



Testing Biopsy and Cytology Specimens for Cancer

Waiting to hear a possible cancer diagnosis can be very stressful. But a better understanding of the tests doctors use to diagnose and classify cancer may relieve some of your stress. Learning about the testing process can also help you understand how test results affect treatment options. It can also help you work with your doctors to make the best decisions about your treatment.

Much of the testing process takes place "behind the scenes." You will have a chance to meet and ask questions of most of your health care team, which may include a surgeon, medical oncologist, radiation oncologist, oncology nurses, and others. You will be able to see what these professionals do. On the other hand, you rarely meet the pathologists, histotechnologists, cytotechnologists, and medical laboratory technologists whose work can tell you whether the cells in your biopsy sample are malignant (cancer) or benign (not cancer).

How is cancer diagnosed?

A cancer diagnosis is nearly always made by an expert looking at cell or tissue samples under a microscope. In some cases, lab tests of the cells' proteins, DNA, and RNA can help tell doctors if cancer is present. These tests may also help in choosing the best treatment options. Tests of cells and tissues can find many other kinds of diseases, too. For example, if doctors are not sure a lump is cancer, they may have the sample tested for cancer and for germs that have been linked to cancer (like HPV, human papilloma virus). The procedure that takes a sample for this testing is called a biopsy, and the tissue sample is called the biopsy specimen. The testing process is sometimes referred to as pathology.

Lumps that might be malignant (cancer) may be found by imaging (radiology) studies or felt as masses (lumps) during a physical exam, but they still must be sampled and looked at under a microscope to find out what they are. Not all lumps are malignant. In fact, most tumors are benign (not cancer). A malignant tumor is able to spread into nearby tissues and even to distant parts of the body. A benign tumor cannot do this.

Overview of biopsy types

Tissue or cell samples can be taken from almost any part of the body. How this is done depends on where the tumor is and what type of cancer is suspected. For instance, the methods used for skin biopsies clearly need to be different from those done for brain biopsies.

Some types of biopsies remove an entire organ. These types are done only by surgeons. Other types of biopsies may remove tumor samples through a thin needle or through an endoscope (a flexible lighted tube). These biopsies are often done by surgeons, but can also be done by other doctors. The most common biopsy types used in cancer diagnosis are discussed in this section. For more complete information, refer to the diagnosis section of our detailed documents that cover specific types of cancer.

Needle biopsy

There are 2 types of needle biopsies:

- Fine needle biopsy (also called fine needle aspiration)
- Core needle biopsy (also called core biopsy)

Fine needle aspiration

Fine needle aspiration (FNA) uses a very thin needle and a syringe to withdraw a small amount of fluid and very small pieces of tissue from the tumor. The doctor can aim the needle while feeling a suspicious tumor or area near the surface of the body. If the tumor is deep inside the body and cannot be felt, the needle can be guided while being watched by imaging procedures such as an ultrasound or CT scan. The main advantages of FNA are that it does not require an incision (cutting through the skin) and that in some cases it is possible to make a diagnosis the same day. The disadvantage is that sometimes this needle cannot remove enough tissue for a definite diagnosis. Although FNA is a type of biopsy, it is also classified as a cytology test (see next section).

Core biopsy

Core biopsy uses needles that are slightly larger than those used in FNA. They remove a small cylinder of tissue (about 1/16 inch in diameter and 1/2 inch long). The core needle biopsy is done using local anesthesia (drugs used to make the area numb) in the doctor's office or clinic. Like FNA, a core biopsy can sample tumors that can be felt by the doctor as well as smaller ones that must be seen using imaging studies. Doctors sometimes use special vacuum tools to get larger core biopsies from breast tissue. (For more information, see our document, *For Women Facing a Breast Biopsy*.) Processing core biopsy samples usually takes longer than processing FNA biopsies.

Excisional or incisional biopsy

With this type of biopsy, a surgeon cuts through the skin to remove the entire tumor (called an excisional biopsy) or a small part of a large tumor (called an incisional biopsy). This is often done using local anesthesia or regional anesthesia (drugs used to make the area numb). If the tumor is inside the chest or abdomen (belly), general anesthesia is used (drugs are used to put the patient into a deep sleep so no pain is felt).

Endoscopic biopsy

An endoscope is a thin, flexible, lighted tube that has a lens or a video camera on the end. It can allow a doctor to look inside different parts of the body. Tissue samples can also be taken out through the endoscope to find out if cancer is present and, if so, the type.

Different endoscopes are used to look at different parts of the body. For example, one type of endoscope is used to look at the inside of the nose and sinuses, the throat, and the voice box (trachea). Another type of endoscope is used to look at the upper part of the digestive tract: the esophagus (the tube that connects the mouth to the stomach), the stomach, and the first part of the intestine.

Some endoscopes are named depending on the part they are used to look at. For instance, a cystoscope is used to look at the urine tube (urethra) and bladder, a hysteroscope to look at the uterus (womb), a bronchoscope to look at the lungs and breathing tubes (bronchi), and a colonoscope to look at the colon.

Laparoscopic, thoracoscopic, and mediastinoscopic biopsy

Laparoscopy is much like endoscopy but uses a slightly different scope (a laparoscope) to look inside the abdomen (belly) and remove tissue samples. A small incision (cut) is made in the abdomen then the laparoscope is passed through this opening to see inside. Procedures like this that look inside the chest are called thoracoscopy and mediastinoscopy.

Laparotomy and thoracotomy

A laparotomy is a type of surgery that involves an incision into the abdomen, usually a vertical cut from upper to lower abdomen. This may be done when there is uncertainty about a suspicious area that cannot be diagnosed by less invasive tests (like a needle biopsy or laparoscopy). During the laparotomy, a biopsy sample can be taken from a suspicious area. The doctor can also look at the size of the area and where it is located. Nearby tissues can be checked, too. General anesthesia is used for this technique (drugs are used to make the patient sleep and not feel pain). A similar operation which opens the chest is called thoracotomy.

Skin biopsies

There are many ways to take a biopsy of the skin. Doctors choose the one best suited to the type of skin tumor suspected. Shave biopsies remove the outer layers of skin and are fine for some basal cell or squamous cell skin cancers, but they are not recommended for suspected melanomas of the skin. *Punch biopsies* or excisional biopsies (as discussed above) remove deeper layers of the skin, and can find out how deeply a melanoma has gone into the skin -- an important factor in choosing treatment for that type of cancer.

Sentinel lymph node mapping and biopsy

Lymph node mapping helps the surgeon know which lymph nodes to remove for an excisional biopsy. Sentinel node mapping and biopsy has become a common way to find out whether the cancer (especially melanoma and breast cancer) has spread to the lymph nodes. This procedure can find the lymph nodes that drain lymph fluid from the area where the cancer started. If the cancer has spread, these lymph nodes are usually the first place it will go. This is why these lymph nodes are called "sentinel" nodes (meaning that they stand watch over the tumor area, so to speak).

To find the sentinel lymph node (or nodes), the doctor injects a small amount of slightly radioactive material into the area of the cancer. By checking various lymph node areas with a machine that detects radioactivity (like a Geiger counter), the doctor can find the group of lymph nodes the cancer is most likely to travel to. Then the doctor injects a small amount of a harmless blue dye into the site of the cancer. After about an hour, a surgeon makes a small incision in the lymph node area that was found with the radioactive test. Those lymph nodes are then checked to find which one(s) turned blue or became radioactive. (Sometimes the dye and the radioactive material may be mixed together, or either part may be used alone.)

When the sentinel node has been found, it is removed (an excisional biopsy) and looked at under a microscope. If the sentinel node does not contain cancer cells, no more lymph node surgery is needed because it is very unlikely the cancer would have spread beyond this point. If cancer cells are found in the sentinel node, the rest of the lymph nodes in this area are removed and looked at, too. This is called a lymph node dissection.

Overview of cytology types

Diagnosing diseases by looking at single cells and small clusters of cells is called cytology or cytopathology. It has become an important part of cancer diagnosis over the past few decades. While the pieces of tissue in biopsy samples may be as small as 1/16 inch or much larger (several inches), the individual cells and the cell clusters in cytology samples are usually too small to see without a microscope. Sometimes, as in some fine needle aspiration (FNA) samples, only one drop of blood or tissue fluid (containing tiny bits of the tumor) is taken. On the other hand, some pleural fluid (from around the lung) or peritoneal fluid (from inside the belly) samples may include a quart or more of fluid.

Compared with tissue biopsy, a cytology specimen usually:

- Is easier to get
- Causes less discomfort to the patient
- Is less likely to result in serious complications
- Costs less

The disadvantage is that, in some cases, a tissue biopsy result is more accurate, though in many cases the cytology fluid may be just as accurate.

Sometimes an excisional biopsy (removing the entire tumor) is the only treatment needed to remove a cancer -- which is a clear advantage. In other cases, a cancer might be better treated by chemotherapy or radiation therapy, and surgery might be done after these treatments. For those types of cancer, a cytology sample, endoscopic biopsy, core needle biopsy, or incisional biopsy might be a better choice. As you can see, choices of tests are not simple -- the doctors consider many factors about the specific type of cancer that is suspected and what organ is affected.

Cytology tests may be used in 2 ways -- for diagnosis or for screening.

A diagnostic test is only used for people who have signs, symptoms, or some other reason to suspect that a particular disease (like cancer) may be present. A diagnostic test finds out if a disease is present and, if so, it precisely and accurately classifies the disease.

A screening test is used to find people who might have a certain disease even before they develop symptoms. A screening test is expected to find nearly all people who are likely to have the disease, but the screening test does not always prove that the disease is present. Often, a diagnostic test is used if a screening test is positive (that is, if something is found on the screening test). Some cytology tests, such as the Pap test (see "Scrape or brush cytology"), are mainly used for screening. Others can accurately identify cancers (see "Scrape or brush cytology" below). When cytology shows cancer, often a biopsy is also done to be sure of any abnormal finding before treatment is started.

Fine needle aspiration biopsy

Fine needle aspiration (FNA) is sometimes considered a cytology test and is sometimes considered a biopsy. It is discussed in the section, "Overview of biopsy types."

Body fluids

Fluids from cavities and spaces in the body can be tested to see if cancer cells are present. Some of the body cavity fluids tested in this way include:

- Urine
- Sputum (phlegm)
- Spinal fluid, also known as cerebrospinal fluid or CSF (from the space surrounding the brain and spinal cord)

- Pleural fluid (from the space around the lungs)
- Pericardial fluid (from the sac that surrounds the heart)
- Ascitic fluid, also called ascites or peritoneal fluid (from the space in the belly)

Scrape or brush cytology

Another cytology technique is to gently scrape or brush some cells from the organ or tissue being tested. The best-known cytology test that samples cells in this way is the Pap test. Pap test samples are taken by using a small spatula and/or brush to remove cells from the cervix (the lower part of the uterus or womb). Other areas that can be brushed or scraped include the esophagus (swallowing tube), stomach, bronchi (breathing tubes that lead to the lungs), and mouth.

What happens to biopsy and cytology specimens after they are removed from the patient?

There are standard procedures and methods that are used with nearly all types of biopsy samples. These procedures are the usual ways that a sample is prepared for use by the doctor. Other procedures, which are described later, may also be done on certain types of samples (such as lymph nodes and bone marrow).

Routine biopsy processing for histology

After the doctor removes the biopsy specimen, it is placed in a container with formalin (a mixture of water and formaldehyde) or some other fluid to preserve it. The container is labeled with the patient's name and other identifying information (hospital number and birth date, for example), site of biopsy (exactly where on the body it was taken from), and then sent to the pathology lab with a paper called a pathology requisition form. This form also identifies who submitted the biopsy, the date the biopsy was taken, information about the patient's symptoms, other abnormal test results, and what type of disease the doctor expects the biopsy may show.

Next, the pathologist or an assistant looks at the specimen without a microscope. In medicine, gross means seen without a microscope. This is what the pathologist observes by simply looking at, measuring, or feeling the tissue. Looking at the whole sample before it is processed further is called the gross examination and includes the tissue sample's size, color, consistency, and other characteristics. The lab staff may even take a picture of the sample as part of the record. The gross examination is important since the pathologist often sees features that suggest cancer. It also helps the pathologist decide which parts of a large biopsy are the most critical to study under a microscope.

For small biopsies, for example, like a punch biopsy or a core needle biopsy, the entire specimen is usually looked at under a microscope. The tissue to be looked at under the

microscope is placed into small containers called cassettes. The cassettes hold the tissue securely while it is processed, and help keep small samples from getting lost. After processing, which may take a few hours but is usually done overnight, the tissue sample is placed into a mold with hot paraffin wax. The wax cools to form a solid block that protects the tissue. This paraffin wax block with the embedded tissue is placed on an instrument called a microtome, which cuts very thin slices of the tissue. These thin slices of the specimen are placed on glass slides, and dipped into a series of stains or dyes to change the color of the tissue. The color makes cells more distinctive when viewed under a microscope. For most biopsy specimens, routine processing as described above is all that is needed. At this point (usually the day after the biopsy was done), the pathologist looks at the tissue under a microscope. Looking at the solid specimens in this way is called histology, which is the study of the structures of cells and tissues.

Intra-operative consultation (frozen section)

Sometimes a surgeon needs information about a tissue sample during surgery to decide about immediate surgical treatment. If the surgeon cannot wait the day or more that it will take for routine processing and histology, he or she will request an intra-operative (during surgery) pathology consultation. This consultation is often called a frozen *section* exam.

How is it done?

When a frozen section exam is done, fresh tissue is sent from the operating room right to the pathologist. Because the patient is often under general anesthesia (kept asleep with drugs) it is important that the tissue be looked at quickly. It usually takes 10 to 20 minutes. The fresh tissue is grossly examined by the pathologist to decide which part of the tissue sample should be looked at under the microscope. Instead of processing the tissue in wax blocks, the tissue is quickly frozen in a special solution that forms what looks like an ice cube around the tissue sample. It is then thinly sectioned (sliced) on a machine called a refrigerated microtome, quickly stained (dipped in a series of dyes), and looked at under the microscope. Although the frozen sections usually do not show features of the tissue as clearly as sections of tissue embedded in wax, they are usually good enough to help the surgeon make decisions about the operation.

When is it done?

To find out if a tumor is cancer: Sometimes the type of operation needed depends on whether the tumor is cancer. For instance, just removing the tumor may be enough to treat a benign (not cancer) tumor, but a more extensive operation (removing more tissue and/or lymph nodes) may be needed if the tumor is cancer. In a case like this, the surgeon may send the tumor for a frozen section exam. This often can give enough information to help the surgeon decide what type of operation, if any, is best for the patient. Sometimes, though, the intra-operative consultation does not give a definite answer and the piece of tissue will need to go through routine or even special processing to get a clear answer. When this happens the surgeon usually stops the operation and closes the surgical incision. When the results are available in a few days, another operation may be needed.

To make sure all of the cancer is removed: Surgical treatment of cancer is often a difficult balance between removing enough tissue to feel that the cancer has been completely removed and leaving enough normal tissue to avoid or minimize damage. If the surgeon is concerned a cancer has not been removed completely, a slice from the edge of the tissue that was removed is sent for a frozen section diagnosis. If there is no cancer in that edge (called a margin), more surgery usually is not needed. But if cancer cells are found, it is assumed that some cancer cells are still present in the tissue left in the patient. If this happens, the surgeon will usually remove more tissue to try to get all the cancer cells and reduce the chance of cancer growing back. If it is not possible to remove more tissue, there may be other options, such as radiation to kill the remaining cancer cells.

Mohs surgery (microscopically controlled surgery)

This procedure is used to treat certain kinds of skin cancer. In Mohs surgery, the surgeon removes a thin layer of the skin that the tumor may have invaded and then checks the sample under a microscope. If cancer cells are seen, deeper layers are removed and checked until the skin samples are found to be free of cancer cells. This process is slow, but it means that more normal skin near the tumor can be saved. This is a highly specialized technique that should only be used by doctors who have been trained in this specific type of surgery and in recognizing skin cancer under a microscope.

Cytology specimen processing

How cytology specimens are processed depends greatly on the type of specimen. Some specimens are smeared directly on glass microscope slides by the doctor who gets the sample. The slides, which are called smears, are then sent to the cytology lab where they are dipped into a series of stains (colored dyes), much like those used for biopsy samples. Other specimens, such as body fluids, cannot easily be placed on a glass microscope slide because they are too dilute (there are too few cells in a large volume of fluid). Cytology labs have ways to concentrate these cells on a glass slide before staining. After processing and staining, the samples are examined under a microscope. The abnormal cells are found and marked with a special pen. A pathologist will then examine the marked cells and decide on a diagnosis.

What do doctors look for under the microscope?

General principles

Over 100 years ago, scientists realized that various tissues and organs look different from each other under a microscope. This is because they are formed by different cell types and because the cells are arranged differently. Even more importantly, it was discovered that the usual appearance of each type of tissue or organ is changed by diseases like cancer. During the past century, this science, known as pathology, has advanced greatly.

Most tissue and cell samples are looked at by pathologists (doctors with special training in diagnosing diseases by lab tests). Sometimes, other doctors will also examine specimens or tissues of organs related to their area of expertise. For example, hematologists often look at blood and bone marrow samples from their patients and some dermatologists will look at their patients' skin biopsy specimens.

Some features that doctors look for under a microscope are important only when found in 1 or 2 types of tissue, while others are more important if found in almost all tissues. Here are a few general concepts explained in less technical terms to help you to better understand how doctors decide whether cancer is present.

Size and shape of the cells

The overall size and shape of cancer cells are often abnormal. They may be either smaller or larger than normal cells. Normal cells often have certain shapes that help them better do their jobs. Cancer cells usually do not function in a useful way and their shapes are often distorted. Unlike normal cells that tend to have the same size and shape, cancer cells often are very different in their sizes and shapes.

Size and shape of the cell's nucleus

The size and shape of the nucleus of a cancer cell is often abnormal. The nucleus is the center of the cell that contains the cell's DNA. The nucleus is surrounded by cytoplasm. Some types of cells can be imagined as looking like a fried egg, in which the central yolk represents the nucleus and the surrounding white is the cytoplasm (this is only a way of imagining cells, and does not truly reflect what cells are made of). Cancer cells typically have a nucleus that is larger than that of a normal cell. And, like the overall cell size and shape, the size and shape of the cell nucleus is usually much the same among normal cells of each tissue, but can vary greatly among cancer cells. Another feature of the nucleus of a cancer cell is that after being stained with certain dyes, it looks darker when seen under a microscope. The nucleus from a cancer cell is larger and darker because it often contains too much DNA.

Arrangement of the cells

The arrangement of normal cells reflects the function of each tissue. For instance, cells can form glands that produce substances that are taken to other parts of the tissue. Gland tissue in the breast, which during breast-feeding can produce milk, is organized into lobules and ducts that carry milk from the lobules to the nipple. Cells of the stomach also form glands, to produce enzymes, acid, and mucus that digest the food and protect the stomach lining.

When cancers develop in the breast, stomach, and many other tissues, the cancer cells do not form glands as they should. Sometimes the cancer cells form abnormal or distorted glands. Sometimes they form cell clumps that do not look like glands at all.

Another feature that shows abnormal interactions by cancer cells is that cancer cells grow into (invade) other tissues. Normal cells stay where they belong within a tissue. The ability of cancer cells to invade reflects the fact that their growth and movement is not coordinated with their neighboring cells. This ability to invade is how cancer spreads to and damages nearby tissues. And, unlike normal cells, cancer cells can metastasize (spread through blood vessels or lymph vessels) to distant parts of the body, too. Knowing this helps doctors recognize cancers under a microscope, because finding cells where they don't belong is a useful clue that they might be cancer.

The type of cancer

There are several basic kinds of cancers, which doctors can further classify into hundreds or even thousands of types, based on how they look under a microscope. Cancers are named according to which type of normal cells and tissues they look like most. For example, cancers that look like glandular tissues are called adenocarcinomas. Other cancers that resemble certain immune system cells are called lymphomas, and those that look like bone or fat tissue are osteosarcomas and liposarcomas, respectively.

Grading the cancer

While identifying the cell type or tissue a cancer looks like, doctors also decide how closely they look like the normal cells or tissues. This is the grade of the cancer. Cancers that look more like normal tissues are called low grade, and those that do not look much like normal tissues are high grade. A high-grade cancer tends to grow and spread faster than a low-grade cancer. Patients with high-grade cancers tend to have a poorer prognosis (outlook).

Special studies in cancer diagnosis

The type and grade of a cancer is usually clear when it is seen under a microscope after routine processing and staining, but this is not always the case. Sometimes the pathologist may need to use other procedures to make a diagnosis.

Histochemical stains

These tests use different chemical dyes that are attracted to certain substances found in some types of cancer cells. An example is the mucicarmine stain, which is attracted to mucus. Droplets of mucus inside a cell that are exposed to this stain will look pink-red under a microscope. This stain is useful if the pathologist suspects, for example, an adenocarcinoma (a glandular type of cancer) in a lung biopsy. Adenocarcinomas can produce mucus, so finding pink-red spots in lung cancer cells will tell the pathologist that the diagnosis is adenocarcinoma.

Besides being helpful in sorting out different kinds of tumors, other types of special stains are used in the lab to identify microorganisms (germs) like bacteria and fungi in tissues. This is important because people with cancer may develop infections as a side

effect of chemotherapy, radiation, or even because of the cancer itself. It is also important in cancer diagnosis because some infectious diseases cause lumps to form which might be confused with a cancer until these histochemical stains prove that the patient has an infection and not cancer.

Immunohistochemical stains

Immunohistochemical (IHC) or immunoperoxidase stains are another very useful category of special tests. The basic principle of this method is that an antibody will attach itself to certain substances (called antigens) that are on or in the cell. Each type of antibody recognizes and attaches to antigens that fit it exactly. Certain types of normal cells and cancer cells have unique antigens. If cells have a specific antigen, they will attract the antibody that fits the antigen. To find out if the antibodies have been attracted to the cells, chemicals will be added that cause the cell to change color only if a certain antibody (and, therefore, the antigen) is present. Our bodies normally produce antibodies that recognize antigens on germs and help to protect us against infections. The antibodies used in IHC stains are different -- they are made in the lab to recognize antigens that are linked to cancer and other diseases.

IHC stains are very useful in identifying certain types of cancers. For example, a routinely processed biopsy of a lymph node may contain cells that clearly look like cancer, but the pathologist may not be able to tell whether the cancer started in the lymph node or whether it started elsewhere in the body and has spread to the lymph nodes. If the cancer started in the lymph node, the diagnosis would be lymphoma. If the cancer started in another part of the body and spread to the lymph node, it might be metastatic cancer. This distinction is very important because treatment depends on the type of cancer (as well as some other factors, too).

There are hundreds of antibodies used for IHC tests. Some are quite specific, meaning that they react only with one type of cancer. Others may react with a few types of cancer, so several antibodies may be tested to decide what type of cancer it is. By looking at these results along with the cancer's appearance after the biopsy specimen is processed, its location, and other information about the patient (age, gender, etc.), it is often possible to classify the cancer in a way that can help the doctor select the best treatment.

Although IHC stains are used most often to classify cells, they also can be used to detect or recognize cancer cells. When a large number of cancer cells have spread to a nearby lymph node, these cells are usually recognized easily when the pathologist looks at the lymph tissue under the microscope using routine stains. But if there are only a few cancer cells in the node, it can be hard to recognize the cells using only routine stains. This is where IHC stains can help. Once the pathologist knows the kind of cancer to look for, he or she can choose one or more antibodies known to react with those cells. More chemicals are added so that the cancer cells will change color and clearly stand out from the normal cells around them. IHC stains are generally not used for looking at tissue from lymph node dissections (which remove a large number of nodes), but they are sometimes used in sentinel lymph node biopsies (see "Sentinel lymph node mapping and biopsy" in the section "Overview of biopsy types").

Another specialized use of these stains is to help distinguish lymph nodes that contain lymphoma from those that are swollen from non-cancerous (benign) lymphocytes (usually as a response to infection). Certain antigens are present on the surface of immune system cells called lymphocytes. Benign lymph node tissue contains many different types of lymphocytes with a variety of antigens on their surface. In contrast, cancers such as lymphoma start with a single abnormal cell, so that the cancer cells that grow from that cell typically share the chemical features of the first abnormal cell. This is especially useful in diagnosing lymphoma. If most of the cells in a lymph node biopsy have the same antigens on their surface, this result supports a diagnosis of lymphoma.

Electron microscopy

The typical medical lab microscope uses a beam of ordinary light to look at specimens. A larger, much more complex, and more expensive instrument called an electron microscope uses beams of electrons. The electron microscope's magnifying power is about 1,000 times greater than that of an ordinary light microscope. This degree of magnification is rarely needed in deciding whether a cell is cancer. But it sometimes helps find very tiny details of a cancer cell's structure that provide clues to the exact type of the cancer. For instance, some cases of melanoma, a highly aggressive skin cancer, may look like other types of cancer when seen under the ordinary light microscope. Most of the time, these melanomas can be recognized by certain immunohistochemical stains. But if those tests don't give a clear answer, the electron microscope may be used to identify tiny bodies inside melanoma cells called melanosomes. This helps to establish the type of cancer, which helps the doctor choose the best treatment plan.

Flow cytometry

This test is often used to test the cells from bone marrow, lymph nodes, and blood samples. It is very accurate in finding out the exact type of leukemia or lymphoma a person has. It also helps to tell lymphomas from non-cancer lymph node diseases. A sample of cells from a biopsy, cytology specimen, or blood specimen is treated with special antibodies and passed in front of a laser beam. Each antibody sticks only to certain types of cells that contain the antigens that fit with it. If the sample contains those cells, the laser will cause them to give off light that is then measured and analyzed by a computer.

Analyzing cases of suspected leukemia or lymphoma by flow cytometry uses the same principles explained in the section on immunohistochemistry. Finding the same substances on the surface of most cells in the sample suggests that they came from a single abnormal cell and are likely to be cancer. On the other hand, finding several different cell types with a variety of antigens means that the sample is less likely to contain leukemia or lymphoma.

Flow cytometry can also be used to measure the amount of DNA in cancer cells (this is called ploidy). Instead of using antibodies to detect protein antigens, cells can be treated with special dyes that react with DNA. If there's a normal amount of DNA, the cells are said to be diploid. If the amount is abnormal, the cells are described as aneuploid.

Aneuploid cancers of most (but not all) organs tend to be more aggressive (they spread faster and are harder to treat) than diploid ones.

Another use of flow cytometry is to measure the S-phase fraction, which is the percentage of cells in a sample that are in a certain stage of cell division called the synthesis (or S) phase. The more cells that are in the S-phase, the faster the tissue is growing and the more aggressive the cancer is likely to be.

Image cytometry

Like flow cytometry, this test uses dyes that react with DNA. But instead of suspending the cells in a stream of liquid and analyzing them with a laser, image cytometry uses a digital camera and a computer to measure the amount of DNA in cells on a microscope slide. Like flow cytometry, image cytometry also can determine the ploidy of cancer cells (see "Flow cytometry").

Genetic tests

Cytogenetics

Normal human cells contain 46 chromosomes (pieces of DNA and protein that control cell growth and function). Some types of cancer have a unique abnormal chromosome. Recognizing the abnormal chromosome helps to identify those types of cancer. This is especially useful in diagnosing some lymphomas, leukemias, and sarcomas. Even when the type of cancer is known, cytogenetic studies may help predict the patient's outlook. Sometimes the studies can even help predict which chemotherapy drugs the cancer is likely to respond to.

Several types of chromosome changes can be found in cancer cells:

- A translocation means part of one chromosome has broken off and is now located on another chromosome.
- An inversion means that part of a chromosome is upside down (now in reverse order) but still attached to the right chromosome.
- A deletion indicates part of a chromosome has been lost.
- A duplication happens when part of a chromosome has been copied, and too many copies of it are found within the cell.

Cytogenetic testing usually takes about 3 weeks, because the cancer cells must grow in lab dishes for about 2 weeks before their chromosomes can be looked at under the microscope.

Fluorescent in situ hybridization

Fluorescent in situ hybridization (FISH) is a newer test that is much like cytogenetic testing. It can find most chromosome changes that can be seen under a microscope in standard cytogenetic tests. It can also find some changes too small to be seen with usual cytogenetic testing. FISH uses special fluorescent dyes that only attach to specific parts of certain chromosomes. FISH can find chromosome changes such as translocations, which are important to help classify some kinds of leukemia. This test can also show when there are too many copies of a certain gene (this is called gene amplification), which can help doctors choose the best treatment for some women with breast cancer. Unlike standard cytogenetic tests, it is not necessary to grow cells in lab dishes before doing FISH. That means FISH results are available much sooner, usually within a few days.

Molecular genetic studies

Other tests of DNA and RNA can be used to find most of the translocations found by cytogenetic tests. They can also find some translocations involving parts of chromosomes too small to be seen under a microscope with usual cytogenetic testing. This type of advanced testing can help classify some leukemias and, less often, some sarcomas and carcinomas. These tests are also useful after treatment to find small numbers of remaining leukemia cancer cells that may be missed under a microscope.

Molecular genetic tests can also identify mutations (abnormal changes) in certain areas of DNA that are responsible for controlling cell growth. Some of these mutations may cause cancers to be especially aggressive in growing and spreading. In some cases, identifying certain mutations can help doctors choose treatments that are more likely to work.

Certain substances called antigen receptors appear on the surface of immune system cells called lymphocytes. Normal lymph node tissue contains lymphocytes with many different antigen receptors, which help the body respond to infection. But some types of lymphoma and leukemia start from a single abnormal lymphocyte. This means all their cells have the same antigen receptor. Lab tests of the DNA on each cell's antigen receptors are a very sensitive way to diagnose and classify these cancers.

Some gene mutations can be inherited from parents and cause a person to have a greater risk of developing certain cancers. Unlike acquired gene mutations that only affect the abnormal cells of the tumor, inherited mutations affect all cells of a person's body. These inherited mutations can often be identified by genetic testing of blood samples. Genetic counseling and testing may be recommended for some people with a strong family history of cancer. Because these tests do not analyze cancer tissue, they are not discussed here. For more information, see our document, *Genetic Testing: What You Need to Know*.

Polymerase chain reaction (PCR): This is a very sensitive molecular genetic test for finding specific DNA sequences, such as those occurring in some cancers. Reverse transcriptase PCR (RT-PCR) is a method used to detect small amounts of RNA, a substance related to DNA that is needed for cells to produce proteins. There are specific RNAs for each protein in our body. RT-PCR can be used to find and classify cancer cells.

RT-PCR tests to detect cancer cells look for the RNA sequences that are responsible for making substances found in cancer cells but not in most normal cells. An advantage of this test is that it can detect very small numbers of cancer cells in blood or tissue samples that would be missed by other tests. RT-PCR is already used routinely for detecting certain kinds of leukemia cells that remain after treatment, but its value for more common types of cancer is less certain. The disadvantage is that doctors are not always sure whether having a few cancer cells in the bloodstream or a lymph node means that a patient will actually develop distant metastases that grow enough to cause symptoms or affect survival. In treating patients with most common cancer types, it is still uncertain whether recognizing a few cancer cells with this test should be a factor in choosing treatment options.

RT-PCR can also be used to sub-classify cancer cells. Some RT-PCR tests measure levels of one or even several RNAs at the same time. By comparing the levels of important RNAs, doctors can sometimes predict whether a cancer is likely to be more or less aggressive (likely to grow and spread) than would be expected based on how it looks under the microscope. Sometimes these tests can help predict whether a cancer will respond to certain treatments.

Gene expression microarrays: These tiny devices are in some ways like computer chips. The advantage of this technology is that relative levels of hundreds or even thousands of different RNA molecules from one sample can be compared at the same time. The results tell which genes are active in a tumor. Recent studies have found that this information can sometimes help predict a patient's prognosis (outlook) or response to certain treatments. Although this is a very active area of research, doctors are still doing studies to learn how this information should guide treatment.

How long does biopsy and cytology testing take?

The uncertainty you feel waiting for biopsy and cytology test results can cause a lot of stress and anxiety. Not knowing when the results will be ready and not understanding why testing sometimes takes longer than expected can cause extra concern.

Routine biopsy and cytology results may be ready as soon as 1 or 2 days after the sample is received in the laboratory. But there are many reasons why some cases take much longer to complete.

More time for processing

Often, there are technical reasons for delays in reporting results. For instance, certain tissues take longer to process than others. Bone and other hard tissues that contain a lot of calcium need special handling. These tissues must be treated with strong acids or other chemicals to remove the minerals so that the tissue becomes soft enough to be thinly sectioned (sliced) on the microtome. This takes extra time. Another technical reason for delay is that the formalin solution used for preserving tissues takes longer to penetrate

samples with lots of fatty tissue (such as breast biopsies). An extra day of fixation (formalin treatment) is sometimes necessary. Large samples, such as those resulting from removal of an entire organ, might also require more than one day for the formalin to penetrate the tissue. If formalin does not completely penetrate the sample, cells may not be clear under the microscope and testing is more difficult and/or less accurate.

Need to look at more tissue

For most large samples, only selected areas are processed and examined under the microscope. After the first sections of tissue are seen under the microscope, the pathologist might want to look at more sections for an accurate diagnosis. In these cases, extra pieces of tissue might need processing. Or the lab may need to make more slices of the tissue that has already been embedded in wax blocks. Either case can add 1 or 2 days to the testing time.

Special stains or tests

Although most cancers can be found by looking at routinely stained sections, sometimes special stains or other tests may be needed for an accurate diagnosis. For example, histochemical stains or immunoperoxidase stains usually delay results for another day. Other advanced studies like flow cytometry, electron microscopy, and molecular pathology techniques can take even longer, sometimes days, before results are ready.

Getting a second opinion

Another important reason for delaying a pathology report is that the pathologist may seek a second opinion from an expert. Unlike some chemical tests done in the lab to measure the amount of a specific substance or look at whether a substance is present or absent, testing tissue or cell samples for cancer is based on the professional opinion of the pathologist who looks at the sample under the microscope. Although the abnormal features of some cancers are obvious, some cases have features that are very hard to recognize. Also, pathologists are often reluctant to diagnose certain very rare types of cancer without a second opinion from an expert who specializes in that area. There are pathology experts specializing in almost every organ system (digestive, head and neck, breast, bone, reproductive, etc.). When hard or rare cases come up, slides are usually sent to experts by overnight mail. Such review can delay the report for several more days.

Other reasons

Finally, patients should realize that delays might occur for reasons that are neither technical nor medical. For example, entering the report into the computer takes time. Some labs send results right to doctors' office computer systems or fax machines, but a hospital mail system or US mail is still often used and can delay the results.

What can you do to learn more about your pathology results?

Pathology results have a key role in making decisions about treatment, and many patients want to learn more about their test results. Ask your doctors to explain these results in a way that you can understand. Focus on how the results influence your treatment options and help predict your outlook. Some pathologists will speak with you to help you understand your pathology reports. But others believe that your oncologist, surgeon, primary care doctor, or other doctors are better able to explain the results because they know more about your overall medical situation. Also, doctors who already know you well are often best able to discuss the complex personal issues affected by your pathology results.

You may request copies of your pathology reports, and you may find it useful to keep a folder or notebook with your pathology, radiology, and other test results. If you see more doctors in the same hospital where your cancer was diagnosed, the new doctors will have access to the original pathology report and other medical records. If consulting doctors (such as for a second opinion) who practice at other facilities, it is usually necessary to send copies of pathology reports and other medical records before your appointment. Usually you can just sign a release form to have the copies sent, but it is a good idea to keep an original copy for yourself that you can share with the new doctor in case a report is not available. You will always want to get back the original for those times you may need it again.

Some cancer centers have a policy requiring that microscope slides of the patient's cancer be reviewed by the pathologists at their own institution. Some pathology labs will give copies of microscope slides to you if you are going to visit another cancer center for a second opinion or consultation. Other labs prefer to mail the slides directly to the consulting cancer center's pathology department.

If you or your doctors have any concerns about your pathology diagnosis, you can have your microscope slides reviewed by a consulting pathologist for a second opinion. Your oncologist or surgeon or the pathologist who first looked at your biopsy or cytology sample can often suggest a consultant with special qualifications in examining samples such as yours. Or you can have your slides sent to the pathology department of a medical school or cancer center you have confidence in.

What information is included in a pathology report?

The pathology report of surgical specimens is often quite long and complex. It is often divided into a number of subheadings.

Identifying information

The general identifying information includes the patient's name, the medical record number issued by the hospital, the date when the biopsy or surgery was performed, and the unique number of the specimen issued in the lab.

Clinical information

The next portion of the report often contains information about the patient that was provided by the doctor who removed the tissue sample. Such information may include a pertinent medical history and special requests made to the pathologist. For example, if a lymph node sample is being removed from a patient already known to have cancer in another organ, the doctor will note the type of the original cancer. This information is often useful in guiding the pathologist's selection of special studies that may be needed to find out whether any cancer in that lymph node is a metastasis from the original cancer or is a new cancer that started in the lymph node.

Gross description

The next part of the report is called the gross description. In medicine, "gross" means visible without a microscope. This is what the pathologist observes by simply looking at, measuring, or feeling the tissue.

For a small biopsy, this description is a few sentences listing its size, color, and consistency. This section also records the number of tissue-containing cassettes submitted for processing.

Larger biopsy or tissue specimens, for example, a mastectomy for breast cancer, will have much longer descriptions including the size of the entire piece of tissue, size of the cancer, how close the cancer is to the nearest surgical margin or edge of the specimen, how many lymph nodes were found in the underarm area, and the appearance of the non-cancer breast tissue. A summary of exactly where tissue was taken from is also included.

For cytology specimens, the gross description is very short and usually notes the number of slides or smears made by the doctor. If the sample is a body fluid, its color and volume are noted.

Microscopic description

This description records what the pathologist sees under the microscope. The appearance of the cancer cells, how they are arranged together, and the extent to which the cancer invades nearby tissues in the specimen are usually included in the microscopic description. For typical cases of common cancers or for benign tissues, a microscopic description may not be included in the report. Results of any other studies done (histochemical stains, flow cytometry, etc.) are noted in the microscopic description or in a separate section.

Diagnosis

The most important part of the pathology report is the final diagnosis. It is, in essence, the "bottom line" of the testing process, although this section may be at the bottom or the top of the page. The patient's doctor relies on this final diagnosis to help decide on the best treatment options. If the diagnosis is cancer, this section will note the exact type of cancer and will usually include the cancer's grade.

Comment

After the final diagnosis is made, the pathologist may wish to add more information for the doctors taking care of the patient. The comment section is often used to clarify a concern or recommend further testing.

Summary

Some pathology reports for cancers contain a summary of findings most relevant to making treatment decisions.

Additional resources

More information from your American Cancer Society

We have selected some related information that may also be helpful to you. These materials may be ordered from our toll-free number, 1-800-227-2345, or found on our Web site, www.cancer.org.

Choosing a Doctor and a Hospital (also available in Spanish)

Talking With Your Doctor (also available in Spanish)

For Women Facing a Breast Biopsy

After Diagnosis: A Guide for Patients and Families (also available in Spanish)

Genetic Testing: What You Need to Know

Surgery (also available in Spanish)

Understanding Chemotherapy (also available in Spanish)

Understanding Radiation Therapy (also available in Spanish)

National organizations and Web sites*

Along with the American Cancer Society, other sources of information and support include:

National Cancer Institute

Toll-free number: 1-800-4-CANCER (1-800-422-6237)

TTY: 1-800-332-8615

Web site: www.cancer.gov

For accurate, up-to-date information on a variety of cancer-related topics for patients, their families, and the general public

College of American Pathologists

Web site: www.MyBiopsy.org

Offers free, comprehensive information on more than 35 of the most common cancers and cancer-related conditions, including breast, colon, lung, and skin. The site includes answers to questions about cancer, lists of available treatment options, a glossary of key terms, and pictures of normal and diseased tissues, among other features.

**Inclusion on this list does not imply endorsement by the American Cancer Society.*

No matter who you are, we can help. Contact us anytime, day or night, for information and support. Call us at **1-800-227-2345** or visit www.cancer.org.

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For additional assistance please contact your American Cancer Society
1 · 800 · ACS-2345 or www.cancer.org