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Cite this as: *BMJ* 2021;373:n1411

<http://dx.doi.org/10.1136/bmj.n1411>

Published: 22 June 2021

## PRACTICE POINTER

# Interpreting a lateral flow SARS-CoV-2 antigen test

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### What you need to know

- The positive predictive value of a positive lateral flow device (LFD) test depends on the underlying likelihood of disease
- When the disease incidence is low, a positive result should be validated by a polymerase chain reaction (PCR) test. However, if your clinical opinion is that covid-19 is likely, then a positive test is likely to be reliable
- LFD testing is not recommended when the person has symptoms of covid-19, as a negative LFD is not sufficient to rule out covid-19
- If a symptomatic patient informs you that they have had a negative covid-19 test, check what type of test was done
- If covid-19 is clinically suspected, a PCR test is recommended, even if the patient has received a negative result from a recent LFD test

Lateral flow devices (LFDs) are being used to test asymptomatic people for covid-19 as part of the approach in the UK and elsewhere to control the spread of the disease and to enable society to reopen.<sup>1-4</sup>

The risks and benefits of using LFDs for widespread testing of asymptomatic people are the subject of ongoing uncertainty and debate.<sup>15-7</sup> Despite concerns about accuracy, LFD tests continue to be widely used. In the week ending 19 May, 4.9 million registered tests were undertaken in England.<sup>8</sup> People taking a test receive advice on what their result means and what they should do; however, the widespread use of these tests means doctors may increasingly be asked about them (for example, when patients with a recent result present to services). This practice pointer considers how to interpret and communicate results from LFD tests based on our current understanding of the tests' performance.

### What is a lateral flow device?

Lateral flow devices can detect the presence of a target substance in a liquid, typically in a single use disposable device. Their use is well established for home pregnancy testing. For covid-19, these devices are detecting a SARS-CoV-2 antigen, consequently sometimes they are termed "rapid antigen tests." A large number of SARS-CoV-2 antigen LFD tests are available internationally.<sup>9</sup> In the UK the Innova test is approved by the Medicines and Healthcare products Regulatory Agency to identify covid-19 in people who do not have symptoms and is the only test widely used.<sup>10</sup> The US drug and device regulator, the Food and Drug Administration, has not approved the Innova test and in June 2021 issued a safety communication warning the public not to use the test

based on concerns that its performance had not been adequately established.<sup>11</sup>

Other LFDs have met minimum standards and are being field tested in the UK.<sup>12</sup> This article focuses on interpreting the Innova LFD; the underlying principles will be similar for the interpretation of other tests.

### What is the UK policy?

Currently the government is making LFDs freely available to all adults and secondary school children (age 11+) in England, with advice to test twice each week to detect cases in people without symptoms.<sup>1</sup> In addition, visitors to care homes in England are also expected to undertake an LFD before their visit. Northern Ireland, Wales, and Scotland are also making these tests widely available, although the recommendations between countries vary.<sup>2-4</sup> Independent of government, some employers are establishing their own testing schemes.

Anyone who has a positive LFD test result is advised to act as if they have symptoms of covid-19—ie, they and their household should isolate and arrange a confirmatory polymerase chain reaction (PCR) test within two days. If the confirmatory test is negative, they are advised that they do not need to isolate.<sup>13</sup> If the confirmatory test is positive, then they are advised to continue to isolate for 10 days.

If people have symptoms of covid-19, they are asked to book a PCR test to rule out covid-19, rather than use an LFD. Typically, people who have had a positive result from a recent PCR test are advised not to participate in regular LFD testing for 90 days.<sup>14 15</sup> People who have been vaccinated are still encouraged to test. The performance of these tests in people who have been vaccinated has not been directly evaluated. Concerns have been raised that people who have been vaccinated may have a lower viral load, therefore will be less likely to test positive, although people who have been vaccinated are also less likely to transmit the virus.<sup>16</sup> Given the LFD is an antigen test, vaccination will not trigger a positive test result.

Recommendations for use of LFDs are changing rapidly. Trials include "test to enable" (eg, testing before attending a large cultural or sporting event) and "test to release" (eg, daily testing of contacts of cases, with a negative test enabling a partial relaxation of the 10 day isolation requirements).<sup>5 17</sup>

### How do LFD tests perform?

Some of the concern around LFDs relates to their ability to correctly pick up cases of SARS-CoV-2 infection, particularly when deployed in home and community testing. Test accuracy is defined in terms

of sensitivity and specificity.<sup>18</sup> Real world performance depends on test characteristics but also the likelihood of disease in the individual and the quality of the testing.

Data on the sensitivity and specificity of the Innova LFD test are limited. A Cochrane review synthesised the current evidence for a wide variety of LFDs.<sup>19</sup> However, all the studies on the Innova LFD included in the review had not been peer reviewed, and some more recent, relevant evaluations were not included as they were published after the end date of the searches (30 September 2020).<sup>20,21</sup> The Cochrane review reports a range of estimates for sensitivity and specificity in different contexts of use.<sup>18</sup> The sensitivity of LFDs (the proportion of people with disease who have a positive test, or the true positive rate), according to the review, ranged from 28% (when used in an outbreak investigation) to 79% (when used by laboratory scientists), and the specificity (proportion of patients without the disease who have a negative test, or the true negative rate) from 99.5% to 99.9%.<sup>19</sup> Recent analysis by Public Health England, not included in the Cochrane review, suggests the specificity may exceed 99.9%.<sup>21</sup>

These estimates of sensitivity and specificity are based on evaluating the LFD tests against a gold standard of PCR.<sup>6</sup> However, the PCR test has limitations as the gold standard test for diagnosing SARS-CoV-2 infection. Firstly, it is not 100% sensitive, meaning that some people will be missed when relying on PCR testing for diagnosis.<sup>18</sup> Secondly, a PCR test can detect very low levels of virus present in a sample, meaning a positive PCR test does not necessarily equate with people being infectious. The median time for which an individual will test positive with a PCR test is the range 22-33 days,<sup>22</sup> longer than the typical infectious period. In a clinical setting this high sensitivity to a low concentration of the virus in the sample may be helpful, by facilitating a diagnosis even if a poor sample is taken or if viral levels are low in the person being tested. But if PCR tests are used to test large numbers of people without symptoms to identify those who are infectious and to prevent further spread, the PCR test will register positive for people who are highly infectious, but it will also register positive for people who recently had the infection but are no longer infectious.

### How sensitive are LFDs at identifying people who are infectious?

In the UK, LFDs are being used primarily to prevent spread of SARS-CoV-2 by finding cases among people who do not have symptoms of covid-19. Key to this is the sensitivity of LFDs in identifying people who are currently infectious. In this context, LFDs' poor ability to detect people who are not infectious (but who recently had the infection) is not a concern. The PCR test, in contrast, identifies those who are currently infectious, and those who were previously infected but are no longer infectious. We need to be mindful of this when assessing data evaluating the accuracy of LFD tests against a PCR reference standard.

Assessing the performance of LFDs in identifying people who are infectious depends on having a good measure of infectiousness. One proxy measure of infectiousness is the Ct value from a PCR test. The Ct value is the number of PCR cycles required to detect the virus, with a low Ct value indicating a large concentration of virus present in the tested sample. The measured Ct value is only likely to be a proxy for the viral load in the patient, as it will depend on the sample quality.<sup>23</sup> Evidence shows a strong correlation between Ct values and in vitro infectiousness<sup>24</sup> and some evidence for increased risk of transmission from patients with lower Ct values.<sup>25</sup> However, no agreed Ct threshold exists for infectiousness. Transmission is also likely to be influenced by other factors (eg, host immunity, social

distancing, and mask wearing) as well as how infectious the case is.

Some of the variation in reported sensitivity is explained by variation in the Ct value.<sup>6,26,27</sup> For example, a large evaluation of community testing in Liverpool (not included in the Cochrane Review) among people without symptoms found relatively high sensitivity when testing people with a lower Ct (>80% for samples requiring fewer than 20 PCR cycles to detect the virus—ie, Ct <20), but very low sensitivity at high Ct values (6% for a Ct value of 30-35).<sup>27</sup> Taking a conservative threshold for an infectious sample (a Ct value ≤25) from the Liverpool study would suggest that LFDs have sensitivity of 67% for identifying a person who is infectious (95% confidence interval 41% to 87%).<sup>27</sup> The Liverpool study (n=5869) was relatively large and reflected real world use, with trained lay testers, and although it was primarily an evaluation of supervised testing rather than home testing, it is more relevant than data from the Cochrane review,<sup>19</sup> which comes exclusively from the early Public Health England (PHE) evaluation.<sup>26</sup>

### Other factors affecting test sensitivity

The Liverpool and PHE evaluations suggested that some of the reported variation in sensitivity could be explained by the quality of the testing undertaken.<sup>26,27</sup> That includes taking the sample, processing it, and reading the test. For example, the PHE evaluation reported higher sensitivity (79%) when the testing was undertaken by laboratory scientists compared with non-scientists (58%).<sup>26</sup> This was based on limited data, but nonetheless raises the possibility that home testing (which increasingly predominates) may be less sensitive than testing performed in supervised test centres.

### What do clinicians need to know to understand a test result?

Test characteristics (sensitivity and specificity) alone are of limited value in interpreting the test result. Knowing the pre-test probability, or the underlying likelihood of an individual having covid-19, is vital for interpreting the test result.

To assess this, inquire about why the test was done, as well as other factors that might influence underlying risk of covid-19, including:

- epidemiological link (eg, contact with a known case or link to an outbreak)
- travel to or residence in an area of higher transmission
- occupational risk
- symptoms suggestive of covid-19
- vaccination status
- history of previous infection.

A good understanding of the local epidemiology (local UK data are available at <https://coronavirus.data.gov.uk/>) can improve interpretation. Where it is known, it may be helpful to shift the pre-test probability up or down based on age or other risk factors. For example, if assessing the result of a student, knowledge of recent outbreaks among students or high infection rates in young adults, would push an estimate up. Conversely, rates of infection tend to be lower in older adults who have fewer social contacts and (in the UK) are now mostly immunised.

Also consider the quality of the testing (eg, who did the test, their familiarity with testing, and whether they used a recognised test). The sensitivity and possibly the specificity may decline if the quality of testing is weaker.

The calculator with this article uses sensitivity, specificity, and pre-test probability to estimate the likelihood that someone with a positive test actually has the infection. It can also be used to estimate other parameters: the negative predictive value (the likelihood that someone with a negative test does not have the infection), as well as the likelihood of having a false positive or a false negative.

## Interpreting a positive test result

Table 1 shows how the post-test probability of being infectious increases as the underlying pre-test probability increases. It also shows how the post-test probability changes as the test characteristics (sensitivity and specificity) change. We have given two scenarios for test performance (boxes 1, 2)—in part to reflect ongoing debate about the accuracy of these tests, but also to reflect possible real differences in performance based on the quality of testing.

Table 1 | Estimates of post-test probability of being infectious after an LFD test under different values of sensitivity and specificity

Pre-test probability (%)	Post-test probability of having covid-19 and being infectious (%)					
	Scenario 1: sensitivity 50%, specificity 99.5% (eg, home testing)		Scenario 2: sensitivity 67%, specificity 99.9% (eg, community testing)		Scenario 3: sensitivity 80%, specificity 99.9% (eg, very high quality testing)	
	Positive test	Negative test	Positive test	Negative test	Positive test	Negative test
0.01	0.99	0.01	6.28	<0.01	7.41	<0.01
0.05	4.86	0.03	25.1	0.02	28.6	0.01
0.10	9.1	0.05	40.1	0.03	44.5	0.02
0.20						
0.50	33.4	0.25	77.1	0.17	80.1	0.10
1.00	50.3	0.51	87.1	0.33	89.0	0.20
5.00	84.0	2.58	97.2	1.71	97.7	1.04
10.0	91.7	5.29	98.7	3.54	98.9	2.18
20.0	96.2	11.2	99.4	7.63	99.5	4.77
50.0	99.0	33.4	99.8	24.8	99.9	16.7

### Box 1: Clinical scenario 1: a positive test result

A 42 year old factory worker has tested positive for SARS-CoV-2 using an LFD. He is well and has not been vaccinated. He has not travelled abroad recently and lives locally. No other cases have been recorded in the factory. The current levels of infection in the community are very low.

Neither epidemiological factors nor symptoms suggest covid-19. The pre-test probability is likely to be very low. Assuming a pre-test probability of 0.05% and relatively good testing (sensitivity 67% and specificity 99.9%), the post-test probability for being infectious would be 25%. Three in four people with this result would have a false positive, but one in four would be a true positive. In keeping with current national recommendations that a positive result from a LFD test requires PCR adjudication, the man should be advised to organise a PCR test within 48 hours of his positive LFD test result.<sup>13</sup> He and his household should continue to isolate pending the results of the PCR test.

### Box 2: Clinical scenario 2: a negative test result

A 16 year old girl presents with a cough, fever, headache, and fatigue. She has been doing twice weekly LFD tests at home before attending school, and these have all been negative. She and her parents assume she cannot have covid-19 because of the negative tests. Two of her close friends tested positive and she has been identified as a close contact.

Clinically you estimate a pre-test probability to be around 40% based on her history. Assuming a pre-test probability of 40%, sensitivity of 50% and specificity of 99.6%, the post-test probability would be 25%. This is too high for the negative result to rule out the diagnosis of covid-19 (ie, two to three out of every 10 children presenting like this would actually have covid-19), and PCR testing would be valuable, ideally undertaken by a healthcare professional to ensure a good sample is taken.

Different values of sensitivity and specificity influence the post-test probability, and the pre-test probability is an important driver of the post-test probability, which underscores the importance of

estimating the underlying likelihood of that person having the disease. When disease levels are lower and the testing is restricted to people who do not have symptoms, the pre-test probability is often likely to be very low, less than 0.1%.

At the lower levels of pre-test probability, the post-test probability will be lower, and false positive results become more likely (box 1). Confirmatory PCR should be undertaken to reduce the risk of false positives. For this reason, in April 2021 NHS Test and Trace recommended that confirmatory PCR testing was reinstated for all positive LFD tests.<sup>28</sup>

Despite this, in some circumstances the pre-test probability may be substantially higher, even when disease levels are low. Current guidelines recommend LFDs should not be used to test people with symptoms of covid-19, but widespread availability means these tests are often used by people with symptoms, and some people may develop symptoms shortly after testing. These people will have an elevated risk of covid-19, which might be substantially higher than the background prevalence in the community. Close contacts will also have an elevated risk of covid-19. If the pre-test probability was 20%, the post-test probability of being infectious given a positive LFD test is likely to exceed 96%.

## Interpreting a negative test

To determine the reliability of a negative LFD test, pre-test probability needs to be taken into account. For most people being tested who do not have symptoms this is likely to be low or very low. A negative LFD test result will reduce the post-test probability of having disease (table 1), but it does not eliminate fully the possibility of infectiousness. If we assume a sensitivity of 50%, a negative test result will approximately halve the post-test probability of disease.



Identify any factors that might indicate a higher pre-test probability. For example, people with symptoms or who have been a contact of case will have an elevated risk of covid-19, potentially greater than the background prevalence in the community, and a negative LFD should therefore be treated with caution. If covid-19 is suspected clinically, arrange a PCR test. More generally, when a patient states they have had a negative covid-19 test, the clinician should check whether this was an LFD test or a PCR test and reinforce the need to isolate if symptomatic until the patient has received a PCR test result.

## Communicating test results

Distilling the complexity and uncertainty surrounding test results is not easy. We suggest that when sharing information about LFDs with the public it is best to be open, share uncertainty, and avoid oversimplifying. In support of this approach, a recent trial found that wording that incorporated uncertainty around SARS-CoV-2 PCR tests led to fewer people interpreting results as definitive, and more people taking a cautious behavioural interpretation (continued self-isolation if symptomatic with a negative test).<sup>29</sup>

A source of concern is that people might interpret a negative result as a “green light” and stop or reduce other protective behaviours. If this attitude is widespread, the benefits of testing, in terms of identifying cases and preventing transmission, could be offset by people relaxing effective measures that stop spread. Very limited empirical evidence supports or refutes these concerns. However, almost 40% of those who used an LFD test in Liverpool reported that they may change their behaviour based on a negative test result—22% reported they would be more likely to engage in low risk activities such as outdoor exercise, and 9% said they would be more likely to visit friends and family, an activity that was actively discouraged at the time of the study because of the higher risk of transmission.<sup>27</sup>

The terms “red light” to describe a positive result (ie, stop all activities and isolate immediately) and “orange light” to describe a negative test (ie, continue to proceed with caution) may be helpful means to guide people’s behaviour.

### Education into practice

- How would you estimate the pre-test probability of a patient being infectious with SARS-CoV-2?
- How would you discuss an LFD test result with a patient who has symptoms of covid-19 and has done an LFD test before presenting?

### How patients were involved in the creation of this article

Four patient and public contributors from the NIHR ARC West Health Systems Panel and the Plain English Panel contributed feedback on how test results should be communicated to patients. One public contributor (Cathy Rice) additionally provided feedback on the clinical cases and reviewed the article before submission.

### How this article was created

This article was produced at speed. We searched Pubmed, Cochrane Covid-19 study register, Google, Google Scholar, and the WHO Global Research Covid-19 database using the terms “covid”, “SARS-CoV-2”, “sensitivity”, “specificity”, “diagnosis”, “test”, “lateral flow”, and “Innova”. This was supplemented by discussion with colleagues and identifying relevant references cited in the identified papers.

Contributors and the guarantor: OM and NM conceived the article. OM drafted the manuscript, PW undertook the literature search, and JW was the contact for public involvement. All authors reviewed the article and helped with the tables and boxes. OM is the guarantor.

Acknowledgments: Patient and public involvement was supported by the National Institute for Health Research (NIHR) Applied Research Collaboration West (NIHR ARC West). We thank our patient and public contributors, in particular Cathy Rice from the NIHR ARC West Health Systems Panel, for their contributions to the article. Noel McCarthy is affiliated to the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Gastrointestinal Infections at University of Liverpool in partnership with Public Health England (PHE), in collaboration with University of Warwick. The views expressed in this article are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

Competing interests *The BMJ* has judged that there are no disqualifying financial ties to commercial companies. The authors declare the following other interests: none.

Further details of *The BMJ* policy on financial interests are here: <https://www.bmj.com/about-bmj/resources-authors/forms-policies-and-checklists/declaration-competing-interests>

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