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## Testing Biopsy and Cytology Specimens for Cancer

Waiting to hear about test results can be very stressful - and sometimes it can take a while to get lab results back. This can be even harder when you're waiting to see if there is a change in a blood result, to know if a tumor or biopsy result is cancer, or to find out if cancer has come back. You may be going through some strong emotions, including disbelief, anxiety, fear, anger, and sadness. It's important to know that it's normal to have those feelings. It may help you to talk with others about it, or you may want to keep it private.

You might be concerned about how long tests results are taking. You might be wondering if waiting for test results will affect when you can start treatment. It's important to know that each patient's situation is different, and any questions you have about your test results can be answered best by your cancer care team.

It can help to have a better understanding of the testing process used to diagnose and classify cancer. Knowing some of this information, and asking your doctor questions, can help you understand how different test results affect treatment options and why some test results may take longer than others. It can also help you know what questions to ask so you can work with your doctors to make the best decisions about your treatment.

You'll probably 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, pharmacists, and many others. You'll be able to see what these professionals do. On the other hand, you rarely meet the pathologists, histotechnologists, cytotechnologists, and medical laboratory technologists who tell you whether the cells in your biopsy sample are cancer or not.

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## How is cancer diagnosed?

Cancer is nearly always diagnosed by an expert who has looked at cell or tissue samples under a microscope. In some cases, tests done on the cells' proteins, DNA, and RNA can help tell doctors if there's cancer. These test results are very important when choosing the best treatment options.

Tests of cells and tissues can find many other kinds of diseases, too. For instance, if doctors are not sure a lump is cancer, they may take out a small piece of it and have it tested for cancer and for infections or other problems that can cause growths that may look like cancer.

The procedure that takes out a piece of the lump, or a sample for testing is called a *biopsy*.

The tissue sample is called the *biopsy specimen*.

The testing process is sometimes referred to as *pathology*.

Lumps that could be cancer might be found by imaging tests or felt as lumps during a physical exam, but they still must be sampled and looked at under a microscope to find out what they really are. Not all lumps are cancer. In fact, most tumors are not cancer.

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# Types of biopsies used to look for cancer

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

Some types of biopsies remove an entire organ. These are done only by surgeons. Other types of biopsies remove tumor samples through a thin needle or through an endoscope (a flexible lighted tube that's put into the body). 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 here. For more details, go to the diagnosis information on specific type of cancer you want to learn about.

## 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, hollow needle attached to a syringe to take out a small amount of fluid and very small pieces of tissue from the tumor. The doctor can aim the needle while feeling the tumor, if it's near the surface of the body. If the tumor is deeper inside the body and can't be felt, the needle can be guided while being watched on an imaging test such as an ultrasound or CT scan.

The main advantages of FNA are that the skin doesn't have to be cut, and in some cases it's possible to make a diagnosis the same day. The disadvantage is that sometimes this needle can't remove enough tissue for a definite diagnosis. Although FNA is a type of biopsy, it's also classified as a [cytology test](#).

## Core biopsy

Needles used in a core biopsy 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 with local anesthesia (drugs are used to make the area numb) in the doctor's office or clinic. Like FNA, a core biopsy can sample tumors that the doctor can feel as well as smaller ones that must be seen using imaging tests.

Doctors sometimes use special vacuum tools to get larger core biopsies from breast tissue. (For more on this, see our [breast biopsy](#)<sup>1</sup> information.)

Processing core biopsy samples usually takes longer than FNA biopsies, so getting the results of those tests also might take longer.

## Excisional or incisional biopsy

In 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 or regional anesthesia (drugs are used to numb the area). 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 they will feel no pain).

## Endoscopic biopsy

An *endoscope* is a thin, flexible, lighted tube that has a lens or a video camera on the end. It allows a doctor to look inside different parts of the body. Tissue samples can also be taken out through the endoscope.

Different types of 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, sinuses, and throat. Another type of endoscope is used to look at the upper part of the digestive tract: the esophagus (the tube that connects the throat to the stomach), stomach, and first part of the intestine.

Some endoscopes are named for the part they are used to look at. For instance, a bronchoscope is used to look inside the lungs and bronchi (breathing tubes), and a colonoscope is used to look inside the colon and rectum (large intestine).

## 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 cut is made in the abdomen, and the laparoscope is passed through it 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 cuts into the abdomen (belly). It's usually a vertical cut from upper to lower abdomen. This may be done when a suspicious area can't be diagnosed with simpler 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 its location. Nearby tissues can be checked, too. General anesthesia is used (drugs are used to put the patient into a deep sleep so they will feel no pain). A similar operation that opens the chest is called *thoracotomy*.

## Skin biopsies

There are many ways to biopsy the skin. Your doctor will 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 usually they aren't used for suspected melanomas of the skin. Punch biopsies or excisional biopsies (as discussed previously) remove deeper layers of the skin, and can be used to 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 biopsy. Sentinel node mapping and biopsy has become a common way to find out whether a cancer (especially melanoma or breast cancer) has spread to the lymph nodes. This procedure can find the lymph nodes that drain lymph fluid from 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 cut in skin to see 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's removed (an excisional biopsy) and looked at under a microscope. If the sentinel node does not contain cancer cells, no more lymph nodes need to be taken out because it's 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*.

## Hyperlinks

1. [www.cancer.org/cancer/breast-cancer/screening-tests-and-early-detection/breast-biopsy.html](http://www.cancer.org/cancer/breast-cancer/screening-tests-and-early-detection/breast-biopsy.html)

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# Types of cytology tests used to look for cancer

Diagnosing diseases by looking at single cells and small clusters of cells is called *cytology* or *cytopathology*. It's an important part of diagnosing some types of cancer.

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, but in many cases the cytology fluid may be just as accurate.

Cytology tests may be used for diagnosis or for screening:

- A **diagnostic test** is only used for people who have signs, symptoms, or some other reason to suspect that they might have a particular disease (like cancer). 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 a screening test doesn't always prove that the disease is present.

Often, a diagnostic test is used if a screening test result is positive (that is, if something is found on the screening test). Some cytology tests, such as the Pap test, are mainly used for screening, while others can accurately identify cancers (see “Scrape or brush cytology” below). When cytology results show cancer, often a biopsy is also done to be sure before treatment is started.

## Fine needle aspiration

Fine needle aspiration (FNA) is sometimes considered a cytology test and is sometimes considered a biopsy. It's discussed in [1Types of biopsies used to look for cancer](#).

## Cytology tests on body fluids

Fluids taken from cavities (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 this way is the Pap test. A small spatula and/or brush is used to remove cells from the cervix (the lower part of the uterus or womb) for a Pap test. Other areas that can be brushed or scraped include the esophagus (swallowing tube), stomach, bronchi (breathing tubes that lead to the lungs), and mouth.

## Hyperlinks

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# What happens to biopsy and cytology specimens?

Standard procedures and methods are used to process nearly all types of biopsy samples. These procedures are the usual ways that a sample is prepared in the lab. Other procedures, which are described later, may also be done on certain types of samples (such as lymph nodes and bone marrow).

## How biopsies are processed routinely

After its removal, the biopsy specimen is put in a container with a mixture of water and formaldehyde (formalin) 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) and the site of biopsy (exactly where on the body it was taken from). It's then sent to the pathology lab. Next, the pathologist or a trained lab assistant looks at the specimen without a microscope. This is called *gross examination*. (In medicine, gross means *seen without a microscope*.) This is done to record what is seen by simply looking at, measuring, or feeling the tissue. The gross examination 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 may see features that suggest cancer. It also helps the pathologist decide which parts of a large biopsy are the most critical to look at under a microscope.

For small biopsies, such as a punch biopsy or a core needle biopsy, the entire



specimen is usually looked at under a microscope. The tissue is put into small containers called *cassettes*. The cassettes hold the tissue securely while it's processed. After processing, which may take a few hours (but is usually done overnight), the tissue sample is put 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 cut into very thin slices using an instrument called a *microtome*. 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 easier to see under a microscope. For most biopsy specimens, this routine processing is all that's 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.

### **Special biopsy processing: Frozen section (intra-operative consultation)**

Sometimes information about a tissue sample is needed during surgery to make immediate decisions. If the surgeon can't wait the day or more that it takes for routine processing and histology, he or she will request an intra-operative (during surgery) pathology consult. This is often called a *frozen section exam*.

#### **How is a frozen section 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's 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 it 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. It's then thinly sectioned (sliced) on a special machine, quickly stained (dipped in a series of dyes), and looked at under the microscope. The frozen sections usually do not show features of the tissue as clearly as sections of tissue embedded in wax, but they are good enough to help the surgeon make decisions during the operation.

#### **When is a frozen section done?**

**To find out if a tumor is cancer:** Sometimes the type of operation needed depends on whether the tumor is cancer (malignant). For instance, just removing the tumor could be

enough to treat a tumor that is not cancer (benign), but more tissue and/or lymph nodes may need to be removed if the tumor is cancer. In a case like this, the surgeon might 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 frozen section doesn't 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 (cut). After the results are back, 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 removed completely and leaving enough normal tissue to limit damage. If the surgeon is concerned that a cancer has not been removed completely, a slice from the edge of the tissue that was removed (called a *margin*) is sent for a frozen section diagnosis. If there are no cancer cells at the margin, more surgery usually isn't needed. But if cancer cells are found, it can be assumed that some are still 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 coming back. If it's not possible to remove more tissue, there may be other options, such as radiation to kill the remaining cancer cells.

### **Process for Mohs surgery (microscopically controlled surgery)**

This procedure is used to treat certain kinds of [skin cancer](#)<sup>1</sup>. In Mohs surgery, the surgeon removes a thin layer of the skin that the tumor might have invaded and then checks the sample under a microscope. If cancer cells are seen, more layers are removed and checked until no cancer cells are found in the skin samples. This process is slow, but it means that more normal skin near the tumor can be saved. Mohs surgery is a highly specialized technique that should only be used by doctors who have been trained to do it.

### **How cytology specimens are processed**

How cytology specimens are processed depends on the type of specimen. Some specimens are smeared on glass microscope slides by the doctor who gets the sample. These slides, which are called *smears*, are then sent to the cytology lab where they're dipped into a series of stains (colored dyes), much like those used for biopsy samples.

Other specimens, such as body fluids, can't be placed on a glass microscope slide easily because they are too diluted (there are too few cells in a large volume of fluid). Procedures are used to concentrate these cells on a glass slide before they are stained.

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 to make a diagnosis.

## How long are pathology specimens kept?

A federal law called CLIA (Clinical Laboratory Improvement Amendments) guides the regulation and certification of clinical labs. To be CLIA accredited, labs must keep human specimens for the minimum amount of time. For instance, CLIA says that labs must keep:

- Cytology slides for at least 5 years
- Histopathology slides for at least 10 years
- Paraffin blocks for at least 2 years

Some states have their own laws that require labs to keep pathology specimens longer than the time specified in the CLIA regulations. And some labs have policies for keeping specimens longer than required by federal or state laws.

## What this means for you

Some people want to get a second opinion on the diagnosis made from their tissue sample (specimen). This is called a pathology review. It means getting another doctor to look at your biopsy tissue and make a diagnosis on what's seen.

Human tissue samples are not discarded right after testing. So, in most cases, if there's enough tissue, the sample can be sent to another doctor or lab.

Sometimes keeping specimens for a longer time can be helpful in other ways. For instance, if a cancer survivor develops a tumor several years after the first one was removed, doctors will want to know if this new tumor is the old one coming back (a recurrence) or a completely new cancer.

This can often be figured out based on the locations of the 2 tumors and by comparing the histopathology slides from both specimens. But sometimes, more tests (such as immunohistochemical stains) that can be done using tissue from the original specimen's paraffin block are also helpful.

## Hyperlinks

1. [www.cancer.org/cancer/skin-cancer.html](http://www.cancer.org/cancer/skin-cancer.html)

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# What do doctors look for in biopsy and cytology specimens?

## General characteristics

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, diseases like cancer change the usual appearance of each type of tissue or organ.

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 (doctors who specialize in blood disorders) often look at blood and bone marrow samples from their patients, and some dermatologists (doctors specializing in skin diseases) will look at their patients' skin biopsy specimens.

Some features that doctors look for under a microscope are important only if they are found in certain types of tissue, while others are more important if they are found in almost all tissues.

Here are a few general concepts explained in less technical terms to help you better understand how doctors decide if 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 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 vary in their sizes and shapes.

## Size and shape of the cell's nucleus

The nucleus is the center of the cell that contains the cell's DNA. The size and shape of the nucleus of a cancer cell is often abnormal. Typically, the nucleus of a cancer cell is larger and darker than that of a normal cell and its size can vary greatly. 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 make substances that are taken to other parts of the tissue. Gland tissue in the breast, which produces milk during breastfeeding, is organized into lobules where the milk is made, and ducts that carry the milk from the lobules to the nipple. Cells of the stomach also form glands, to make enzymes and acid that digest the food, as well as mucus that helps 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 don't look like glands at all.

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 don't 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).

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# Tests used on biopsy and cytology specimens to diagnose cancer

The type and grade of a cancer is usually clear when the cells are seen under a microscope after routine processing and staining, but this is not always the case. Sometimes the pathologist needs 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. For example the mucicarmine stain 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](#)<sup>1</sup> 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 treatment, or even because of the cancer itself. It's also important in cancer diagnosis because some infectious diseases cause lumps to form which might be confused with a cancer until histochemical stains prove that the patient has an infection and not cancer.

## Immunohistochemical stains

Immunohistochemical (IHC) or immunoperoxidasestains are another very useful category of special tests. The basic principle of this method is that an immune protein called 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 are added that make the cells change color only if a certain antibody (and, therefore, the antigen) is present.

Our bodies normally make antibodies that recognize antigens on germs and help protect us against infections. The antibodies used in IHC stains are different. They're 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's often possible to classify the cancer in a way that can help 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 to look 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 our [review of biopsy types](#).)

Another specialized use of these stains is to help distinguish lymph nodes that contain lymphoma from those that are swollen from increased numbers of normal white blood cells (usually as a response to infection). Certain antigens are present on the surface of white blood cells called *lymphocytes*. Benign (non-cancerous) lymph node tissue contains many different types of lymphocytes with a variety of antigens on their surface. In contrast, cancers like [lymphoma](#)<sup>2</sup> start with a single abnormal cell, so 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.

Some IHC stains can help recognize specific substances in cancer cells that influence a patient's prognosis and/or whether they are likely to benefit from certain drugs. For example, IHC is routinely used to check for estrogen receptors on breast cancer cells. Patients whose cells have these receptors are likely to benefit from hormone therapy drugs, which block the production or effects of estrogens. IHC can also help determine which women with breast cancer are likely to benefit from drugs that block the growth-promoting effects of abnormally high levels of HER2 protein.

## Electron microscopy

The typical medical lab microscope uses a beam of ordinary light to look at specimens. A larger, much more complex 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 under the ordinary light microscope. Most of the time, these melanomas can be recognized by certain IHC stains. But if those tests don't give a clear answer, the electron microscope may be used to identify tiny structures inside melanoma cells called *melanosomes*. This helps establish the type of cancer and helps in choosing the best treatment plan.

## Flow cytometry

Flow cytometry is often used to test the cells from bone marrow, lymph nodes, and blood samples. It's very accurate in finding out the exact type of [leukemia](#)<sup>3</sup> or



[lymphoma](#)<sup>4</sup> a person has. It also helps tell lymphomas from non-cancer diseases in the lymph nodes.

A sample of cells from a biopsy, cytology specimen, or blood specimen is treated with special antibodies. Each antibody sticks only to certain types of cells that have the antigens that fit with it. The cells are then passed in front of a laser beam. If the cells now have those antibodies, the laser will make them give off light that's then measured and analyzed by a computer.

Analyzing cases of suspected [leukemia](#)<sup>5</sup> or [lymphoma](#)<sup>6</sup> 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.
- 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 (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 grow and spread faster 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.

## Genetic tests

## Cytogenetics

Normal human cells have 46 chromosomes (pieces of DNA and protein that control cell growth and function). Some types of cancer have one or more abnormal chromosomes. Recognizing abnormal chromosomes 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 tests may help predict the patient's outlook. Sometimes the tests 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 in the cell.

Sometimes, an entire chromosome might be gained or lost in the cancer cells.

For cytogenetic testing, cancer cells are grown in lab dishes for about 2 weeks before their chromosomes can be looked at under the microscope. Because of this, it usually takes about 3 weeks to get results.

## Fluorescent in situ hybridization

Fluorescent in situ hybridization (FISH) is a lot 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 linked to pieces of DNA that only attach to specific parts of certain chromosomes. FISH can find chromosome changes like translocations, which are important to help classify some kinds of leukemia.

Finding certain chromosome changes is also important in determining if certain targeted drugs might help patients with some types of cancer. For example, FISH can show when there are too many copies (called *amplification*) of the HER2 gene, which can help doctors choose the best treatment for some women with breast cancer.

Unlike standard cytogenetic tests, it's not necessary to grow cells in lab dishes for FISH. This means FISH results are available much sooner, usually within a few days.

### **Molecular genetic tests**

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 control cell growth. Some of these mutations may make cancers especially likely to grow and spread. In some cases, identifying certain mutations can help doctors choose treatments that are more likely to work.

Certain substances called *antigen receptors* are 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 these cancer cells have the same antigen receptor. Lab tests of the DNA of each cell's antigen receptor genes are a very sensitive way to diagnose and classify these cancers.

**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 (or RT-PCR) is a method used to detect very small amounts of RNA. RNA is a substance related to DNA that's needed for cells to make proteins. There are specific RNAs for each protein in our body. RT-PCR can be used to find and classify cancer cells.

An advantage of RT-PCR 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 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 will grow enough to cause symptoms or affect survival. In treating patients with most common cancer types, it's still not clear whether finding 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 RNAs from one sample can be compared at the same time. The results tell which genes are active in a tumor. This information can sometimes help predict a patient's prognosis (outlook) or response to certain treatments.

This test is sometimes used when a cancer has spread to several parts of the body but doctors aren't sure where it started. (These are called cancers of unknown primary.) The RNA pattern of these cancers can be compared with the patterns of known types of cancer to see if they match. Knowing where the cancer started is helpful in choosing treatment. These tests can help narrow down the cancer type, but they are not always able to tell the exact type of cancer with certainty. (To learn more about this type of cancer, see [7Cancer of Unknown Primary](#)<sup>8</sup>.)

**DNA sequencing:** For the past couple of decades, DNA sequencing has been used to identify people who have inherited genetic mutations that greatly increase their risk of developing certain types of cancer. In this case, the testing generally uses DNA from blood cells of either patients who already have certain cancers (such as [breast cancer](#)<sup>9</sup> or [colon cancer](#)<sup>10</sup>) or from the blood of their relatives who do not have any known cancer but may be at increased risk.

Doctors have started using DNA sequencing of some cancers to help predict which targeted drugs are most likely to work in individual patients. This practice is sometimes called "personalized oncology" or "precision oncology." At first, DNA sequencing was done for only one gene or for a few genes that were known to be most often affected for certain types of cancer. Recent progress has made it possible to sequence many more genes, or even all of the genes from a cancer (although this is still not done routinely). This sequence information sometimes shows unexpected mutations in genes that are affected less often, and may help the doctor choose a drug that otherwise would not have been considered and avoid other drugs that are unlikely to be helpful.

## Hyperlinks

1. [www.cancer.org/cancer/lung-cancer.html](http://www.cancer.org/cancer/lung-cancer.html)
2. [www.cancer.org/cancer/lymphoma.html](http://www.cancer.org/cancer/lymphoma.html)

3. [www.cancer.org/cancer/leukemia.html](http://www.cancer.org/cancer/leukemia.html)
4. [www.cancer.org/cancer/lymphoma.html](http://www.cancer.org/cancer/lymphoma.html)
5. [www.cancer.org/cancer/leukemia.html](http://www.cancer.org/cancer/leukemia.html)
6. [www.cancer.org/cancer/lymphoma.html](http://www.cancer.org/cancer/lymphoma.html)
7. [www.cancer.org/cancer/cancer-unknown-primary.html](http://www.cancer.org/cancer/cancer-unknown-primary.html)
8. [www.cancer.org/cancer/breast-cancer.html](http://www.cancer.org/cancer/breast-cancer.html)
9. [www.cancer.org/cancer/colon-rectal-cancer.html](http://www.cancer.org/cancer/colon-rectal-cancer.html)

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## Reasons for delays in getting your biopsy and cytology test results

The uncertainty you feel while 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 gets to the lab. But there are many reasons some take much longer to complete.

### Processing time

Often, there are technical reasons for delays in reporting results. For instance, certain types of body 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). 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). So, an extra day of fixation (formalin treatment) is sometimes necessary. Large samples, such as when an entire organ is removed, might also require more than one day for the formalin to soak into the tissue. If formalin does not penetrate the sample completely, cells might 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 to make an accurate diagnosis. For example, histochemical or immunohistochemical stains usually delay results for another day. Other advanced tests like flow cytometry, electron microscopy, and molecular genetic tests 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 want to get a second opinion from an expert. Unlike some chemical tests done in the lab to measure the amount of a specific substance or to 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 have features that are very hard to recognize. Also, pathologists are often reluctant to diagnose certain very rare types of cancer without getting 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 or as digital images. This review can delay the report for several 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.

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## How to learn more about your pathology results

Pathology results have a key role in decisions made about treatment, and many patients want to [learn more about their test results](#)<sup>1</sup>. Ask your cancer care team to explain your 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 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 ask for 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 you see consulting doctors (such as for a second opinion) who practice at other facilities, it's usually necessary to send copies of pathology reports and other medical records before your appointment. In most cases you can just sign a release form to have the copies sent, but it's a good idea to keep an original copy for yourself to share with the new doctor in case a report is not available. You'll 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 mail the slides directly to the consulting cancer center's pathology department. Again, you'll probably have to sign forms to get this done.

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 like yours. Or you can have your slides sent to the pathology department of a medical school or cancer center you have confidence in.

## Hyperlinks

1. [www.cancer.org/treatment/understanding-your-diagnosis/tests/understanding-your-pathology-report.html](http://www.cancer.org/treatment/understanding-your-diagnosis/tests/understanding-your-pathology-report.html)

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# What information is included in a pathology report?

The [pathology report](#)<sup>1</sup> of surgical specimens is often quite long and complex. It's 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 done, and the unique number of the specimen (which is assigned in the lab).

## Clinical information

The next part of the report often contains patient information that was provided by the doctor who removed the tissue sample. This may include a medical history and special requests made to the pathologist.

For example, if a lymph node sample is being removed from a patient 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 tests that may be needed to find out whether any cancer in that lymph node is a metastasis (spread) from the original cancer or 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 seen without a microscope. This is what the pathologist sees by simply looking at, measuring, and feeling the tissue sample.

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, such as 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 (edge) of the specimen, how many lymph nodes were found in the underarm area, and the appearance of the non-cancer 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 is a description of what the pathologists see when they look in 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. Results of any other studies done (histochemical stains, flow cytometry, etc.) may be noted in the microscopic description or in a separate section.

### **Diagnosis**

The most important part of the pathology report is the final diagnosis. This is the “bottom line” of the testing process, although this section may be at the bottom or the top of the page. The 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 want 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.

## Hyperlinks

1. [www.cancer.org/treatment/understanding-your-diagnosis/tests/understanding-your-pathology-report.html](http://www.cancer.org/treatment/understanding-your-diagnosis/tests/understanding-your-pathology-report.html)

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# To learn more

## National organizations and websites\*

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 Website: [www.cancer.gov](http://www.cancer.gov)<sup>1</sup>

- 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** Website: [www.cap.org](http://www.cap.org)<sup>2</sup>

- Offers a free video and information written for patients on how to read pathology reports.

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

## Hyperlinks

1. <http://www.cancer.gov>
2. <http://www.cap.org>

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