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How Biopsy and Cytology Samples Are Tested for Cancer

After a biopsy or cytology sample is taken, it must be prepared in a lab and then tested by a pathologist. Some procedures and methods are standard for almost all samples, while some types of samples (such as lymph nodes and bone marrow) require additional special procedures.

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How Biopsy and Cytology Samples Are Processed

There are standard procedures and methods that are used with nearly all types of biopsy or cytology samples. Some types of samples need additional processing.

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Routine biopsy processing

After a biopsy sample (sometimes called a **specimen**) is removed, it is usually put in a container with a mixture of water and formaldehyde (formalin) or some other fluid to preserve it. Sometimes part of the sample might be sent fresh (without any preservative) to the pathology department, so that certain tests that require fresh tissue (like cytogenetics – see below) can be done.

The container is labeled with the patient's name and other information (such as hospital number and birth date) and the site of biopsy (exactly where on the body it was taken from).

Gross examination

study of the structures of cells and tissues.

Frozen section (intra-operative consultation)

Sometimes during surgery, the surgeon might need information about a tissue sample to help make important decisions, such as how much of an organ to remove. In such situations, the surgeon will ask for 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 biopsy tissue is sent from the operating room right to the pathologist. Because the patient is often still under general anesthesia (kept in a deep sleep so they don't feel pain), it's important that the tissue is looked at quickly (usually within 10 to 20 minutes).

The pathologist will do a gross examination of the fresh tissue to decide which part of it should be looked at under a 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 sliced into very thin sections on a special machine, quickly stained (dipped in a series of dyes), and then looked at under a microscope.

The frozen sections typically do not show features of the tissue quite as well as sections of tissue embedded in wax, but they are usually good enough to help the surgeon make decisions during the operation.

Why might a frozen section be done?

Frozen sections are typically done during an operation, when the surgeon needs information right away that can help guide what type of operation should be done. There might be different reasons for this.

To find out if a tumor is cancer: Sometimes the type of operation the surgeon needs to do depends on whether the tumor is cancer (malignant). For instance, just removing a tumor might be enough if a frozen section shows that it's not cancer (benign). But the surgeon might need to remove a wider margin of tissue around the tumor, as well as nearby lymph nodes, if the tumor is cancer. In a case like this, a frozen section exam often can help the surgeon decide which type of operation is best.

Sometimes, though, the frozen section might not give a definite answer, so the biopsy tissue will still need to go through routine or even special processing. When this

happens, the surgeon usually stops the operation and closes the surgical incision (cut). Once the results are back, another operation may be needed.

To help make sure all of the cancer has been removed: Surgery to treat cancer is often a difficult balance between removing enough tissue to get all of the cancer, and leaving as much normal tissue as possible to help limit side effects from the surgery.

If the surgeon is concerned that a cancer hasn't been removed completely, a slice from the edge of the tissue that was removed (called a **margin**) can be sent for a frozen section diagnosis.

If there are no cancer cells at the margin (known as a **negative margin**), typically no more tissue needs to be removed. Having a negative margin doesn't guarantee that the cancer won't come back, although it makes it less likely.

If cancer cells are found at the margin (known as a **positive margin**), it's likely that at least some cancer cells weren't removed. If this happens, the surgeon will usually remove more tissue from the area to try to get all the cancer cells and lower the chances of the cancer coming back. If it's not possible to remove more tissue, there may be other treatment options, such as using radiation to kill the remaining cancer cells.

Process for Mohs surgery (microscopically controlled surgery)

[Mohs surgery](#)¹ (also known as **Mohs micrographic surgery**, or MMS) can be used to treat certain kinds of [skin cancer](#)². In this procedure, the surgeon removes a thin layer of the skin that the tumor might have invaded. The sample is then frozen and sectioned, and then checked under a microscope. If cancer cells are seen, another layer is removed and checked. This process is repeated until no cancer cells are found in the skin samples.

This process is slow, often taking several hours, but it means that more normal skin near the tumor can be saved. This can help the area look better after surgery.

Mohs surgery is a highly specialized technique that should only be done by doctors who have been trained to do it.

Processing cytology samples

How cytology specimens are processed depends on the type of sample.

Some samples 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.

In some situations, a portion of the sample might be stained right away for a preliminary interpretation (called **rapid on-site evaluation**). This might be done, for example, to help determine which lab tests should be done on the specimen.

Other samples, such as body fluids, can't be smeared on a glass microscope slide easily because they're too diluted (that is, there are too few cells in a large amount of fluid). These types of samples need to be concentrated before the cells are put on a glass slide and stained.

After processing and staining, the samples are examined under a microscope, typically by a **cytotechnologist**. 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?

Clinical labs (including pathology labs) are regulated and certified based on a federal law called CLIA (Clinical Laboratory Improvement Amendments). To be CLIA93.97 Tm 0 0 0 rg /GLIAS

Some people might want to get a second opinion on the diagnosis made from their biopsy or cytology tissue sample. Getting another doctor to look at your sample to either confirm the diagnosis or suggest another one is called a **pathology review**.

The processed samples (slides and blocks) are kept for a certain amount of time (although other parts of the sample are typically discarded). Because of this, the samples can often be sent to another doctor or lab.

Sometimes keeping samples for a longer time can be helpful in other ways. For instance, if someone who had cancer develops a new tumor several years after the first one was removed, doctors often want to know if this tumor is the original cancer coming back (a recurrence) or a new cancer.

This can often be figured out by comparing the histopathology slides from the new tumor to the original tumor. In some cases, it might also be helpful to do more tests (such as immunohistochemical stains) using tissue from the original specimen's paraffin block.

Hyperlinks

1. www.cancer.org/cancer/types/skin-cancer/skin-biopsy-treatment-procedures/mohs-surgery.html
2. www.cancer.org/cancer/types/skin-cancer.html

Reference

Centers for Medicare & Medicaid Services. Clinical Laboratory Improvement Amendments (CLIA). Cms.org. Accessed at <https://www.cms.gov/medicare/quality/clinical-laboratory-improvement-amendments> on April 5, 2024.

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What Do Doctors Look for in Biopsy and

Cytology Samples?

After your biopsy and cytology samples have been prepared, a doctor called a **pathologist** will determine if the cells in the sample are cancer and, if so, provide information about the cancer to help your doctors determine the right treatment options for you.

Here is information about what pathologists look for when they analyze your biopsy or cytology samples.

- [General characteristics](#)
- [The type of cancer](#)
- [Grading the cancer](#)

General characteristics

Various tissues and organs look different from each other under a microscope. This is because they are formed by different types of cells and because the cells are arranged differently. Even more importantly, diseases like cancer can change the usual appearance of each type of cell, 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 samples 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 to help you better understand how doctors decide if cancer is present.

Size and shape of the cells

Cancer cells are often abnormal in terms of their size and shape. 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 don't function in a useful way, and their shapes

are often distorted. Unlike normal cells, which 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. Typically, the nucleus of a cancer cell is larger than that of a normal cell, and its size and shape can vary greatly. And after being stained with certain dyes, the nucleus of a cancer cell looks darker than a normal nucleus. 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, gland tissue in the female 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 other glandular tissues, the cancer cells no longer form normal glands like they should. Sometimes they form abnormal or distorted glands. Sometimes they form clumps of cells that don't look like glands at all.

While normal cells stay where they belong within a tissue, cancer cells often grow into (invade) nearby tissues. The ability of cancer cells to invade reflects the fact that their growth and movement isn't properly coordinated with their neighboring cells. This ability to invade is how cancer spreads into 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. Knowing this helps doctors recognize cancers under a microscope, because finding certain types of cells where they don't belong is a useful clue that they might be cancer.

The type of cancer

Different kinds of cancer are often referred to by the organ or part of the body they started in. But cancer types are also named according to which type of normal cells and tissues they look like most.

There are several basic types of cancer, which doctors can further classify into hundreds or even thousands of types, based on how they look under a microscope. For

example, cancers that look like glandular tissues are called **adenocarcinomas**. Other cancers that look like certain immune system cells are called **lymphomas**, and those that look like connective tissue (such as bone or fatty tissue) are **sarcomas**.

Grading the cancer

For many types of cancer, doctors also determine how closely the cancer cells and the growth patterns 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** (or **well differentiated**), while those that don't look much like normal tissues are called **high grade** (or **poorly differentiated**). For some types of cancer, grading might be done using a number scale, such as from 1 to 3, with grade 1 being a low-grade cancer and grade 3 being a high-grade cancer.

High-grade cancers tend to grow and spread faster than low-grade cancers. This might affect a person's treatment options, as well as their prognosis (outlook).

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Tests Used on Biopsy and Cytology Samples to Diagnose and Classify Cancer

When diagnosing cancer, the pathologist will look at the cells in the biopsy or cytology samples under a microscope. This is likr some typshea0l1lgedat3 0 0 1ly303 gs ()Tj 0 ae7u2ndehe 3

- [Flow cytometry](#)
- [Image cytometry](#)
- [Electron microscopy](#)
- [Genetic and genomic tests](#)

Here are some of the more common lab tests and procedures done on biopsy or cytology samples. To learn more about the tests that might be done for a specific kind of cancer, see the testing section of our information on that [cancer type](#)¹.

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 or 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. Adenocarcinoma cells can produce mucus, so finding pink or red spots in lung cancer cells exposed to this stain will tell the pathologist that the diagnosis is adenocarcinoma.

Besides being helpful in sorting out different types of tumors, other 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

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 biopsy of a lymph node might contain cells that clearly look like cancer cells, but the pathologist might not be able to tell whether the cancer started in the lymph node or whether it started elsewhere in the body and then spread to the lymph nodes. This distinction is very important because if the cancer started in the lymph node, the diagnosis would most likely be a lymphoma, whereas if the cancer started in another part of the body and spread to the lymph node, it would be a different type of cancer. The treatment might be very different depending on the type of cancer (as well as some other factors).

There are many antibodies that can be used for IHC tests. Some are very specific, meaning that they react only with one type of cancer. Others may react with a few types of cancer, so several antibodies might be tested to decide what type of cancer it is. By looking at these results, along with how the cancer looks under a microscope, where it is in the body, and other information, it's often possible to classify the cancer in a way that can help doctors decide the best treatment.

Although IHC stains are used most often to classify cells, they also can be used to find cancer cells. For example, if there are only a few cancer cells in a lymph node, it can be hard for the pathologist to see the cells using only routine stains. But if the pathologist knows what type of cancer they're looking for, they can choose an IHC stain that uses one or more antibodies known to react with those types of cancer cells, which will help any cancer cells stand out if they are there. IHC stains are sometimes used in sentinel lymph node biopsies, but are generally not used to look at tissue from lymph node dissections, which remove a large number of nodes. (See [How Is a Biopsy Done?](#)) 12 Tf 0 07.6

blood samples. It's very accurate in finding out the exact type of [leukemia](#)⁵ or [lymphoma](#)⁶ a person has. It can also help tell lymphomas from non-cancer diseases in the lymph nodes.

For this test, a sample of cells from a biopsy, cytology, or blood sample is treated with special antibodies. Each antibody sticks only to certain types of cells that have the antigens that fit with it. The antibodies are linked to chemicals that are fluorescent (that is, they give off light of a certain color when exposed to a laser beam).

The cells are put into a stream of liquid that is passed through a laser beam, and an instrument measures the color and brightness of the light each cell gives off. A computer collects and analyzes the data to help doctors recognize and classify cancers.

Along with analyzing suspected cases of 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 of DNA 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.

Electron microscopy

The typical microscope uses a beam of light to look at specimens. An **electron microscope** is a larger, much more complex instrument that 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 to determine if a cell is cancer. But it sometimes can help find very tiny details of a cancer cell's structure that provide clues to the exact type of the cancer.

For instance, some melanoma skin cancers may look like other types of cancer under an 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 diagnose the type of cancer and might help in choosing the best treatment plan.

Genetic and genomic tests

Different types of lab tests might be done on the biopsy or cytology samples to learn more about the changes in genes (or patterns of changes in several genes) in cancer cells. These types of tests might help determine which type of cancer a person has. In some situations, they might also be helpful in determining treatment options.

Cytogenetic tests

In some cancers, the cells have one or more abnormal chromosomes (long strands of DNA that contain our genes, which control cell growth and function). Recognizing abnormal chromosomes can help identify these cancers. This is especially useful in diagnosing cancers such as lymphomas, leukemias, and sarcomas.

Even when the type of cancer is known, cytogenetic tests may help predict a person's outlook. Sometimes the tests can even help predict which treatments are likely to be helpful.

Several types of chromosome changes might be found in cancer cells. For example:

- A **translocation** means part of one chromosome has broken off and is now attached to another chromosome.
- An **inversion** means that part of a chromosome is now in reverse order but is still attached to the right chromosome.
- A **deletion** means part of a chromosome has been lost.
- A **duplication** means there are extra copies of part of the DNA in a chromosome.

Sometimes, an entire chromosome might be gained or lost in the cancer cells. (Normal human cells have 46 chromosomes.)

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 at least this long to get results.

Fluorescent in situ hybridization (FISH)

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 can also be important in determining if certain targeted drugs might be helpful in treating some types of cancer. For example, FISH can show when there are too many copies of the HER2 gene (known as *HER2 amplification* or *HER2 overexpression*). This can help doctors determine if drugs that target HER2 might be helpful in people with cancers that have too much HER2, such as some breast, stomach, and other cancers.

Unlike with standard cytogenetic tests, the cancer cells don't need to be grown in lab dishes for FISH. This means FISH results are available much sooner, usually within a few days.

Molecular tests

Some newer types of sensitive lab tests can also find changes in the DNA, RNA, or proteins in cancer cells. These tests can be used to help determine the type of cancer a person has, as well as if certain treatments are likely to be helpful. Some of these tests can also be used to see if treatment is working, or to look for signs that cancer has come back.

Testing for changes in a person's cancer cells to help determine the best care (including treatment) is sometimes referred to as [biomarker testing](#)⁷. Using the results of these types of tests to help plan treatment for each person is known as [precision or personalized medicine](#)⁸.

Polymerase chain reaction (PCR): This is a very sensitive molecular test for finding specific DNA sequences, such as those occurring in some cancers.

Reverse transcriptase PCR (RT-PCR) is a method used to detect very small amounts of RNA in a sample. 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.

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.

It's less clear, though, if RT-PCR is useful for more common types of cancer. Doctors aren't always sure if finding a few cancer cells in the blood or in a lymph node means that a person's cancer will grow and spread enough to cause symptoms or affect their survival. In treating people with most common cancer types, it's still not clear if 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 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 profiling: These tests compare the levels of many different RNAs from one sample at the same time. The results tell which genes are active (expressed) in a tumor, rather than just looking for changes in individual genes.

This pattern of gene activity can sometimes help predict a person's prognosis (outlook), which can help determine further treatment options. For example, for some early-stage breast cancers, this type of testing can show how likely it is the cancer will come back after surgery (and possibly radiation). This can help determine if chemotherapy should be part of further treatment.

This type of test can also be helpful when a cancer is found in different parts of the body, but doctors aren't sure where it started. (This is called a [cancer of unknown primary, or CUP](#)⁹.) 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.

DNA sequencing: Some types of tests can determine the actual sequence of DNA – the order of the chemicals that make up its code – to look for gene mutations (changes) inside cells.

This type of testing has been available for the past couple of decades, where it has been used mainly to identify people who have inherited gene mutations that greatly increase their risk of developing certain types of cancer.

Over time, doctors have learned more about the gene changes in cancer cells, and the technology behind DNA sequencing has improved to make it easier to do. Newer, **next generation sequencing (NGS) tests** are now used to look for gene changes in some types of cancers that can help predict which treatments, such as targeted drugs, are likely (or not likely) to work for each person.

DNA sequencing might be done to look at only a small number of genes for certain types of cancer. But as more gene changes are discovered in cancer cells (and as medicines are developed to target cells with these gene changes), sequencing is now likely to include more genes, or even all of the genes in a cancer cell (although this is still not done routinely). Sometimes DNA sequencing information might even show an unexpected gene mutation, which might help the doctor choose a drug that otherwise would not have been considered.

Getting the results of molecular tests

Some types of molecular tests might be done in the pathology lab at the center where you're getting your care, but others might need to be sent out to a central lab for testing. Depending on the test, it might take at least a couple of weeks to get these results.

Once the results are back, a member of your health care team should go over them with you. Ask them to explain the results in a way you can understand, including how they might affect your treatment options and help predict your outlook.

Hyperlinks

1. www.cancer.org/cancer/types.html
2. www.cancer.org/cancer/diagnosis-staging/tests/biopsy-and-cytology-tests/biopsy-types.html
3. www.cancer.org/cancer/types/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-hormone-receptor-status.html
4. www.cancer.org/cancer/types/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-her2-status.html
5. www.cancer.org/cancer/types/stomach-cancer/detection-diagnosis-staging/how-diagnosed.html

