtube.com/watch?v=JlxWQHiC7vY)
  » This video, from the Smithsonian Channel, explains PCR procedures to gather DNA from evidence.
• Two-Minute Science Lesson: How Forensic DNA Testing Works
  (youtube.com/watch?v=qZeYu76bOsQ)
  » This video, from the Innocence Project, is a simple explanation of how DNA is collected and analyzed to determine the innocence or guilt of a suspect.

• Catching Killers: DNA Profiling: The Serial Killer Next Door
  (youtube.com/watch?v=oXwnUFrsjPo)
  » This video, from the Smithsonian Channel, explains how familial DNA could be used to solve a case.

• The Case for Innocence
  (pbs.org/wgbh/pages/frontline/shows/case/etc/video.html)
  » This video excerpt, from a PBS Frontline documentary, discusses DNA databanks and ethics.

Now that students have been introduced to how DNA can be used to assist police in solving crimes, the focus will shift to DNA databanking. DNA databanks may be public or private institutions. In 2013, the Supreme Court ruled that police could take a DNA sample from a suspect in a serious crime. Within the criminal justice system, DNA fingerprints are collected from criminals and are stored to compare DNA from a crime scene to DNA of a suspect. The practice of taking a DNA sample from a suspect or a convicted criminal raises many ethical questions.

Students should review several articles to gain a clear picture of the DNA databank controversy in the justice system and the general population. Students will review these articles using a jigsaw activity. Divide the class into five groups. Give each group the rubric with which students will be graded and one article to review and discuss. Sample articles that students may view online are as follows:

• Ethical-legal problems of DNA databases in criminal investigation
  (jme.bmj.com/content/26/4/266.full.pdf)
  » From the Journal of Medical Ethics

• Population biobanking in selected European countries and proposed model for a Polish national DNA bank
  (ncbi.nlm.nih.gov/pmc/articles/PMC3334487)
  » From the Journal of Applied Genetics
• Balancing the Risks and Benefits of Genomic Data Sharing: Genome Research Participants’ Perspectives  
(ncbi.nlm.nih.gov/pmc/articles/PMC3318928) 
  » From the journal Public Health Genomics
• Swabbing Students: Should Universities Be Allowed to Facilitate Educational DNA Testing?  
(ncbi.nlm.nih.gov/pmc/articles/PMC3390747) 
  » From The American Journal of Bioethics
• Minors or suspects? A discussion of the legal and ethical issues surrounding the indefinite storage of DNA collected from children aged 10–18 years on the National DNA Database in England and Wales  
(msl.sagepub.com/content/52/4/187.long) 
  » From the journal Medicine, Science and the Law
• In DNA Ruling, a Cruel Blow to Scriptwriters  
(nytimes.com/2013/06/05/arts/television/supreme-courts-dna-ruling-tests-the-scriptwriters-art.html) 
  » From The New York Times
• State and Federal DNA Database Laws Examined  
(pbs.org/wgbh/pages/frontline/shows/case/revolution/databases.html) 
  » From PBS Frontline

Give students 12 to 15 minutes to silently read and identify the main points of the articles. Ask each group to take 7 to 10 additional minutes to agree on the main points of its assigned article. Next, form new collaboration groups by choosing one member from each original group. Each collaboration group will have every article represented by one student. In the collaboration group, give students 5 to 8 minutes to explain the main points of the article. Other students may ask questions for clarification within the collaboration group. Collaboration group discussion will take 25 to 30 minutes.

Finally, provide 10 to 12 minutes for discussion among the entire class. Help students formulate the big ideas in DNA databanking. Give each student a copy of the student worksheet for this activity that contains the letter rubric. Each student will write a letter to a state or federal official, such as the president, the governor or a senator, that shares his or her concerns and/or solutions with regard to DNA databanking.

**ASSESSMENT**

Students may be assessed in their discussion participation using the discussion rubric in the student worksheet. The letter also may be evaluated with the letter rubric. After you review
each letter, it may be sent to the political leaders to whom it is addressed if the student chooses to send it.

**EXTENSION**

This activity may be extended by using the interactive GENE BOY webpage (www.dnai.org/geneboy/), to analyze DNA sequences that are generated by the page or entered by students. These sequences may be compared against the National Library of Medicine’s BLAST database (blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the organisms to which they may belong.
# Why Bank My DNA?

## Student Worksheet

## Discussion Rubric

<table>
<thead>
<tr>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
</table>
| **Discussion Participation** | • Offers well-timed, thoughtful comments  
• Comments respectfully on others’ ideas  
• Questions are appropriate and thought-provoking  
• Body language and behavior demonstrates respect and attention  
• Listens to others’ comments and questions | • Offers occasional thoughtful comments  
• Comments on others’ ideas  
• Questions may provoke others to comment or question  
• Listens most of the time to others  
• Inconsistently responds to others’ comments | • Participates when questioned  
• Restates information without adding new ideas  
• Asks questions that do not fit current discussion  
• Listens to some remarks but ignores others  
• Appears to drift through discussion | • Does not participate or offers negative/inappropriate comments  
• Behavior demonstrates lack of involvement in discussion |
| **Resource Usage** | • Has read research materials thoroughly and with a critical eye  
• Clearly references research materials in discussions  
• Appropriately uses research materials to support ideas/comments | • Has read research materials, with basic understanding  
• Occasionally references research materials to support comments | • Has skimmed research materials, with little understanding  
• Rarely uses research materials to support ideas thoughtfully | • Did not read research materials  
• Fails to reference research materials  
• Unable to form comments that are on topic |
# LETTER RUBRIC

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<td>• Follows some pieces of business letter format</td>
<td>• Does not follow business letter format</td>
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<tr>
<td></td>
<td>• Features all 7 parts of a business letter:</td>
<td>• Features 5 or 6 parts of a business letter:</td>
<td>• Features few, if any, parts of a business letter:</td>
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<td>» Sender’s address</td>
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<td><strong>Structure</strong></td>
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<td>• Simple paragraphs and sentences</td>
<td>• Poorly constructed paragraphs and sentences</td>
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<td>• Several run-on sentences or fragments</td>
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<td>• Few to no errors in capitalization, grammar, punctuation or spelling</td>
<td>• Few to several errors in capitalization, grammar, punctuation or spelling</td>
<td>• Many errors in capitalization, grammar, punctuation or spelling</td>
</tr>
<tr>
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<td>• Content vaguely links to reference materials</td>
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<td>• Accurate information</td>
<td>• Some factual information</td>
<td>• Little to no factual information</td>
</tr>
<tr>
<td></td>
<td>• Clear presentation of writer’s point of view</td>
<td>• Vague presentation of writer’s point of view</td>
<td>• No clear focus presentation of writer’s point of view</td>
</tr>
</tbody>
</table>
From *Sherlock Holmes* to *CSI*, fictional dramatization of crime investigation has increased public interest in the science of crime investigation. And while these dramatizations tend to overstate the speed and accuracy of forensic techniques, advances in forensic science are helping investigators solve cases in some amazing ways. Advances in forensic science, particularly in DNA and chemical analysis, not only are helping find and convict the guilty, but also are helping exonerate innocent people and reunite families long separated by war or disaster.

Forensic science is the application of science and technology to establish facts in legal cases. It includes a wide range of techniques and sciences. Forensic medicine is the application of medical knowledge to legal issues. It establishes medical facts in civil and criminal law cases. It includes investigations of murder and sexual assault as well as identification of war and disaster victims, investigation of child abuse, employer drug screening programs, paternity investigation of unexpected and accidental deaths, and other cases in which medical expertise is required.

**MEDICAL EXAMINER**

A medical examiner is a medical doctor, usually qualified as a *forensic pathologist*, in charge of death investigations. He or she typically is appointed or employed by the state. A coroner may or may not be a medical doctor, typically is elected and also is responsible for investigating unusual
or suspicious deaths. Most states and local jurisdictions in the United States have changed to a medical examiner system. Some jurisdictions still use a coroner or a hybrid system. North Carolina uses a medical examiner system, although coroners still exist in a few rural counties. According to the Guidelines, Rules, and Statutes of the North Carolina Office of the Chief Medical Examiner, a medical examiner’s “primary purpose is to detect, analyze, and document the medical aspects of certain types of deaths so that deaths can be better understood scientifically, legally, and socially.” (Visit www.ocme.dhhs.nc.gov/rules/guidelines to read the full document.)

A medical examiner is required to investigate and personally view the body in all homicides, suicides, accidents, drug overdose cases and unexpected or unwitnessed deaths in his or her jurisdiction. This includes situations in which a person dies in a medical facility of an unknown cause. A medical examiner must authorize the removal of a dead person’s body from the location where it is first found. He or she reviews all the evidence, including the autopsy, laboratory findings, medical history and evidence from the scene of death, in order to complete the death investigation and determine the official cause of death.

The North Carolina Department of Health Statistics and the Office of the Chief Medical Examiner provide detailed analyses of deaths by age, race and county. This enables policymakers and health workers to make informed decisions about prevention programs and policies. In North Carolina, the medical examiner system investigated 9,724 deaths in 2007. It is important to remember that the medical examiner system investigates only a small percentage of all deaths that occur. Only 13% of the 75,803 deaths that occurred in North Carolina in 2007 were investigated by medical examiners.

**FORENSIC TOXICOLOGY**

Forensic toxicology is the investigation of drugs and poisons in the body in cases of illegal drug use, drug abuse, poisoning and death. Forensic toxicologists use lab tests to determine the presence and possible
role of alcohol, drugs (including medications and illicit drugs) and poisons in death investigations and other legal cases. In a death investigation, the pathologist collects specimens for analysis by the toxicology laboratory. Blood samples almost always are examined. Depending on the case, other tissues and body fluids, such as urine, oral fluid (saliva), vitreous humor (eye), gastric contents (stomach), liver, brain and even hair also may be examined.

Just as with other diagnostic tests, it is critical to collect, label, store and transport the sample correctly to ensure accurate results. In a death investigation or other legal case, it is essential to maintain a documented chain of custody so test results can be submitted as evidence in court. In North Carolina, all medical examiner cases submit specimens to the central forensic toxicology laboratory.

The specific lab tests done on the samples depend on the case and suspected cause of death due to symptoms and/or evidence found at the death scene. Initial screening can test for more than 300 different drugs and potential poisons. Once a toxin is detected in a sample, more advanced tests are used to confirm the finding and measure the amount or concentration within the body.

The first step in testing is to prepare the sample for analysis by separating the part of interest from the rest of the sample.

For example, a blood sample is spun in a centrifuge to separate the plasma from the blood cells and platelets. The plasma is kept, while the cells are discarded. Next, different methods of extraction may be applied to separate and concentrate the toxins for analysis. The sample then can be put in a gas or liquid chromatograph-mass spectrometer. Different chemicals in the sample move through the chromatograph at different speeds and thus exit at different times.

As each chemical exits the chromatography column, it enters the mass spectrometer, where it is hit with a beam of electrons. This causes the chemical to ionize, or separate into charged particles. These particles are streamed through an electric or magnetic field that changes their direction, in a process known as deflection. The mass and charge of the particles determine the magnitude of the deflection. The sorted ions continue to a detector, which graphs the amount of each ion. Each different chemical breaks up into a different set of particles and thus has a different pattern in the graph. To identify the chemicals in the sample, the forensic toxicologist compares the pattern detected to a database of the patterns of known chemicals. Current databases include spectra for more than 700,000 different compounds.

A challenge to this process is that people who make illegal drugs constantly are making slight changes to the chemistry of
their drugs in an attempt to evade detection and identification of the compounds and laws that specify particular chemicals. Forensic toxicologists work with other researchers to identify and characterize these compounds, while medical researchers document their effects on the human body and health.

The gas chromatograph-mass spectrometer, or GC-MS, needs to be calibrated carefully before each use to ensure accuracy. It is expensive (can cost more than $250,000) and requires advanced training to maintain and operate. For that reason, specimens in North Carolina are sent to be analyzed by the forensic toxicology laboratory of the Office of the Chief Medical Examiner rather than by local laboratories. New liquid chromatograph-mass spectrometers are advancing forensic toxicology. These instruments enable analysis with a smaller sample, and the samples require less advance preparation (fewer extraction steps). Unlike the gas chromatograph, the liquid chromatograph doesn’t require high temperatures to volatilize the substances. This allows temperature-sensitive materials to be analyzed.

Beyond identifying the chemicals found in a specimen, the forensic toxicologist must quantify the amount found and analyze the likely contribution of that quantity to the death that is under investigation. The postmortem redistribution of drugs makes this analysis more challenging. After a person dies, many drugs diffuse from solid organs, the stomach, the intestines and muscle tissues into the blood and within blood vessels. As a result, different blood samples taken from different places in the body or at different times after death can produce completely different results.

The postmortem concentration of the drug in the blood often is much higher than it would have been if the blood sample had been taken before or at the time of death. This can make a death appear as though it was caused by an overdose of a drug when the amount of drug ingested actually was within the therapeutic range. The problem is further complicated because the rate of diffusion is different for different drugs and under different conditions. Forensic pathologists and toxicologists are studying postmortem redistribution in order to better
understand this phenomenon and interpret toxicological test results.

Hair could become important in forensic analysis because it holds the promise of providing information about ingestion of drugs or heavy metals over time. In addition, taking a hair sample might be preferred because it is much less invasive than obtaining a urine or blood sample. However, a significant limitation of using hair samples for forensic analysis is that chemicals that enter hair through environmental exposure, such as secondhand smoke, dust or contact with a contaminated surface, can’t be reliably differentiated from drugs that are ingested by the subject.

Dr. Jeri Ropero-Miller, of RTI International, in Research Triangle Park, N.C., is studying this problem. RTI’s research with testing for cocaine in hair has demonstrated that there currently are no effective methods — not even 10 weeks of daily shampooing — that can wash cocaine off hair when the cocaine has been acquired through environmental contamination. Studies have shown than a reliable distinction currently cannot be made between hair contaminated with cocaine in the laboratory and hair from cocaine users. These studies analyze the breakdown products produced by the body as it metabolizes cocaine, known as metabolites. This research has important implications in guidelines for drug screening programs.

**FORENSIC PATHOLOGISTS**
Forensic pathologists are medical doctors who specialize first in pathology, then in forensic pathology. Forensic pathologists carry out autopsies to document the cause and contributing factors to a death. They also may document injuries in accidents or assaults for legal purposes. They work closely with the entire medical examiner team to investigate and document the cause of death. They collect samples for forensic toxicologists to analyze. They collect and analyze critical evidence, such as blood or body fluids from an attacker found on a victim’s body.

**FORENSIC ANTHROPOLOGY**
Forensic anthropology is the application of physical anthropology and archeological techniques to recover, identify and investigate skeletal remains. Forensic anthropologists typically are not medical doctors but instead are trained as physical anthropologists, bioarchaeologists or primatologists. Most forensic anthropologists work as faculty and researchers in universities and act as consultants to law enforcement and medical examiners. A forensic anthropologist is needed when a body or part of a body is found skeletonized or in an advanced state of decay, when a body is found in a natural area or when assistance is needed in identifying bodily remains. In some cases, forensic anthropologists may be needed to determine whether bones belonged to a human or another type of animal. In addition to working on death
investigations in the U.S., forensic anthropologists have taken an important role in identifying victims and documenting mass graves in cases of disaster, mass murder and genocide.

Training in archeology helps forensic anthropologists find, document and decipher the position a person’s body was in when he or she died, as well as what might have happened afterward due to weather and disturbance by animals. Forensic anthropologists use archeological techniques to find and document the presence and relation of other items, such as clothing and possessions, at the scene of a death. Their training in physical anthropology helps them determine the age, sex, height and medical history of a person by analyzing his or her skeleton.

For example, the approximate age of a child at the time of death might be determined by the ossification of bones and eruption of adult teeth. The approximate age of an older person might be determined based on degeneration, wear and signs of arthritis. Sex is determined by the shape of the pelvic bones (wider in females) and the size of muscle attachment points in the skull (larger in males). Height can be estimated by reconstructing the skeleton even if some of its bones are missing. The bones also can provide clues about a person’s health before his or her death. A forensic anthropologist can note signs of malnutrition, how previous bone injuries healed, possible joint replacements and evidence of dental work. Various bone features can give clues as to a person’s ancestry as well. Signs of weathering along with knowledge of the weather in the location where a body is found can provide clues as to how long ago a person died. All this information can be combined and matched to databases of missing people to identify the remains. A forensic anthropologist also documents evidence related to how a person died, including unhealed bone fractures and other signs of trauma on the body and clues from the scene where his or her body was found.

An interesting new technique is the use of isotope analysis to determine where a person was born and spent his or her early years, as well as where he or she might have lived more recently. This analysis also can provide clues about a person’s diet, which can help identify the remains of someone who died far from home. For example, strontium is incorporated into the bones of humans no matter where they live through their drinking water — but different locations have different ratios of strontium’s different isotopes. The isotopes found in teeth, which form early in life, give clues as to where a person was born and spent the first few years of his or her life. Because bone tissue gradually is replaced throughout life, the ratio of isotopes in bone gives clues as to where a person lived during the last 10 to 15 years of life. Hair and fingernails can give clues as to
where a person lived during the final year of life. However, while isotope analysis provides clues that can help identify someone, it also has limitations. For example, a person might drink bottled water from different locations and eat foods from all over the world, which can create a confusing or misleading isotope signature.

**FORENSIC ODONTOLOGY**

Forensic odontology is the application of dental science to forensic cases. Dental evidence often is important for identifying crime victims if a body is in an advanced state of decay. By comparing a body's teeth to dental records, a positive identification can be made. Forensic dentists also analyze evidence from bite marks. Evidence that a bite mark matches a person's teeth has been used to convict people of rape and murder in the past. More recently, however, DNA evidence has exonerated some of these people and has cast doubt as to the accuracy of this technique.

**WILDLIFE FORENSIC SCIENCE**

Wildlife forensic science applies forensic techniques to cases involving nonhuman biological evidence. It includes both evidence from plants and animals in crimes with human victims and evidence in cases involving illegal wildlife trade and cruelty to animals.
LEARNING OUTCOMES
• Students will define mass spectroscopy.
• Students will identify and define ion, charge and mass.
• Students will define and calculate the mass-to-charge ratio.
• Students will use the basic facts to identify compounds using a mass spectra.

KEY VOCABULARY
• Mass spectra
• Liquid chromatograph-mass spectrometer (LC-MS)
• Mass-to-charge ratio

TIME REQUIRED
• Approximately 10 minutes of teacher prep time
• Approximately 90 minutes of class time for discussion and spectra analysis
MATERIALS REQUIRED
For 15 Lab Sets
• Coffee filter cut into 15 rectangular strips
• Pencil
• Washable marker
• Water
• 15 cups or beakers
• 3 copies of unknown cards
• 15 sets of known chemicals

BACKGROUND INFORMATION
A key tool used by a forensic pathologist is the liquid chromatograph-mass spectrometer, or LC-MS. One common use of this instrument is the analysis of a urine sample. The first step is to separate chemicals that may be drugs or toxins from the normal components of urine. The urine sample is mixed with a reagent and placed in a centrifuge. Depending on the reagent, either the top or bottom layer will have the chemicals of interest. This layer is kept and the other is discarded. The process then is repeated with different reagents until the waste has been removed. The remaining liquid sample, which is a mixture of chemicals, is placed in the chromatograph. The liquid chromatograph separates the chemicals so each can be analyzed separately.

The analysis of each chemical occurs in the mass spectrometer. First, the sample is bombarded and ionized, which means its electrons are removed so it will have a positive charge. The sample is broken into many different positive ions. The sample then enters the analyzer, which may be a magnetic field in which the ions move at different angles based on the mass of the particle. Finally, the particle enters the detector, where the abundance of a given mass/charge particle is identified. The relative abundance, or mass-to-charge ratio, is marked on the graph. Each chemical has a unique mass/charge signature pattern called a mass spectrum. Thus, when an unknown chemical is analyzed in the LC-MS, the mass spectrum then should be compared to a library of known chemicals. For more information about how to interpret mass spectra, visit Michigan State University’s Mass Spectrometer webpage, www.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectropy/MassSpec/massspec1.htm.

TEACHING NOTES
This activity is designed for students to understand the basic process that occurs in liquid chromatography-mass spectrometry. The students will be given an unknown chemical to
identify against a library of 10 known chemicals. Cut the unknown cards so each student can have 1 unknown card. A basic understanding of chemistry would be beneficial for students. Being able to calculate the atomic mass of a chemical from the chemical formula of a given ion is helpful. You may choose specific mass spectra for specific chemicals using RTI International’s Forensics Database, at forensicdb.org. Several of these are provided with the student worksheet.

**PROCEDURE**

Ask students, “What do you need to do to get a job?” Provide time for the students to discuss what must be done. Students may list the following: fill out an application, be interviewed, provide references, complete a drug test. If the students do not list drug test/screening, explain that many jobs require this on a regular basis. A drug screening is done on a urine sample, or “aliquot,” which is split into two portions. One portion is tested to see if there is evidence of certain drugs or toxins. If it is positive for these chemicals, the second portion is run through an LC-MS or GC-MS (gas chromatograph-mass spectrometer) to verify the presence and identity of the drug.

Chromatography is the separation of a mixture of chemicals. Once separated, these chemicals are sent into the mass spectrometer. Ask students to brainstorm what a mass spectrometer might do. If the students need assistance, help the students break the word down into its parts. Mass indicates the mass of the chemical is involved, spectro- means a range or continuum and -meter indicates a device to measure. From this simple analysis, the basic understanding of a mass spectrometer can be developed.

Before a specific chemical can be identified, the waste must be washed away. (This process is described in the Background Information section above.) The remaining sample often is a mixture of chemicals, which must be separated. A liquid chromatograph is one method for doing this. It takes the liquid and allows different chemicals to exit at different times. This can be demonstrated to the students through coffee filter chromatography.

Divide students into pairs. Give each pair a cup filled with a half an inch of water. On a coffee filter strip, have students draw a pencil line parallel to the short end of the rectangle and 1 inch above the short end. Place a dot of the washable marker on the pencil line. Next, have students put the pencil line above the water. If the coffee filter will not stand up away from the side of the cup, tape the opposite end of the coffee filter to a pencil and lay the pencil across the top of the cup. After a few minutes the washable marker components will
begin to travel up the coffee filter. Explain to the students that just as the marker components travel at different rates, so do the chemicals in the chromatograph.

The mass spectrometer then will analyze each chemical. First, the sample will be ionized, which means it will have a charge. This can be created by electrons bombarding the sample, which creates a positively charged sample. The ions then are separated by the analyzer. Try to visualize spraying water onto balls rolling down a hill in an attempt to change their path. The path of a bowling ball barely would be affected, the path of a tennis ball could be affected somewhat and the path of a Ping-pong ball would be affected significantly. When a sample enters the analyzer, chemicals with smaller atomic masses have a larger change in path, while those with larger atomic masses have smaller changes in path. The ionized samples then enter the detector, where the relative abundance and the mass-to-charge ratio is graphed for the sample. This creates a mass spectrum, which can be used as a chemical fingerprint to identify chemicals.
The common chemicals that are scanned in a urine drug screening are found in the chart below. (Visit dig.pharm.uic.edu/faq/2011/Feb/faq1.aspx for more information.)

<table>
<thead>
<tr>
<th>CHEMICAL NAME</th>
<th>CHEMICAL FORMULA</th>
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<tr>
<td>Amphetamine</td>
<td>C₉H₁₃N</td>
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<tr>
<td>Codeine</td>
<td>C₁₈H₂₁NO₃</td>
</tr>
<tr>
<td>Diacetylmorphine (heroin)</td>
<td>C₂₁H₂₃NO₅</td>
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<tr>
<td>Methamphetamine</td>
<td>C₁₀H₁₅N</td>
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<tr>
<td>Morphine</td>
<td>C₁₇H₁₉NO₃</td>
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<tr>
<td>Oxycodone</td>
<td>C₁₈H₂₁NO₄</td>
</tr>
<tr>
<td>Tetrahydrocannabinol (marijuana)</td>
<td>C₂₁H₃₀O₂</td>
</tr>
</tbody>
</table>

Explain to students that in this activity they will be taking the position of a bench chemist at a clinical laboratory who is analyzing a urine sample for a drug screen. Explain that teams of students will be given an unknown spectrum and a selection of known mass spectra. The students will be expected to match the unknown chemical to a known chemical and explain why this match was chosen. Students may choose to use math and/or vocabulary to explain the testing vocabulary.

After completing the student worksheet, give students the opportunity to discuss their findings with the class.

**ASSESSMENT**
Students may complete the student worksheet. Collect unknown spectra from students and pass out another unknown spectrum with a list of known spectra for the students to identify.
### Key for Unknown Spectra

<table>
<thead>
<tr>
<th>CHEMICAL #</th>
<th>CHEMICAL NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amphetamine</td>
</tr>
<tr>
<td>2</td>
<td>Methamphetamine</td>
</tr>
<tr>
<td>3</td>
<td>Tetrahydrocannabinol (marijuana)</td>
</tr>
<tr>
<td>4</td>
<td>Codeine</td>
</tr>
<tr>
<td>5</td>
<td>Diacetylmorphine (heroin)</td>
</tr>
<tr>
<td>6</td>
<td>Morphine</td>
</tr>
<tr>
<td>7</td>
<td>Oxycodone</td>
</tr>
</tbody>
</table>

### Printable Cards for Unknown Spectra

<table>
<thead>
<tr>
<th>CHEMICAL #</th>
<th>MASS SPECTRUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Mass Spectrum Graph" /></td>
</tr>
</tbody>
</table>
EXTENSION
This activity may be extended by learning more about mass spectroscopy at RTI International's Forensic Science Education website. Visit https://www.forensiced.org/training/alltraining.cfm and click on the Fundamentals of Mass Spectrometry used in Toxicology course.
Many companies require a urine drug screen. Clinical laboratories hire chemists to run the samples through a liquid chromatograph-mass spectrometer, or LC-MS, for analysis. Today you will be given an unknown spectrum to identify.

**PROCEDURE**

**Unknown Chemical #: _____**

1. Which chemical do you think this is?

2. What do the numbers mean on the x-axis?

3. Why are the peaks at different heights?
4. What is the last peak on the right of the graph?

DISCUSSION QUESTIONS

1. How do you calculate the atomic mass of the chemical you identified?

2. What steps does a sample go through in the liquid chromatograph-mass spectrometer?
## Known Chemicals

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Chemical Formula</th>
<th>Mass Spectrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>C&lt;sub&gt;9&lt;/sub&gt;H&lt;sub&gt;13&lt;/sub&gt;N</td>
<td><img src="image" alt="Amphetamine Mass Spectrum" /></td>
</tr>
<tr>
<td>Codeine</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td><img src="image" alt="Codeine Mass Spectrum" /></td>
</tr>
<tr>
<td>Diacetylmorphine (heroin)</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;NO&lt;sub&gt;5&lt;/sub&gt;</td>
<td><img src="image" alt="Diacetylmorphine Mass Spectrum" /></td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Chemical Formula</td>
<td>Mass Spectrum</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>$C_{10}H_{15}N$</td>
<td><img src="image" alt="Methamphetamine Mass Spectrum" /></td>
</tr>
<tr>
<td>Morphine</td>
<td>$C_{17}H_{19}NO_3$</td>
<td><img src="image" alt="Morphine Mass Spectrum" /></td>
</tr>
<tr>
<td>Oxycodone</td>
<td>$C_{18}H_{21}NO_4$</td>
<td><img src="image" alt="Oxycodone Mass Spectrum" /></td>
</tr>
<tr>
<td>CHEMICAL NAME</td>
<td>CHEMICAL FORMULA</td>
<td>MASS SPECTRUM</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Tetrahydrocannabinol (marijuana)</td>
<td>$C_{21}H_{30}O_2$</td>
<td><img src="image" alt="Mass Spectrum" /></td>
</tr>
</tbody>
</table>
SECTION 5
UNCERTAINTY IN MEDICAL TESTING
Imagine it is summer, but you are feeling terrible. You woke up in the middle of the night with a fever and every muscle aches. You have a sore throat and a headache and are beginning to cough and wheeze. Is it flu? Or is it something else? You have a summer job as a camp counselor but your boss takes one look at you and suggests you go to the doctor rather than exposing all the children to your germs. Your doctor examines you carefully and says, “Flu would be unusual this time of year, but we’ll do a rapid flu test just to be sure.” Fifteen minutes later the test comes back positive and the doctor writes you a prescription for an antiviral medication.

Because your mom has chronic pulmonary disease, the doctor suggests she visit her health provider and possibly take antiviral medication to prevent herself from getting the flu as well. Your mom wonders if the rapid flu test might have been a false positive. You wonder, “What is a false positive, and what are the chances of getting one?”

Medical tests, including both diagnostic and forensic tests, usually are very accurate. But as with any type of testing, they have a certain amount of inherent error. Because diagnostic tests have important consequences for making a treatment plan, it is important for medical practitioners and patients to understand the probability of different types of errors in different situations. Similarly, it is important for forensic scientists to explain clearly the
limits of accuracy in forensic tests. Most people, even medical practitioners, underestimate the chances of errors in diagnostic test results. In some cases this isn’t all that important, but in other cases an incorrect test result can have serious health or legal consequences. For that reason, it often is important to repeat tests and have multiple lines of evidence when making a diagnosis or solving a crime.

**TYPES OF ERROR**

To understand error analysis in medical testing, we first need to discuss some vocabulary with specific meanings in this context. In diagnostic testing (and in statistics in general), a **true positive** is when a test shows positive for a condition or disease that truly is present. A **false positive** is when a test shows something that isn’t really there. For example, a false positive strep test would show a person has strep throat when, in fact, he or she does not. A **true negative** is when a test shows negative for a condition that really isn’t present. A **false negative** is when a test fails to detect a condition that a person really has.

<table>
<thead>
<tr>
<th>Person actually has strep throat</th>
<th>Person does not actually have strep throat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test result is positive</strong></td>
<td><strong>Test result is negative</strong></td>
</tr>
<tr>
<td><strong>TRUE POSITIVE</strong></td>
<td><strong>FALSE NEGATIVE</strong></td>
</tr>
<tr>
<td><strong>TEST IS CORRECT</strong></td>
<td><strong>TEST IS INCORRECT</strong></td>
</tr>
<tr>
<td><strong>FALSE POSITIVE</strong></td>
<td><strong>TRUE NEGATIVE</strong></td>
</tr>
<tr>
<td><strong>TEST IS INCORRECT</strong></td>
<td><strong>TEST IS CORRECT</strong></td>
</tr>
</tbody>
</table>

False negatives and false positives create further problems. In the case of a false positive strep test, the doctor might prescribe an antibiotic that the patient doesn’t actually need. While this might not be a big problem, it does have additional negative consequences. The incorrect test result may cause the doctor to miss the diagnosis of a different disease that needs a different medication. In addition, the patient unnecessarily is subjected to the cost and side effects of the antibiotic and, in a few cases, might have an allergic reaction to the antibiotic. Unnecessary use of antibiotics also is linked to the development of antibiotic-resistant microbes, which causes an overall increase in the difficulty of treating infections. In the case of a false negative strep test, the doctor does not prescribe an antibiotic that the patient actually does need. While some patients’ immune systems will throw the infection off without medication, other patients will get sicker, leading to heart and kidney damage in some cases. For this reason, a doctor might order repeat or additional tests if the test results don’t match the clinical picture.

The U.S. Food and Drug Administration, or FDA, requires companies that develop and manufacture diagnostic tests to determine the sensitivity and specificity of these tests. **Sensitivity** is the measure of how often a test will detect a condition when the condition is present. If you take 100 people who are known with certainty to have a medical condition and the test
detects 96 cases, the test has a sensitivity of 96%. To show this as a mathematical formula:

\[
\text{Sensitivity} = \frac{\text{TruePositives}}{\text{TruePositives} + \text{FalseNegatives}}
\]

When we use the values from the example above, the formula looks like this:

\[
\text{Sensitivity} = \frac{96}{96 + 4}
\]

**Specificity** is the measure of how often a test will give negative results for a condition when the condition is not present. If you take 100 people who are known with certainty not to have a medical condition and the test gives 2 false positives and 98 correct negatives, the test has a specificity of 98%. To show this as a mathematical formula:

\[
\text{Specificity} = \frac{\text{TrueNegatives}}{\text{TrueNegatives} + \text{FalsePositives}}
\]

When we use the values from the example above, the formula looks like this:

\[
\text{Specificity} = \frac{98}{98 + 2}
\]

Ideally, all tests would be 100% sensitive and 100% specific. In other words, they would catch all cases of the disease and produce no false positives. In practice, no tests are this accurate. In order to make the best decision about further tests and treatment, the patient and doctor need to know the chances of a false positive or false negative. Although it seems counterintuitive, the chances of a false positive or false negative depend not only on the accuracy of the test but also on the prevalence of the disease condition in the population. This can vary with the way the test is being used. For example, if a test is being given to everyone in a population as a screening tool, the population prevalence is lower than if the test is being given only to patients who have symptoms.

**EXAMPLE SCENARIOS**

To see how the prevalence of a disease affects the chance of a false positive or false negative test result, let’s examine two scenarios.

**Scenario #1: High Prevalence**

It is flu season in the United States (between October and May). The prevalence of the flu in people who visit the doctor with respiratory illness is 40%. The doctor is using a rapid flu test with a sensitivity of 70% and a specificity of 90%. The tree diagram on the next page is a good way to analyze this situation.

Of the 340 people who get a positive result (280 true positives + 60 false positives), only 82% actually have the flu. The other 18% have a false positive result. In other words, if you get a positive result in this scenario, your result is correct 82% of the time — and wrong the other 18% of the time. Likewise, 660 people got a negative test result (120 false negatives + 540 true negatives). Of these, 540 instances, or 81%,
are correct, while 19% are incorrect (false negatives). This means that 19% of the time in this scenario, negative test results are incorrect and the person really does have the flu.

**Scenario #2: Low Prevalence**

It is summer in the U.S., and flu prevalence is low. Only 5% of the people who visit the doctor with respiratory illness actually have the flu. The doctor is using the same rapid flu test, with a sensitivity of 70% and specificity of 90%. The tree diagram below is a good way to analyze this situation.

In Scenario #2, 130 people get a positive result but only 35 are true positives. If you receive a positive result in this scenario,
it is correct only 27% of the time. The other 73% of positive test results are false positives. In this scenario, 870 people get negative test results. Of these negatives, 855 instances, or 98%, are correct negative results.

Comparing Scenarios
Looking at Scenarios #1 and #2, we can see that in low prevalence situations, negative results are likely to be correct but positive results include a high rate of false positives. Screening tests, such as mammograms for breast cancer, PSA tests for prostate cancer and many newborn screening tests, often are done in very low prevalence situations. In these situations, a negative result usually is correct, but the high rate of false positives means that a positive test always should be followed up by further testing to see whether it was a true positive.

Sources of Error
While medical and laboratory professionals work extremely hard to minimize errors in lab test results, these errors still occur at every stage, from collecting and labeling patient specimens to reporting results. Some errors in results are due to the natural variability of biological processes. For instance, a patient with a weak immune system might produce a very low number of antibodies to a disease. This means that a test relying on antibodies to determine infection will miss the disease in this person.

Error analysis allows laboratories to figure out where in their process problems are occurring and to change the process in order to minimize errors. For example, laboratories may change a form or institute a new or different checklist for specimen collection. Hospital and reference laboratories regularly test their accuracy by analyzing known samples. This enables them to detect problems with procedures or instruments, which they then can fix. Finally, medical professionals and patients need to understand the limits of accuracy in any one diagnostic test and be sure to use the patient’s total clinical presentation and additional tests to confirm diagnoses.

Resources
Math is Fun
The False Positives and False Negatives page of the Math is Fun website contains a great explanation and many sample problems. Visit mathsisfun.com/data/probability-false-negatives-positives.html.

Lab Tests Online
Lab Tests Online is produced by the American Association for Clinical Chemistry and has clear, peer-reviewed, explanatory materials for many common laboratory tests. For an excellent article on sources of error and how these are minimized in laboratories, visit labtestsonline.org/understanding/features/reliability.
Centers for Disease Control and Prevention
The Rapid Diagnostic Testing for Influenza page of the CDC website presents information on the sensitivity and specificity of rapid flu tests and how they affect the rate of false negatives and false positives. Visit cdc.gov/flu/professionals/diagnosis/rapidlab.htm.

Medical False Positives and False Negatives (Conditional Probability)
This article, by Stan Brown of Oak Road Systems, explains why the prevalence of a disease is so important in understanding diagnostic test results. Visit tc3.edu/instruct/sbrown/stat/falsepos.htm.
WHAT IS A FALSE POSITIVE?

TEACHER ACTIVITY OVERVIEW

LEARNING OUTCOMES

• Students will define true positives, true negatives, false positives and false negatives.
• Students will explain the difference between sensitivity and specificity.
• Students will solve statistical problems involving false positives.
• Students will explain uncertainty in medical testing results in relation to the prevalence of the tested condition in the population.

KEY VOCABULARY

• True positive
• True negative
• False positive
• False negative
• Sensitivity
• Specificity

TIME REQUIRED

• Approximately 10 minutes of teacher prep time
• Approximately 90 minutes of class time for lab and discussion
BACKGROUND INFORMATION
“Your Pap screening test is positive.” “Your strep test results are negative.” “It might be a false positive, so we’ll have to do some more tests.”

Patients hear statements like these from doctors every day. What do they mean? Millions of medical tests are done each month in the United States. While most of these tests are accurate, even a small percentage of erroneous test results causes anxiety, missed diagnoses and extra expenses. This activity is designed to get students thinking about the issues involved with medical testing.

It is important for the public to understand — and for medical professionals to be able to explain — the inherent uncertainty in test results. Moreover, the extent of the uncertainty depends not only on the test itself, but also on the underlying prevalence of the condition in the population being tested. In order to discuss the issues involved with medical test results, students need to understand some specialized terms. “Positive” generally means that the condition being tested for has been found to be present. A true positive is a positive test result for a person who actually has the condition being tested for. A false positive is a positive test result for a person who does not actually have the condition. A true negative is a negative test result for a person who does not actually have the condition. A false negative is a negative test result for a person who actually has the condition.

These possibilities are often illustrated in a table, such as this one for a strep throat test:

<table>
<thead>
<tr>
<th>Person actually has strep throat</th>
<th>Test result is positive</th>
<th>Test result is negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>True positive</td>
<td>TRUE POSITIVE TEST IS CORRECT</td>
<td>FALSE NEGATIVE TEST IS INCORRECT</td>
</tr>
<tr>
<td>False negative</td>
<td>FALSE POSITIVE TEST IS INCORRECT</td>
<td>TRUE NEGATIVE TEST IS CORRECT</td>
</tr>
</tbody>
</table>

Sensitivity is the measure of how often a test will detect a condition when the condition is present. It is calculated by dividing the number of true positives by the sum of the number of true positives and false negatives. To show this as a mathematical formula:

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$
Specificity is a measure of how often the test results are negative when the patient does not have the condition that is being tested for. It is calculated by dividing the number of true negatives by the sum of the number of true negatives and false positives. To show this as a mathematical formula:

\[ \text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}} \]

Sensitivity and specificity typically are expressed as percentages. Remember to multiply by 100 to convert the result from a decimal to a percentage. Sensitivity and specificity do not depend on the prevalence of the disease condition, but they do depend on the specific test.

**TEACHING NOTES**

This activity is designed to provide students with an opportunity to understand the uncertainty inherent in medical diagnostic testing. Students will look at the test results for a population to determine the sensitivity and specificity of the test. They will use given sensitivity and specificity values for a diagnostic test and information about the prevalence of the disease within a population to determine the number of true positives, true negatives, false positives and false negatives.

**PROCEDURE**

Begin by flipping a coin. Ask students the probability that the coin will be heads. It is 50%, regardless of how many times the coin is flipped. Every time the coin is flipped the probability it will be heads is 50%.

Draw 3 doors on the board or show the students the Advanced Monty Hall activity from the Shodor website (shodor.org/interactivate/activities/AdvancedMontyHall). Explain that behind one door is a prize, while behind the others is a “zonk,” or a silly item such as a pig or goat. Ask a student to choose the door that he or she thinks the prize is behind.

Show the student that a zonk is behind one of the other doors. Now, ask if student wants to switch his or her choice to the third door. The student is more likely to get the prize if he or she chooses the third door rather than the original, first choice. This is called conditional probability because the second choice is dependent on the first choice.

The statistical proof that changing doors is more likely to win the prize requires a large number of trials. Students would need to simulate this 100 or more times to see the statistical proof. If you use the Advanced Monty Hall activity, you can run the simulation 1,000
times in just a few seconds for the both scenario where the student changes the door choice and the scenario where he or she does not. Conditional probability is the basis for the statistics behind these medical statistics.

**Scenario #1**
Continue the discussion by introducing a medical testing scenario with which the students have experience. For example: “You have a scratchy sore throat so you go to the doctor to find out what is wrong. The doctor gives you a rapid strep test and takes a swab for a throat culture. The rapid strep test comes back negative, and the doctor tells you to go home and get some rest. You go home but your throat and lymph nodes swell, you develop a high fever and you experience a lot of pain when swallowing. Two days later, your doctor calls to say your throat culture came back positive and you have strep throat. The doctor orders you the proper medication. Afterward, you wonder how could you have a strep throat when your first test came back negative.” Provide time for the students to share their thoughts about the results.

Explain to students that the rapid strep test provided a false negative. Use the definitions below to explain the four possible outcomes for the test.

- True positive: A positive test result for a person with the illness
- False positive: A positive test result for a healthy person
- True negative: A negative test result for a healthy person
- False negative: A negative test result for a person with the illness

In the example above, the strep test provided a false negative for the person who had strep. Statistically, the chance of a false positive or false negative can be analyzed using sensitivity and specificity for a particular test. Most rapid strep tests have a sensitivity of 95%, and the specificity of the rapid strep test is 98%. (Visit medicinenet.com/rapid_strep_test/page3.htm for more information.) Different tests — even ones for the same medical condition — have different sensitivities and specificities. These also may differ under different environmental conditions.

**Scenario #2**
In this scenario, the manufacturer reports that sensitivity of the strep test is 95% and the specificity is 98%. Let’s look at a population of 1,000 people who visit a clinic complaining of a sore throat. Of this population, 30% of people actually have strep throat. The tree diagram on the next page is a good way to analyze this situation.
This information also can be presented in a chart form. Below is an example of the blank chart that can be used for any set of data.

<table>
<thead>
<tr>
<th>People who actually have illness</th>
<th>People who tested positive</th>
<th>People who tested negative</th>
<th>Total people</th>
</tr>
</thead>
<tbody>
<tr>
<td>People with strep throat</td>
<td>TRUE POSITIVE TEST IS CORRECT</td>
<td>FALSE NEGATIVE TEST IS INCORRECT</td>
<td>WHO HAVE ILLNESS</td>
</tr>
<tr>
<td>People without strep throat</td>
<td>FALSE POSITIVE TEST IS INCORRECT</td>
<td>TRUE NEGATIVE TEST IS CORRECT</td>
<td>WHO DO NOT HAVE ILLNESS</td>
</tr>
<tr>
<td>Total tests</td>
<td>POSITIVE TESTS</td>
<td>NEGATIVE TESTS</td>
<td>OVERALL POPULATION</td>
</tr>
</tbody>
</table>

If you wanted to solve the strep throat problem with the chart, it would begin like this:

<table>
<thead>
<tr>
<th>People who tested positive</th>
<th>People who tested negative</th>
<th>Total people</th>
</tr>
</thead>
<tbody>
<tr>
<td>People with strep throat</td>
<td></td>
<td>$1,000 \times 0.3 = 300$</td>
</tr>
<tr>
<td>People without strep throat</td>
<td></td>
<td>$1,000 \times 0.7 = 700$</td>
</tr>
<tr>
<td>Total tests</td>
<td></td>
<td>$1,000$</td>
</tr>
</tbody>
</table>
The number of people who tested positive and negative then can be calculated:

<table>
<thead>
<tr>
<th>People who tested positive</th>
<th>People who tested negative</th>
<th>Total people</th>
</tr>
</thead>
<tbody>
<tr>
<td>People with strep throat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$300 \times 0.95 = 285$</td>
<td>$300 \times 0.05 = 15$</td>
<td>$300$</td>
</tr>
<tr>
<td><strong>285</strong> TRUE POSITIVES</td>
<td><strong>15</strong> FALSE NEGATIVES</td>
<td></td>
</tr>
<tr>
<td>People without strep throat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$700 \times 0.02 = 14$</td>
<td>$700 \times 0.98 = 686$</td>
<td>$700$</td>
</tr>
<tr>
<td><strong>14</strong> FALSE POSITIVES</td>
<td><strong>686</strong> TRUE NEGATIVES</td>
<td></td>
</tr>
<tr>
<td>Total tests</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$299$</td>
<td>$701$</td>
<td>$1,000$</td>
</tr>
</tbody>
</table>

This table is an alternative to a tree diagram. You can use this table to determine that the overall chance of incorrect results in this scenario is 2.9% (29 false results ÷ 1,000 total results × 100). If you have a positive result in this scenario, the chance it is a false positive is 4.7% (14 false positive results ÷ 299 positive results × 100).

Explain that students will need to work with a “patient” to discuss medical test results and the statistics behind the testing. Students will work with a partner to define vocabulary words in reference to the testing. They also will use math to explain the testing vocabulary.

After they complete the student worksheet, give students the opportunity to discuss this information with the class. Allow students to voice their views on testing and what the statistics mean.

**ASSESSMENT**
Students may complete the student worksheet.

**EXTENSION**
This activity may be extended by researching the statistics for a diagnostic test purchased at a local pharmacy, such as pregnancy test or urinalysis.
Medical test results do not always provide a clear or accurate picture of a patient’s medical condition. Consider this example: A patient receives a positive ELISA test, which is used to screen blood donations for HIV. For a couple weeks until her next doctor’s appointment, the patient worries she is a carrier of HIV. However, once she goes back to the doctor and further tests are completed, the tests come back negative. The patient is shown to not be a carrier of HIV. Still, she wonders why and how she could have received the initial, positive test result.

**PROCEDURE**

The sensitivity of the ELISA test is 97.7%. The specificity of the test is 92.6%. (Visit [amstat.org/publications/jse/v3n2/rossman.html](http://amstat.org/publications/jse/v3n2/rossman.html) for more information.) Based on past data, 0.5% of the United States population carries HIV.
1. What are the terms for the 4 possible test results? Complete the following chart.

<table>
<thead>
<tr>
<th>Test result is positive</th>
<th>Test result is negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person actually has the disease</td>
<td></td>
</tr>
<tr>
<td>Person does not actually have the disease</td>
<td></td>
</tr>
</tbody>
</table>

2. What is sensitivity?

3. What does a sensitivity of 97.7% mean?

4. What is specificity?
5. What does a specificity of 92.6% mean?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

6. In a sample population where 0.5% of people have HIV, how many people out of 100,000 have HIV? How many people out of 100,000 do not have HIV?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

7. How many true positives will the test show?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

8. How many false positives will the test show? If a person has a positive test result in this scenario, what is the chance it is a false positive?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
9. How many true negatives will the test show?

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________

10. How many false negatives will the test show?

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________

11. In some parts of Africa, the prevalence of HIV is 10%. If the prevalence is 10%, how many people are infected? How many are not infected?

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________

12. Calculate the number and percentage of true and false positives and negatives for a population where the prevalence is 10%.

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________

__________________________________________________________________
DISCUSSION QUESTIONS

1. What is the difference between a true positive and a false positive?

2. Explain the problem with the following statement: “A positive test results means the patient definitely has the disease.”
3. How are sensitivity and specificity different?