TEST

Acid-fast stain

An acid-fast stain uses a microscope to look for certain bacteria, especially mycobacteria, in a body sample.

WHAT IT MEASURES
Acid-fast bacteria visible under a microscope

SAMPLE
Usually sputum; sometimes body fluid, urine, stool, or tissue

FASTING NEEDED?
No, unless another collection procedure requires it

HOW LONG IT TAKES
Minutes for sputum collection; longer for procedures

RESULTS IN
Often same day; timing varies by laboratory

USED FOR
Presumptive diagnosis and treatment monitoring of mycobacterial infection

MAIN LIMITATION
Misses low bacterial levels and cannot identify the species

Overview

An acid-fast stain is a laboratory test that looks for bacteria that keep a special dye even after being washed with acid. These are called acid-fast bacilli , or AFB.

Mycobacteria are the main group of acid-fast bacteria. This group includes Mycobacterium tuberculosis , which causes tuberculosis (TB), and nontuberculous mycobacteria, which can also cause infection.

The test is often performed on sputum, the mucus coughed up from deep in the lungs. It can also be used on urine, stool, body fluids, or tissue collected during a biopsy.

After staining the sample, a laboratory professional examines it under a microscope. Some laboratories use fluorescent dyes that make possible acid-fast bacteria easier to see.

An acid-fast stain provides an early result, but it does not usually identify the exact species. Culture and molecular tests are often needed to confirm what is present.

Why the test is ordered

Your healthcare team may order an acid-fast stain when your symptoms, imaging, medical history, or exposure history suggest a possible mycobacterial infection.

For a possible lung infection, reasons may include:

The stain may also be used when mycobacteria are suspected in a lymph node, bone, joint, urinary tract, digestive tract, brain lining, or another body site.

During treatment for pulmonary TB, repeat sputum stains may help show whether the number of visible bacteria is falling. A stain alone cannot prove that treatment has cured the infection because it may detect dead as well as living bacteria.

- A cough that is not improving
- Coughing up blood
- Unexplained fever, night sweats, or weight loss
- An abnormal chest X-ray or CT scan
- Close contact with someone who has infectious TB
- A weakened immune system with concerning respiratory symptoms

Who should have the test

This test is generally for people being evaluated for active mycobacterial disease or monitored during treatment. It is not a routine screening test for people without symptoms.

TB blood tests and TB skin tests look for an immune response to TB bacteria. They do not show whether bacteria are present in sputum and cannot, by themselves, distinguish inactive TB infection from active TB disease.

An acid-fast stain may be appropriate if you cannot produce sputum naturally. In that situation, a clinician may discuss induced sputum, bronchoscopy, or another way of collecting a sample.

How to prepare

No fasting is usually needed when you are coughing up sputum naturally. Your collection kit or laboratory may ask for an early-morning sample before eating, drinking, brushing your teeth, or using mouthwash. Follow the instructions you receive.

Rinsing your mouth with plain water may reduce contamination from food and material in your

mouth. The sample needs to be sputum from deep in your lungs, not saliva.

Tell the healthcare team about antibiotics like amoxicillin or doxycycline you are taking or have recently taken. Treatment can reduce the number of bacteria in the sample.

Preparation is different if the sample will be collected by bronchoscopy, biopsy, or another procedure. You may need to stop eating and drinking beforehand or receive instructions about medicines, especially medicines that affect bleeding. Do not stop a prescribed medicine unless the team arranging the procedure tells you to.

What happens during the test

For a sputum sample, you will usually take deep breaths and cough forcefully into a sterile container. You may need to provide more than one sample because bacteria are not always spread evenly through sputum.

If you cannot cough up enough sputum, you may breathe in a salty mist that helps bring mucus up from the lungs. This is called sputum induction.

A bronchoscopy may be considered when adequate sputum cannot be obtained or another lung problem needs investigation. During this procedure, a thin flexible tube is passed through the nose or mouth into the airways. Sedation and numbing medicine are commonly used.

Samples from other body sites may be collected through a urine or stool container, a needle procedure, a lumbar puncture, or a biopsy. The time and preparation depend mainly on how the sample is collected. The staining itself happens in the laboratory.

Understanding your results

A result is usually reported as AFB seen or no AFB seen . Positive samples may also be graded according to how many acid-fast organisms were visible. Grading systems vary by laboratory.

A positive stain means acid-fast organisms were seen. It does not confirm that they are Mycobacterium tuberculosis . Nontuberculous mycobacteria and, less commonly, other acid-fast organisms can look similar under the microscope.

A negative stain means no acid-fast organisms were seen in the examined portion of the sample. It does not rule out TB or another mycobacterial infection. There may be too few bacteria to see, or the sample may not have come from the affected area.

There is no universal reference range beyond whether organisms are seen. Reporting and interpretation vary by laboratory, age, sex, pregnancy status, medicines, specimen type, and

clinical context. Molecular testing and culture results are usually interpreted alongside the stain.

What affects the results

The most important influences relate to the sample and the number of bacteria present:

Food, coffee, ordinary exercise, kidney function, age, and pregnancy do not directly change the staining reaction. They may still affect collection instructions, the likely diagnosis, or whether a procedure is suitable.

- Sample quality: Saliva contains less material from the lungs than a deep sputum sample
- Uneven bacteria: One portion of sputum may contain bacteria while another does not
- Site of infection: Infections outside the lungs often contain fewer detectable organisms
- Recent treatment: Antibiotics active against mycobacteria may lower the number of bacteria
- Previous treatment: Dead acid-fast bacteria may remain visible after they can no longer grow
- Immune status: Some people, including those with weakened immunity, may have fewer bacteria in sputum despite active disease
- Collection and processing: Concentrating the sample may improve the chance of finding organisms

Accuracy and limitations

Acid-fast microscopy is fast, but it needs a relatively large number of bacteria in the examined sample. It can miss infections with low bacterial levels. Negative stains are especially common in early disease, disease outside the lungs, and some people with weakened immune systems.

When the stain is positive, acid-fast material is usually present. However, the test cannot reliably tell TB bacteria from nontuberculous mycobacteria. Stain-like debris, sample contamination, or transfer of material between specimens can occasionally produce a misleading positive result.

A molecular test can look specifically for genetic material from the M. tuberculosis group. Some molecular tests also check for markers of resistance to particular antibiotics. These tests are generally more useful than a stain for rapidly confirming TB.

Culture attempts to grow living mycobacteria. It can identify the species and allow drug-susceptibility testing, but results take much longer. Culture and molecular testing may still be needed whether the stain is positive or negative.

Risks and complications

The stain itself has no direct physical risk because it is performed on a sample.

Coughing up sputum is generally low risk. Induced sputum can cause temporary coughing, wheezing, or chest tightness and is performed with appropriate supervision.

Bronchoscopy, needle collection, lumbar puncture, and biopsy have procedure-specific risks. These may include bleeding, infection, discomfort, reactions to sedation, or injury to nearby structures. The team arranging the procedure should explain the relevant risks beforehand.

What happens next

If acid-fast organisms are seen in a respiratory sample, the laboratory may perform or continue molecular testing and culture. While TB is being assessed, temporary airborne precautions and public health follow-up may be needed. This does not mean the stain has already confirmed TB.

If the stain is negative but concern remains, your healthcare team may wait for culture, review molecular test results, request additional samples, or investigate another cause. This is routine follow-up because a negative stain cannot exclude infection.

Contact your healthcare team promptly if you receive a positive result or if symptoms continue to worsen. Seek urgent medical help for severe trouble breathing, confusion, blue or grey lips, fainting, or coughing up a significant amount of blood.

Common questions

Can I drink coffee before an acid-fast stain?

Usually, yes. However, an early-morning sputum sample may need to be collected before eating or drinking. Do not drink coffee if you have been told to fast for bronchoscopy, sedation, or another procedure.

Does the time of day matter?

It can. Sputum collected soon after waking may contain more material that gathered overnight.

Follow the timing instructions from your laboratory, especially if several samples are requested.

Can I exercise beforehand?

Ordinary exercise does not change how bacteria take up the stain. If exercise makes you short of breath or triggers coughing, ask whether you should avoid it before sputum collection.

Is a positive acid-fast stain the same as a positive TB test?

No. It shows that acid-fast organisms were seen, but it does not identify the species. A molecular test or culture is needed to determine whether TB bacteria are present.

Does a negative result rule out TB?

No. The stain can miss low numbers of bacteria. Culture, molecular testing, imaging, and other clinical information may still be needed.

How long will the full results take?

The stain may be reported the same day or within a few days, depending on the laboratory. Molecular testing may also be rapid, while mycobacterial culture commonly takes several weeks.

Sources

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