TEST

Sputum gram stain

A sputum Gram stain uses a microscope to look for bacteria and inflammatory cells in mucus coughed up from the lungs.

WHAT IT MEASURES
Bacteria and inflammatory cells visible in coughed-up lung mucus

SAMPLE
Sputum coughed up from the lower airways

FASTING NEEDED?
No

HOW LONG IT TAKES
Usually 5 to 15 minutes; longer if sputum is induced

RESULTS IN
Usually same day; timing varies by laboratory

USED FOR
Supporting diagnosis and early treatment of bacterial lung infection

MAIN LIMITATION
Saliva contamination can hide or mimic lower-airway bacteria

Overview

A sputum Gram stain is a laboratory test performed on sputum, the thick mucus brought up by a deep cough. Sputum comes from the lower airways and lungs. It is different from saliva or spit, which mainly comes from the mouth.

In the laboratory, a thin layer of the sample is placed on a glass slide and treated with colored dyes. Different bacteria take up the dyes differently. Under a microscope, laboratory staff can assess the bacteria's color, shape, and arrangement.

The test can provide an early clue about whether bacteria may be present. It may also help suggest which broad group of bacteria is involved while a sputum culture is being completed. A culture grows organisms from the sample so they can be identified more precisely and sometimes tested against antibiotics like amoxicillin or doxycycline .

A Gram stain cannot, by itself, confirm the cause of every respiratory illness. The result must be considered alongside your symptoms, examination, imaging, and other tests.

Why the test is ordered

A sputum Gram stain may be ordered when a bacterial infection of the lower airways is suspected. This can include some cases of pneumonia, an infection involving the lung tissue, or worsening infection in someone with long-term lung disease.

The test may be especially useful when an illness is severe, initial treatment has not worked as expected, or there is concern about bacteria that may resist commonly used antibiotics. It may also be ordered for someone receiving hospital care or someone who can produce a good-quality sputum sample.

Many coughs do not require this test. Viruses commonly cause respiratory infections, and sputum testing may not help in a mild, uncomplicated illness.

Who should have the test

A healthcare professional may recommend a sputum Gram stain if you have symptoms and other findings that could fit a lower respiratory infection, such as a productive cough, fever, breathlessness, or an abnormal chest X-ray.

It may also be considered if you:

Not everyone can produce sputum on request. Young children, people with a weak cough, and people with a dry cough may need another collection method or a different test.

A standard sputum Gram stain does not reliably test for tuberculosis. Suspected tuberculosis requires special collection procedures and tests designed for acid-fast bacteria. Fungal and viral infections also need different tests.

- Have severe pneumonia or need hospital care
- Have a long-term lung condition with a suspected bacterial flare-up
- Are not improving as expected with treatment
- Have previously had resistant bacteria in respiratory samples
- Have a weakened immune system or another reason an unusual infection is possible

How to prepare

Fasting is not usually required. Follow the collection instructions from your clinic or laboratory because procedures can differ.

If possible, collect the sample before starting antibiotics. Antibiotics can reduce the number of visible bacteria, although testing may still be worthwhile after treatment has begun. Do not delay prescribed treatment just to collect a sample unless your healthcare team specifically tells you to.

An early-morning sample may be easier to produce because mucus can collect overnight. You may be asked to rinse your mouth with plain water first. This helps remove food and loose material without using an antiseptic mouthwash that might affect organisms in the sample.

Drink enough water in the hours before collection unless you have been told to limit fluids. Hydration may make thick mucus easier to cough up.

What happens during the test

You will receive a clean, sterile container. After taking a few deep breaths, you will cough deeply from your chest and bring sputum into the container. The aim is to collect thick mucus from the lower airways, not clear saliva.

The sample may look thick, cloudy, yellow, green, rust-colored, or blood-streaked. Color alone does not identify whether bacteria are present or which organism is responsible.

If you cannot produce sputum, a clinician may use nebulized saline. This is a fine mist of salty water that you breathe in to loosen mucus and trigger coughing. This is called an induced sputum sample and may take longer.

The container is sealed and sent promptly to the laboratory. Staff examine the stained slide under a microscope and may also use part of the sample for culture or other tests.

Understanding your results

A report may describe bacteria as Gram-positive or Gram-negative . Gram-positive bacteria usually appear purple after staining, while Gram-negative bacteria usually appear pink or red. The report may also describe their shape, such as round bacteria called cocci or rod-shaped bacteria called bacilli.

Laboratory staff also look for white blood cells. Large numbers can support the presence of inflammation, but they do not prove that a specific bacterium is causing an infection.

Squamous epithelial cells are cells from the surface of the mouth. Many of these cells may suggest that the sample contains mostly saliva rather than sputum from the lungs. The laboratory may report such a sample as poor quality or unsuitable for culture.

Possible reports include:

"No organisms seen" does not rule out infection. There may be too few bacteria to see, antibiotics may have reduced their number, or the illness may be caused by something that does not show well on a routine Gram stain.

There is no universal normal range for this test. Reporting and interpretation vary by laboratory, age, sex, pregnancy status, medicines, and clinical context. Antibiotic use and sample quality usually have a greater direct effect than age, sex, or pregnancy.

- A predominant type of bacteria: One bacterial pattern is seen more than others
- Mixed respiratory flora: Several types of bacteria normally found in the mouth are present
- No organisms seen: No bacteria were visible on the examined slide
- Poor-quality specimen: The sample likely contains too much saliva

What affects the results

Sample quality is the most important issue. Bacteria naturally live in the mouth and throat, so saliva can add organisms that are not causing a lung infection. It can also dilute sputum and make significant bacteria harder to find.

Other factors include:

Some organisms are not seen reliably with a routine Gram stain. These include bacteria without a typical cell wall, acid-fast bacteria associated with tuberculosis, and organisms present mainly inside cells. Separate cultures, molecular tests, or special stains may be needed.

- Antibiotics: Even a few doses may reduce visible or growable bacteria
- Collection technique: A shallow cough often produces saliva instead of lung mucus
- Transport delays: Organisms may die or multiply if the sample is not handled promptly
- Low numbers of bacteria: A small amount may be missed on the portion placed on the slide
- Oral bacteria: Normal mouth organisms can resemble bacteria involved in infection
- Recent mouth care: Antiseptic mouthwash may reduce organisms in the sample
- Difficulty producing sputum: A small or watery sample may not represent the lower airways

Accuracy and limitations

A Gram stain is fast, but it is not perfectly accurate. It is most informative when the sample clearly comes from the lower airways and contains one dominant bacterial type alongside inflammatory cells.

The test can miss a bacterial infection. This may happen when bacteria are sparse, antibiotics were started before collection, the wrong part of the sample was examined, or the organism does not stain well.

It can also suggest infection when the visible bacteria are only mouth contamination or harmless organisms living in the airways. Some people with chronic lung disease carry bacteria without those bacteria causing the current symptoms.

A sputum culture is generally better for identifying a specific bacterium and testing which antibiotics may work. Molecular tests can be better for certain viruses and hard-to-grow bacteria. A chest X-ray or other imaging can show lung changes, but it cannot identify the organism by itself.

Risks and complications

Producing a sputum sample is low risk. Deep coughing can briefly cause throat irritation, breathlessness, dizziness, or chest discomfort.

Induced sputum can sometimes cause wheezing or prolonged coughing, particularly if you have asthma or another airway condition. It is usually performed where staff can monitor you and stop if needed.

What happens next

A preliminary Gram stain result may be available before the sputum culture. Your healthcare team will interpret it with your symptoms, oxygen level, examination, imaging, and any treatment already started.

A culture may take several days and can identify an organism that was not clear on the stain. If a bacterium grows, the laboratory may test its response to antibiotics. Treatment may then be continued, changed, or stopped based on the full clinical picture.

A poor-quality result may lead to routine follow-up, another sputum collection, or a different test. This does not necessarily mean anything went wrong in the laboratory.

Seek prompt medical advice if breathing symptoms are worsening, you develop persistent

blood in your sputum, or you are becoming more unwell while waiting for results. Seek emergency help for severe trouble breathing, blue or gray lips, new confusion, fainting, severe chest pain, or coughing up a large amount of blood.

Common questions

Can I drink coffee before a sputum Gram stain?

Fasting is usually unnecessary, and coffee does not directly change the staining reaction. If possible, collect the sample before breakfast or coffee and rinse your mouth with plain water to reduce food and drink residue.

Does the time of day matter?

An early-morning sample is often preferred because mucus may collect overnight. A sample collected later can still be useful if it contains sputum from a deep cough.

Can I exercise beforehand?

Exercise does not usually alter the result. However, avoid strenuous activity if you are breathless or unwell. Follow any instructions from the collection site.

What if I cannot cough anything up?

Tell the person arranging the test. You may be offered induced sputum using nebulized saline, or a different sampling method may be more appropriate.

Should I stop antibiotics before the test?

Do not stop or delay prescribed antibiotics unless your healthcare team tells you to. Ideally, the sample is collected before the first dose, but testing may still provide useful information afterward.

Why was my sample rejected?

The laboratory may reject a sample that is mostly saliva, too small, leaking, incorrectly labeled, or delayed in transport. You may be asked to provide another sample using a deeper cough.

Sources

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