TEST

Sputum direct fluorescent antibody (DFA) test

A sputum DFA test uses fluorescent antibodies to look for a specific germ in mucus coughed up from the lungs.

WHAT IT MEASURES
Fluorescent antibodies attached to a specific germ

SAMPLE
Deep-cough sputum, sometimes induced sputum

FASTING NEEDED?
No

HOW LONG IT TAKES
Usually 10-20 minutes; induced sputum takes longer

RESULTS IN
Same day to two days, varying by laboratory

USED FOR
Helping diagnose a specific respiratory infection

MAIN LIMITATION
Poor-quality sputum or low germ levels can cause false negatives

Overview

A sputum direct fluorescent antibody, or DFA, test looks for a specific germ in sputum. Sputum is thick mucus coughed up from the lungs and lower airways. It is not the same as saliva.

The laboratory adds antibodies made to attach to a particular bacterium, fungus, or other organism. These antibodies carry a fluorescent dye. If they attach to the target germ, trained laboratory staff can see a characteristic glow under a special microscope.

A DFA test must be aimed at a named organism. It is not a general test for every cause of cough or pneumonia. For example, fluorescent antibody methods may be used to look for Pneumocystis jirovecii , a fungus that can cause pneumonia in people with weakened immune systems. DFA has also been used for certain bacteria, including Legionella , although molecular tests, culture, or urine antigen testing may be preferred in some situations.

Test availability differs between laboratories. Some samples are sent to a specialist or public health laboratory.

Why the test is ordered

Your healthcare professional may order a sputum DFA test when your symptoms, medical history, imaging, or exposure history suggest a particular respiratory infection.

It may help:

DFA is usually part of a wider assessment. Other tests may include a chest X-ray, blood tests, sputum culture, a molecular test such as PCR, or a urine antigen test.

- Detect a specific organism directly in respiratory mucus
- Support or rule out one possible cause of pneumonia
- Provide a quicker result than culture for some organisms
- Guide decisions about further tests or treatment

Who should have the test

This test may be considered if you have signs of a lower respiratory infection and there is a reason to suspect an organism that the available DFA method can detect.

It can be particularly useful when an infection such as Pneumocystis is being considered in someone whose immune system is weakened by an illness or medicine. Testing for Legionella may be considered in selected cases of pneumonia or during a public health investigation.

Sputum DFA is not generally used to screen people who feel well. It is also not the right test for every cough. Your symptoms, immune health, recent travel or exposures, medicines, and local laboratory services help determine whether it is appropriate.

How to prepare

Fasting is not usually needed. You can generally eat and drink unless you have been given different instructions for an induced sputum procedure or another test scheduled at the same time.

Tell the healthcare team about antibiotics like amoxicillin or doxycycline , antifungal medicines, inhalers, or other treatments you have recently used. Do not stop prescribed medicine unless you have been specifically told to do so.

You may be asked to rinse your mouth with water before collecting the sample. This reduces food particles and material from the mouth. Avoid adding tap water, saliva, or anything else to

the specimen container.

If you have asthma or another condition that makes your airways sensitive, mention this before induced sputum collection.

What happens during the test

You will receive a sterile container. After taking several deep breaths, you will cough deeply from your chest and spit the sputum into the container. A good sample is usually thick or cloudy rather than clear and watery.

If you cannot cough up enough sputum, staff may use a nebulizer to help. A nebulizer creates a mist, often containing a salty solution, that you breathe in. This loosens mucus and encourages a deep cough. This is called induced sputum collection.

If sputum still cannot be collected, another type of respiratory sample may be needed. Bronchoscopy is one option. During bronchoscopy, a thin flexible tube is passed into the airways to collect fluid. This is a separate procedure with its own preparation and risks.

In the laboratory, the sample is spread onto a slide. Fluorescent antibodies are added, and the slide is washed and examined under a fluorescence microscope. The staining pattern, shape, and location of any glowing organisms are assessed.

Understanding your results

Results are usually reported as detected, not detected, negative, positive, inadequate, or indeterminate. The wording varies by laboratory and organism.

A positive or detected result means the laboratory saw material matching the specific organism targeted by the test. This may support a diagnosis when it fits your symptoms and other findings. It does not show whether unrelated germs are also present.

A negative or not-detected result means the target was not seen in that sample. It does not always rule out infection. The sample may contain too few organisms, or it may have come mainly from the mouth rather than the lungs.

An inadequate result means there was not enough suitable sputum or the sample contained too many mouth cells and too little lower-airway material. Another sample or a different test may be recommended.

There is no universal numerical reference range for sputum DFA. Interpretation varies by laboratory, the organism being tested for, and your clinical context. Age, sex, pregnancy status,

and medicines may affect the chance of infection or how the result is used, even though they do not usually change what fluorescent staining means.

What affects the results

Sample quality is one of the biggest influences. Saliva can dilute or replace mucus from the lower airways, making the test negative even when infection is present.

Other factors include:

Food, coffee, exercise, kidney function, pregnancy, and a recent blood transfusion do not directly change fluorescent antibody staining. However, anything that affects your ability to produce a deep sputum sample can affect its usefulness.

- Low numbers of organisms: Early infection or a small affected area may produce too little material to see
- Recent treatment: Antibiotics or antifungal medicines can reduce the number of organisms in sputum
- Collection method: Induced sputum and samples collected during bronchoscopy may contain more useful material than an ordinary cough sample
- Transport and storage: Long delays, incorrect temperatures, drying, or leakage can damage the sample
- Laboratory technique: Slide preparation, staining quality, microscope settings, and staff experience can affect interpretation
- Background staining: Mucus, cells, debris, or unrelated organisms may glow and be mistaken for the target

Accuracy and limitations

There is no single accuracy figure for "sputum DFA." Performance differs greatly by the organism, antibody reagent, sample type, number of organisms present, and laboratory experience.

The test can miss an infection when the sample is mostly saliva, the germ is present in low numbers, or treatment has already reduced the amount present. For some infections, a sample collected during bronchoscopy is more likely to find the organism than ordinary sputum.

The test can also report a positive result when the target infection is not present. This may happen because of non-specific fluorescence, cross-reaction with another organism,

contamination, or an error when reading the slide.

A molecular test such as PCR often finds smaller amounts of genetic material and may be better when maximum detection is important. However, very sensitive molecular tests can sometimes detect an organism that is present without causing the current illness.

Culture may be better when the laboratory needs a living organism for identification, antibiotic testing, or public health investigation. For suspected Legionella , guidelines generally favour lower-respiratory culture or molecular testing together with a urine antigen test in appropriate cases. The urine test mainly detects Legionella pneumophila serogroup 1 and does not cover every type.

Risks and complications

Coughing up sputum is non-invasive and usually has no significant complications. Forceful coughing may briefly cause throat irritation, dizziness, chest discomfort, or shortness of breath.

Induced sputum can trigger wheezing or narrowing of the airways, especially if you have asthma. Staff monitor you and can stop the procedure if needed. Bronchoscopy has additional risks, but it is not part of a routine sputum DFA collection.

What happens next

Your healthcare professional will interpret the result alongside your symptoms, examination, imaging, and other laboratory findings.

A positive result may lead to treatment directed at the organism or to confirmatory testing. A negative result may need no further action if infection is unlikely and you are improving. If concern remains, another sputum sample, PCR, culture, urine testing, or a sample collected by bronchoscopy may be considered.

Waiting for a routine result is usually appropriate if your condition is stable. Seek urgent medical advice if breathing or fever is getting worse, you cannot keep fluids down, or you are becoming unusually weak.

Call emergency services for severe difficulty breathing, blue or grey lips, new confusion, fainting, or severe chest pain. Do not wait for a sputum result if these symptoms occur.

Common questions

Can I drink coffee before the test?

Usually, yes. Fasting is not normally required. If possible, collect the sample before eating or drinking when your laboratory specifically requests this, and follow any instructions for an induced sputum procedure.

Does the time of day matter?

Not always. An early-morning sample can sometimes contain more mucus that collected overnight, but the best timing depends on the organism and laboratory instructions.

Can I exercise beforehand?

Exercise does not directly alter DFA staining. Avoid strenuous activity if you are short of breath, dizzy, feverish, or have been advised to rest.

What if I can only produce saliva?

Tell the person collecting the sample. Saliva is less useful because it comes mainly from the mouth. You may be asked to try again or have sputum induced with a nebulizer.

Will antibiotics make the result negative?

They can reduce the number of some organisms and increase the chance of a negative result. Do not delay or stop prescribed treatment unless your healthcare professional tells you to.

Is DFA the same as PCR?

No. DFA uses fluorescent antibodies to show the target under a microscope. PCR looks for the organism's genetic material and may detect smaller amounts.

Sources

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