TEST

Sputum stain for mycobacteria

A sputum stain uses a microscope to look for acid-fast bacteria, including those that can cause tuberculosis.

WHAT IT MEASURES
Acid-fast bacteria in mucus coughed up from the lungs

SAMPLE
Sputum coughed up from the lungs

FASTING NEEDED?
No

HOW LONG IT TAKES
Usually 10 to 20 minutes

RESULTS IN
Same day to two days, varying by laboratory

USED FOR
Diagnosis and treatment monitoring

MAIN LIMITATION
Negative stains can miss low numbers of mycobacteria

Overview

A sputum stain for mycobacteria looks for certain bacteria in mucus coughed up from your lungs. Mycobacteria include Mycobacterium tuberculosis , which causes tuberculosis (TB), and nontuberculous mycobacteria found in water and soil.

The test is often called an acid-fast bacilli stain, AFB stain, or smear microscopy. "Acid-fast" describes how these bacteria hold onto a laboratory dye after being washed with acid. "Bacilli" means rod-shaped bacteria.

A laboratory spreads a small amount of sputum onto a glass slide, applies special stains, and examines it under a microscope. A fluorescent stain or a traditional stain such as Ziehl-Neelsen may be used.

The stain gives a relatively quick result, but it cannot usually identify the exact species. Culture and molecular tests are normally needed to confirm whether TB or another mycobacterium is present.

Why the test is ordered

The test may be ordered when symptoms, an examination, or a chest image suggests possible infection in the lungs. Relevant symptoms can include a persistent cough, sputum production, coughing up blood, fever, night sweats, or unexplained weight loss. These symptoms have many possible causes and do not by themselves mean that you have TB.

The stain can help with several questions:

A positive smear can suggest that many mycobacteria are present and that a person with pulmonary TB may be more likely to spread bacteria through the air. The result alone does not prove TB or determine whether someone is contagious.

- Whether acid-fast bacteria are visible in your sputum
- Whether further TB testing and infection precautions are needed
- Whether the number of visible bacteria is changing during treatment
- Whether nontuberculous mycobacteria could be involved

Who should have the test

You may be offered this test if pulmonary TB or another mycobacterial lung infection is being considered. This may follow symptoms, an abnormal chest X-ray or CT scan, contact with someone who has infectious TB, or a medical condition that raises the risk of active infection.

The test may also be used during treatment. In that setting, repeated stains and cultures can show how the amount of bacteria is changing. Dead bacteria can sometimes remain visible on a stain, so a positive result during treatment does not always mean that live bacteria remain.

Testing practices differ by location and clinical situation. Several sputum samples are often collected because mycobacteria may not appear in every sample.

How to prepare

Fasting is not normally required. Follow the collection instructions from your laboratory or clinic, especially if you are collecting samples at home.

Sputum is mucus from deep in the lungs. It is not the same as saliva or nasal mucus. Before collecting it, you may be asked to rinse your mouth with plain water. Avoid adding water, mouthwash, or anything else to the container.

An early-morning sample is often requested because mucus can collect overnight. If several samples are needed, you will be told when to collect each one.

Tell the healthcare team about antibiotics or medicines being used to treat TB or other mycobacterial infections. Do not stop prescribed medicine to improve the chance of a positive test.

What happens during the test

You will receive a sterile container with a secure lid. Collection should take place in the area recommended by the clinic, often outdoors or in a specially ventilated room. Deep coughing can release bacteria into the air if an infection is present.

You will usually take several deep breaths and then cough deeply from your chest. The sputum is spat directly into the container without touching its inside. The container is then sealed and labelled.

Collection commonly takes 10 to 20 minutes, although producing sputum can take longer. If you cannot cough up a sample, you may be offered sputum induction. This involves breathing a salty mist that helps loosen mucus. In some situations, a sample may instead be collected during bronchoscopy, a procedure that passes a thin camera into the airways.

At the laboratory, the specimen is concentrated when appropriate, placed on a slide, stained, and examined under a microscope.

Understanding your results

Results may be reported as "AFB seen," "AFB not seen," positive, negative, or with a grade describing roughly how many organisms were visible.

A negative stain means no acid-fast bacteria were seen in the examined material. It does not rule out TB or another mycobacterial infection. There may be too few bacteria to see, or the tested portion may not contain them.

A positive stain means acid-fast bacteria were visible. It does not show whether they are M. tuberculosis , a nontuberculous mycobacterium, or occasionally another organism that stains in a similar way. Molecular testing and culture provide more specific information.

A culture attempts to grow living mycobacteria. It is slower than a stain but can detect smaller numbers, identify the species, and support testing for antibiotic resistance. A nucleic acid amplification test, or NAAT, looks for genetic material and can identify TB more quickly than

culture.

There is no universal numeric reference range for this test. Reporting and interpretation vary by laboratory and clinical context. Age, sex, pregnancy status, medicines, and other health conditions can affect how the result is used, even though they do not usually change the staining reaction itself.

What affects the results

The quality of the sputum sample is especially important. A sample made mainly of saliva may contain little material from the lungs and can produce a negative result even when bacteria are present deeper in the airways.

Other factors include:

Food particles, excessive saliva, a leaking container, or delays in storing and transporting the sample correctly can also reduce its usefulness.

A TB skin test, TB blood test, previous BCG vaccination, coffee, and ordinary exercise do not make mycobacteria appear on the stain.

- Low numbers of bacteria: Early, mild, or less cavity-forming lung disease may be smear-negative
- Number and timing of samples: Mycobacteria may appear in one specimen but not another
- Treatment or active antibiotics like amoxicillin or doxycycline : These can reduce the number of bacteria in sputum
- Difficulty producing sputum: This is common in children and some adults
- Reduced immune response: Some people with weakened immune systems have fewer bacteria visible on a smear despite active disease
- Environmental mycobacteria: Water or collection contamination can introduce nontuberculous species
- Recent treatment: Dead acid-fast bacteria may remain visible after treatment has started

Accuracy and limitations

Smear microscopy is quick, but it needs a fairly large number of bacteria in the examined sample. It therefore misses some cases that are later found by molecular testing or culture.

Missing infection is more likely when the sample is poor, the bacterial count is low, or disease is outside the lungs.

A positive stain can also give the wrong impression about the cause. It detects acid-fast organisms but usually cannot distinguish TB from nontuberculous mycobacteria. Stain debris and other organisms can occasionally be mistaken for mycobacteria, although laboratory quality checks reduce this risk.

Molecular testing is better when a rapid answer about M. tuberculosis is needed. Some molecular tests can also identify specific forms of antibiotic resistance. Culture remains important because it can find smaller amounts of living bacteria, identify the species, and allow broader drug-resistance testing.

No single smear result should be interpreted on its own. Symptoms, imaging, exposure history, molecular results, cultures, and response to treatment all provide additional information.

Risks and complications

Producing a sputum sample is non-invasive. Deep coughing may briefly cause throat irritation, chest discomfort, dizziness, or tiredness.

Sputum induction can cause coughing, wheezing, or shortness of breath, particularly in people with sensitive airways. It is performed with monitoring and appropriate infection-control measures.

If bronchoscopy is needed, it has separate risks and preparation requirements. These should be explained before the procedure.

What happens next

A negative result is usually reviewed alongside a molecular test and culture. You may need to provide more samples even if the first stain is negative. Cultures can take much longer than stains because mycobacteria grow slowly.

If acid-fast bacteria are seen, the laboratory may perform or recommend rapid molecular testing. The healthcare or public health team may contact you promptly about additional testing and temporary steps to reduce possible spread. A positive smear is not, by itself, confirmation of TB.

Attend routine follow-up to review final culture and identification results. Contact the clinic promptly if you cannot produce the requested samples or if your symptoms worsen.

Seek urgent medical assessment for worsening shortness of breath or repeated coughing of blood. Call emergency services for severe breathing difficulty, a large amount of coughed-up blood, fainting, confusion, or blue or grey lips.

Common questions

Can I drink coffee before the test?

Usually, yes. Fasting is not normally needed. If possible, collect the sample before eating or drinking and follow any specific instructions from the laboratory.

Does the time of day matter?

An early-morning sample is often preferred because sputum can collect overnight. Samples collected at other times can still be useful.

Can I exercise beforehand?

Ordinary exercise does not alter the stain. You do not need to exercise to produce sputum, and strenuous activity may make deep coughing more uncomfortable.

What if I only produce saliva?

Tell the person supervising the collection. Saliva is less useful because the test needs mucus from deep in the lungs. You may be asked to try again or have sputum induced.

Does a positive result mean I have TB?

Not necessarily. The stain can detect TB bacteria and nontuberculous mycobacteria but usually cannot tell them apart. Molecular testing and culture are needed for identification.

Why do I need more than one sample?

The number of bacteria in sputum can change from one sample to another. Testing several samples reduces the chance of missing mycobacteria that were absent from the first specimen.

Sources

- Centers for Disease Control and Prevention. Clinical and Laboratory Diagnosis for Tuberculosis .
- Lewinsohn DM, Leonard MK, LoBue PA, et al. Official ATS/IDSA/CDC Clinical Practice Guidelines: Diagnosis of Tuberculosis in Adults and Children . Clinical Infectious Diseases . 2017;64(2):e1-e33.

- World Health Organization. WHO operational handbook on tuberculosis, Module 3: Diagnosis-rapid diagnostics for tuberculosis detection . 2021 update.
- Caulfield AJ, Wengenack NL. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques . Journal of Clinical Tuberculosis and Other Mycobacterial Diseases . 2016;4:33-43.