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Testing for SARS-CoV-2: interpretation of PCR and serology tests

Aims

This guide describes the various diagnostic tests that are available to detect the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection resulting in coronavirus disease (COVID-19), or an immune response to this infection, to help clinicians make more informed decisions based on the results of these tests. No particular specialist knowledge is assumed. This guide is not a recommendation for a particular testing policy.

General considerations of testing

As with any clinical test it is important to understand exactly what a test can tell you and how reliable a test result might be. Tests are used in different ways:

Diagnostic Tests

Tests are frequently used to inform the diagnosis of disease. When a patient presents with a particular spectrum of symptoms or signs suggesting a certain disease, then a test can be extremely informative in establishing a diagnosis. To be useful, a diagnostic test must meet certain criteria, notably **sensitivity** and **specificity**.

Sensitivity is a measure of how good the test is in correctly identifying the presence of the disease in all those who actually have it. The sensitivity of a test is the proportion of people who test positive using the test out of all those tested who actually have the disease. So a test with 90% sensitivity, will detect 90 people out of 100 tested who actually have the disease. A negative diagnostic test result in an individual who actually has the disease is a false negative.

	Disease present
Test positive	90
Test negative	10
Total	**100**

False negative

Specificity is a measure of how well the test distinguishes those who do not have the disease from those who do. It is the proportion of negative test results obtained out of all those who really do not have the disease. So, when testing 100 people who do not have the disease being tested, if the test gives a negative result in 99 cases out of 100 people it has a specificity of 99%. In other words, 1% of the tests give a false positive result.

	Disease absent
Test positive	1
Test negative	99
Total	**100**

False positive

An ideal test is both highly sensitive and specific – correctly picking up almost everyone with a disease and with a very low false positive rate. However, in practice, there is often a trade off between sensitivity and specificity and a test can still be useful even if it does not meet high levels of both of these criteria. For example, D-dimer is normally undetectable in the blood and is produced after a clot has formed and is in the process of being broken down. The D-dimer test is therefore very sensitive for the detection of venous thrombosis and hence pulmonary embolism. However, it is not very specific as the level can be elevated in a number of other conditions, notably, infection or inflammation. The D-dimer test is said to have high sensitivity but low specificity.

This example works because the disease (pulmonary embolism) has a trait (raised D-dimer) that **is almost always present and the test looks for that trait**. If the trait is not present, the disease is unlikely to be present.

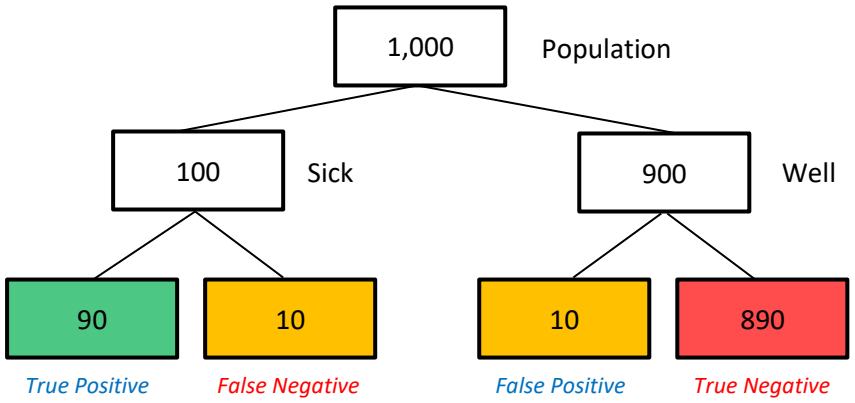
In the event of finding a positive test result (raised D-dimer), a more specific test has to be used – such as Doppler imaging of lower leg veins or a computed tomography (CT) pulmonary angiogram. But the negative test result means these more invasive techniques do not need to be employed.

Diagnostic tests also have a predictive value, ie how reliable is the result in ruling in or ruling out the diagnosis? This is measured as the **positive and negative predictive values** of a test. The positive predictive value tells you how likely it is that the patient has the disease if the test result is positive. The negative predictive value tells you how likely it is that a negative test result means the patient does not have the disease. These measures depend on the sensitivity and specificity of a test and also the **prevalence** of the disease in the population.

For example, take a disease with a prevalence of 10% and a test that has a sensitivity of 90% and a specificity of 99%. In 1,000 people a 10% population prevalence indicates that 100 will have the disease. With a sensitivity of 90% the test will identify true positives only 90% of the time, so the test will identify 90 people – but it is only 99% specific, so it will also show 10 people as positive who do not have the disease. So out of this total of 100 positive tests, the likelihood that the test correctly identifies a patient with the disease is 90/100 or 90%.

	Disease present	Disease absent	**Total**
Test positive	90	10	**100**
Test negative	10	890	**900**
Total	**100**	**900**	**1,000**

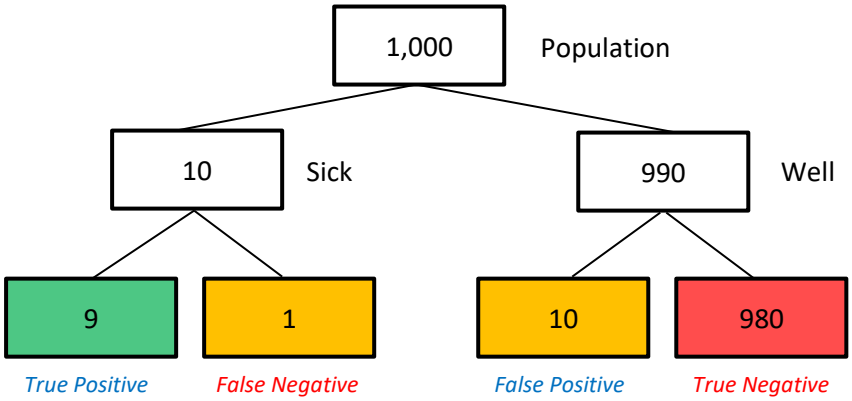
Positive predictive value	90%
Negative predictive value	99%



If the prevalence of the disease is now less, say 1%, only 10 people will have it. The test will identify true positives 90% of the time, ie 9 patients. As it is only 99% specific, there will be 1% false positives, ie 10 patients. So out of the 19 positive tests, only 9 will actually have the disease, and the likelihood that the test correctly identifies a patient with the disease is 9/19 (47%).

	Disease present	Disease absent	**Total**
Test positive	9	10	**19**
Test negative	1	980	**981**
Total	**10**	**990**	**1,000**

Positive predictive value	47%
Negative predictive value	99.9%



Thus, as the prevalence of a disease declines, the positive predictive value of the test will also decline. Correspondingly, the negative predictive value of the test will increase as the prevalence declines.

Further considerations of interpreting test results in the light of prevalence of a disease and its application to testing for SARS-CoV-2 have been described.[1]

Screening Tests

Tests can also be used as screening tools – to detect patients with a disease who do not have any visible symptoms. An example is screening for bowel cancer, where testing is used to detect small amounts of blood in stool as a potential marker for a bowel malignancy. This is sensitive test that will pick up tumours at an early stage when treatment is most effective. It is not 100% specific as other things, even eating red meat, can give false positives. A more invasive test – colonoscopy – is required to establish a firm diagnosis. A screening test can be made more specific by offering it to sections of the population where prevalence is known to be highest, so that bowel cancer screening is only offered to those aged 50 to 74 years, increasing the test's positive predictive value.

Application to SARS-CoV-2 diagnosis and screening

Diagnostic testing

Diagnostic testing for SARS-CoV-2 should take place in the population of patients suspected to have a high chance of having COVID-19 – that is, patients who have the recognised features of the disease, such as persistent cough, fever or loss of taste or smell. In the summer months other causes of these symptoms are relatively rare, so the prevalence of COVID-19 in those with these symptoms is likely to be reasonably high. If the diagnostic test has high specificity (ie correctly reports almost all of those who do not have SARS-CoV-2 infection with a negative test result) the positive predictive value (PPV) of the test in such patients will correspondingly be high. Hence, the test will have a high likelihood of correctly identifying infected patients out of those with symptoms. However, the sensitivity of the test is not 100% (ie not all those who actually have SARS-CoV-2 infection are reported with a positive test result), so a negative test will not exclude infected patients with 100% certainty.

Screening testing

It is estimated that about 40% of infections with SARS-CoV-2 are asymptomatic and people would not be identified by the presence of symptoms as potentially being infected.[1] Asymptomatic patients have as high a viral load as symptomatic patients.[2] How long such patients remain infectious remains unclear, but they may shed viable virus for up to 14 days.[3] Identifying such individuals allows them to isolate, interrupting transmission, which is of particular use in high-risk settings such as hospitals or care homes where nosocomial transmission is a constant threat. A reliable screening test would also be useful in the period of about 48 hours prior to symptoms developing when patients are infectious. Widespread testing could identify both asymptomatic and presymptomatic individuals and break the chain of transmission.

The optimal frequency for population screening is not easy to estimate. Weekly testing in any population (eg hospital workforce) would still allow a period of time during which an individual might be at work and potentially infectious. Nor is it always clear what the true prevalence of the infection might be in the population.

The Scottish Government publishes a weekly estimate of the number of infected people in Scotland, based on modelling forecasts which includes symptomatic and asymptomatic individuals. In addition, the Office for National Statistics, in collaboration with the University of Oxford, carries out regular PCR testing of households in Scotland regardless of the presence or absence of symptoms. These data are now published regularly. As the number of infected people rises and falls, so the positive predictive value of the test varies. More details are available in a recent publication.[1]

Contact tracing

Contract tracing is a key measure to limit viral transmission. Individuals identified as contacts of a known case are much more likely to have acquired the infection (even if asymptomatic). This increases the positive predictive value of the test. In superspreading events, where a large number of individuals become infected from one case, backward tracing will also identify a population with a much higher likelihood of infection, in whom the positive predictive value of a test for SARS-CoV-2 is much higher. Identifying those individuals will be important in limiting onward transmission.

Available tests for SARS-CoV-2 and their interpretation

Only tests carried out by NHS and Lighthouse laboratories have been considered – tests offered by private companies have not been evaluated.

A series of questions on available tests for SARS-CoV-2 and their interpretation have been addressed. For each question, key sources of secondary evidence were used – in particular, an evidence appraisal report by Health Technology Wales,[2] and a rapid review by the European Network for Health Technology Assessment (EUnetHTA).[3] A high-level search of the literature was also conducted. The answers to the questions are not based on a systematic review of the literature, and the included evidence sources were not critically appraised. Further information on types of tests available and their limitations and opportunities is available in the document Coronavirus (COVID-19): Scotland's Testing Strategy, available on the Scottish Government website.

RT-PCR testing

There are a number of real-time reverse-transcription polymerase chain reaction (RT-PCR) tests being used in Scotland to diagnose SARS-CoV-2 infections, including the test being used by the Public Health England (PHE) respiratory virus unit at Colindale. These tests have been validated by both PHE and the Scottish laboratory performing the assay.

RT-PCR assays are used to identify the presence of SARS-CoV-2 virus in clinical samples. This type of assay is sometimes called an 'antigen test'. This is misleading as it is not a test for any antigen. Genuine antigen tests for SARS-CoV-2 are being developed, although they are not yet in routine clinical use within the UK. Although their sensitivity is not as high as a PCR test, they may find use in community mass testing programmes.

What does an RT-PCR test detect?

The test detects viral ribonucleic acid (RNA). The assays target a section of the SARS-CoV-2 genetic material which is specific to the new virus. The presence of viral RNA does not automatically mean that viable virus particles are present, ie it does not determine 'infectiousness' of the person tested. The test is typically reported as positive or negative or sometimes that the viral RNA is detected at a low level. The test does provide some information as to the amount of viral RNA detected – the PCR involves a number of reaction cycles, and a higher amount of viral RNA present means the test will become positive at a lower number of cycles. The cycle number at which the threshold of detection is passed is known as the cycle threshold (C_t) value – the smaller this value, the more viral RNA was present in the sample. Research is underway to understand any relationship between the C_t value and the infectious nature of the sample.

When a sample only just reaches the threshold positive value – ie it has a C_t value close to the cut off, additional tests can be performed to establish whether this is a truly positive result. The sample can be rerun on a different platform and/or using a different segment of the viral genome as a target. If this rerun test is also positive, the result will be returned as positive. If it is negative, the report will state: "Initial low positive result did not confirm on subsequent testing. Please repeat if high clinical suspicion of current infection". This approach will substantially reduce the numbers of false positives and hence increase the positive predictive power.

NHS laboratories are able to perform confirmatory repeat sample testing. This is not available in the additional testing facilities provided by Lighthouse laboratories. They may be able to report the C_t value of the test to guide decision making on whether a test is likely to reflect 'real' infection. In the future, an interpretive comment may be appended. If required, advice from a clinical virologist should be sought to assist decision making.

How quickly will I get a result?

Results take around 24 hours from the time the sample is received by the laboratory. This allows for extraction of the viral RNA from the sample and PCR reaction runtime.

A number of newer platforms can provide more rapid testing. The Cepheid system can provide test results in about 30 minutes and SARS-CoV-2 testing can be combined with rapid detection of other respiratory pathogens such as influenza and respiratory syncytial virus.

What does a positive RT-PCR test result mean?

It means viral RNA is present in the swab site. It does not necessarily mean that the person is infectious, or has an 'active infection'. The result has to be interpreted in the context of the clinical condition of the person (do they have symptoms of COVID-19 or not), whether or not they have had any previous tests (why and when), if they are a contact of a known case of COVID-19, and if they are living/working in an environment where COVID-19 is currently active.

In a person with symptoms compatible with COVID-19 a positive test result is highly likely to indicate that SARS-CoV-2 is the cause of the illness.

In a person with symptoms not compatible with COVID-19, a positive test result indicates either that they have an atypical presentation of COVID-19, or that they have recently had COVID-19 and parts of the virus are still detectable, but it is not the cause of the current illness.

In a person with no symptoms, a positive test result indicates that:

- they have asymptomatic infection, or
- they are presymptomatic and may develop symptoms in the next few days, or
- they have had a recent infection and viral RNA is still detectable, or
- it is a false positive.

From the test result alone, it is not possible to determine which of these four scenarios apply to the person.

Where should swabs for diagnostic testing be taken from and what type of sample (nasal swab, saliva, sputum) is most useful?

Studies have shown the level of SARS-CoV-2 in the upper respiratory tract declines after the first few days of symptoms and it is found in higher levels in either the throat or the nose depending on the individual, thus a combined throat/nose swab is

recommended. The timing and quality of the swabbing process can significantly affect the amount of virus collected. Storage conditions and time of transport to the lab will also affect test results. Testing the upper respiratory tract when the infection is in the lower part may give a false negative result.

Bronchoalveolar lavage samples from ventilated or severely ill patients tend to have higher viral loads and may provide a positive test result when upper respiratory tract samples are negative. However, routine collection of such samples is not possible, and induction of sputum is not recommended because of the risks associated with aerosolising respiratory secretions.

The Health Technology Wales report found 18 studies that compared samples taken from different parts of the body for RT-PCR testing.[2] Most of these studies reported taking swab samples from the upper respiratory tract. Other common samples included saliva, sputum and stool/rectal swabs. Due to the different study designs, it was difficult to compare detection rates between studies or sample sites. Table 1 presents the range of detection rates reported for different samples sites to give a sense of the variability in detection rates. Health Technology Wales tentatively concluded that the type of sample and area of the body sampled may have an effect on RT-PCR diagnostic accuracy and test results, but were unable to determine what those effects would be.

Table 1: SARS-CoV-2 detection rates using RT-PCR sampling from different sites[2]

Sample site	N studies	Lowest detection rate	Highest detection rate
Bronchoalveolar lavage fluid	2	80%	95%
Pharyngeal	13	4.2%	100%
Nasopharyngeal	2	18.9%	84.1%
Nasal	2	75%	81%
Oropharyngeal	2	7.6%	62.5%
Throat wash	1	29.2%	-
Lingual	1	36.3%	-
Saliva	6	6.3%	100%
Sputum	6	49.2%	100%
Plasma/blood	6	0%	72%
Urine	8	0%	1%
Faeces and/or rectal swab	9	12.1%	66.7%
Tears/conjunctival swab	3	3.3%	16%
Fibrobronchoscope brush biopsy	1	46%	-

Evidence-based guidelines from the Infectious Diseases Society of America (IDSA) make two recommendations relating to sampling location.[4] The first recommendation states:

"*The IDSA panel suggests collecting nasopharyngeal, or mid-turbinate or nasal swabs rather than oropharyngeal swabs or saliva alone for SARS-CoV-2 RNA testing in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19 (conditional recommendation, very low certainty of evidence).*"

This recommendation is based on 13 primary studies. Specimen types/locations reported in these studies were categorised as nasopharyngeal, mid-turbinate, nasal, throat, or saliva. The guideline group considered indirect evidence from influenza and other respiratory viruses when suggesting that nasal swabs and mid-turbinate swabs had similar sensitivity to nasopharyngeal swabs, and that these swabs were preferable to saliva samples. Table 2 presents sensitivity and specificity estimates for different sample sites reported in the guideline, and Table 3 applies these estimates to a hypothetical population of 1,000 people.

The guideline authors noted several concerns about the primary studies behind this recommendation, including samples being collected at different times from the same patient, lack of reporting of timing of sample collection relative to symptom onset, and variation in definitions of a positive test result.

The guideline authors also noted the differing levels of invasiveness associated with particular test sites/types. For example, saliva collection or a nasal swab would be less invasive than nasopharyngeal sampling. The type of sampling would also have implications for the level of personal protective equipment (PPE) required by healthcare providers when collecting the samples.

Table 2: Test accuracy estimates for different sample types at a population prevalence of 10%[4]

Sample type	Sensitivity (95% confidence interval (CI))	Specificity (95% CI)
Oral	56% (35% to 77%)	99% (99% to 100%)
Nasal	76% (59% to 94%)	100% (99% to 100%)
Nasopharyngeal	97% (92% to 100%)	100% (99% to 100%)
Saliva	85% (60% to 94%)	100% (99% to 100%)
Mid-turbinate	100% (93% to 100%)	100% (99% to 100%)

Table 3: Test results per 1,000 hypothetical patients tested based on diagnostic accuracy data from Table 2, at a prevalence of 10%[4]

	True positive	False negative	True negative	False positive
Oral	56	44	891	9
Nasal	76	24	900	0
Nasopharyngeal	97	3	900	0
Saliva	85	15	882	18
Mid-turbinate	100	0	900	0
Consequence	Patient has COVID-19 and is correctly treated	Patient has COVID-19 but does not receive treatment	Patient does not have COVID-19 and returns to daily life	Patient is incorrectly diagnosed with COVID-19

The second IDSA recommendation on sampling states:[4]

"*The IDSA panel suggests a strategy of initially obtaining an upper respiratory tract sample (eg, nasopharyngeal swab) rather than a lower respiratory sample for SARS-CoV-2 RNA testing in hospitalized patients with suspected COVID-19 lower respiratory tract infection. If the initial upper respiratory sample result is negative, and the suspicion for disease remains high, the IDSA panel suggests collecting a lower respiratory tract sample (eg, sputum, bronchoalveolar lavage fluid, tracheal aspirate) rather than collecting another upper respiratory sample (conditional recommendations, very low certainty of evidence).*"

This recommendation is based on three cohort studies that performed both an upper and lower respiratory tract sample collection consecutively on the same patients. The evidence suggested that testing lower respiratory tract samples increased sensitivity of testing for SARS-CoV-2 with RT-PCR, reducing the number of false negative results (see Table 4). Table 5 reports the results of applying the diagnostic accuracy estimates from Table 4 to a hypothetical group of 1,000 patients.

Table 4: Test accuracy estimates for upper and lower respiratory tract sampling[4]

	Sensitivity (95% CI)	Specificity (95% CI)
Upper respiratory tract (URT) sample	76% (51% to 100%)	100% (99% to 100%)
Lower respiratory tract (LRT) sample	89% (84% to 94%)	100% (99% to 100%)

Table 5: Results per 1,000 patients tested based on diagnostic accuracy data from Table 4, at a prevalence of 40% and 80%[4]

	True positive	False negative	True negative	False positive
Prevalence 40%				
URT sample	304	96	600	0
LRT sample	356	44	600	0
Prevalence 80%				
URT sample	608	192	200	0
LRT sample	712	88	200	0
Consequence	Patient has COVID-19 and is correctly treated	Patient has COVID-19 but does not receive treatment	Patient does not have COVID-19 and returns to daily life	Patient is incorrectly diagnosed with COVID-19

Current recommended samples include: upper respiratory tract sample(s): a viral nose swab and a viral throat swab in one collection tube OR single swab used for throat then nose in one collection tube OR a nasopharyngeal aspirate in a universal transport pot. Lower respiratory tract sample, if obtainable, ie sputum in a universal container.

Refer to Health Protection Scotland (HPS) guidance for sampling and laboratory investigations for more detail.

Is self testing as accurate as testing based on samples taken by a healthcare professional?

Studies in the Health Technology Wales report most frequently used sample collection by healthcare professionals; only one study involved self sampling by healthcare workers who swabbed their own nasopharynx and oropharynx.[2] No studies compared the reliability of sample collection by the tested subject with sample collection by a healthcare worker.

The IDSA guideline on molecular testing for SARS-CoV-2 makes one recommendation relating to sample collection by patients or healthcare professionals:[4]

"*The IDSA panel suggests that nasal and mid-turbinate (MT) swab specimens may be collected for SARS-CoV-2 RNA testing by either patients or healthcare providers, in symptomatic individuals with upper respiratory tract infection (URTI) or influenza like illness (ILI) suspected of having COVID-19 (conditional recommendation, low certainty of evidence).*"

The guideline notes that the three cohort studies on self collection involved patients collecting a sample in the presence of a healthcare worker. Data on self collection in asymptomatic patients were not available. Although data were limited, collection of nasal or mid-turbinate samples by patients and healthcare workers appeared to result in similar detection rates. Self collection of nasopharyngeal samples was not thought to be a likely option.

Two primary studies reported on utility of self-collected samples for testing, but did not compare self collection with samples collected by healthcare professionals.[5,6] The first study involved patients collecting oropharyngeal swabs, saliva and dried blood spot samples at home while being supervised through a telehealth video appointment with a clinician.[6] One hundred and fifty-three patients returned their kits for analysis. Of the samples collected, 96% of saliva, 97% of oropharyngeal swabs, and 93% of dried blood spots, were of sufficient quality for laboratory testing. All of the orophayrngeal samples and 99% of saliva samples had C_t values that indicated sufficient nucleic acid was present for RNA-PCR testing.

The second study reported development and validation of an at-home finger-prick dried blood spot collection kit for antibody testing.[5] The study authors report 100% sensitivity and specificity using the at-home, self-collection method with people across the USA.

What is the evidence for diagnostic testing based on blood, urine or faeces?

Using the PCR test, viral RNA has been detected very occasionally in blood or urine and fairly reliably from stool. Live virus has very occasionally been recovered from stool. There is currently no evidence to suggest that faeces are a significant route of transmission.

The Health Technology Wales report included detection rates from studies that explored the use of RT-PCR testing of blood/plasma, urine or faeces (Table 1).[2] Detection rates were consistently low in studies that sampled urine. Results from blood/plasma samples were mixed, with some studies reporting very low detection rates and others reporting detection rates comparable to other samples from the same population.

Two additional primary studies were identified that reported detection rates for blood, anal or urine samples.[7,8] One study reported viral detection rates of 2.8%, 0.8% and 10.1% for serum, urine, and stool samples, respectively.[7] SARS-CoV-2 RNA was detected in 10.5% of patient blood samples and 39.3% of anal samples. The second study described a retrospective analysis of pharyngeal swabs, blood and anal swabs using RT-PCR.[8] This study found that detection of RNA in serum, urine and stool samples was intermittent which would make testing these samples less reliable in clinical practice.

How sensitive and specific is an RT-PCR test?

It is extremely rare for a laboratory test to be 100% sensitive and 100% specific. Using real-time PCR is the best technology for detecting respiratory viruses.

A meta-analysis of 19 diagnostic studies (n=1,502) reported a pooled sensitivity for RT-PCR of 89% (95% CI 81% to 94%, I^2=90%), where repeat RT-PCR was used as the reference test.[9] Sensitivity of RT-PCR was negatively associated with the proportion of elderly patients (p=0.01). Outside of China, prevalence of COVID-19 ranged from 1.0% to 22.9%, which resulted in PPVs ranging from 47.3% to 96.4% and negative predictive values (NPV) ranging from 96.8% to 99.9%. In the UK, when the COVID-19 prevalence was estimated at 22.9%, the PPV was 96.4% and NPV 96.8% (symptomatic individuals).

Specificity of the PCR test is more difficult to gauge. Operationally, it is probably about 99.9% specific – ie 1 in 1,000 tests will be a false positive.

The Health Technology Wales report also identified five studies that assessed the use of loop-mediated isothermal amplification assays (LAMP) for the diagnosis of COVID-19.[2] These primary studies reported sensitivity ranging from 74.7% to 100% and specificity ranging from 87.7% to 100%, using RT-PCR as the reference test.

The analytical sensitivity of a real time RT-PCR is not the only factor to be considered when testing clinical samples. The quality and timing of the clinical sample also needs to be taken into account, particularly when the amount of viral material present in a patient sample will change during the course of infection.

Does a positive RT-PCR test result mean the person is infectious to other people?

A positive test result in isolation cannot determine this because it does not detect whole viable (infectious) virus particles. The result has to be interpreted in the context of the clinical condition of the person (do they have symptoms of COVID-19 or not), whether or not they have had any previous tests (why and when), if they are a contact of a known case of COVID-19, and if they are living/working in an environment where COVID-19 is currently active.

How long after an infection does a patient remain infectious?

This is an area of active research. The only way of measuring potential infectiousness is by showing the presence of live virus by culture. This is currently a research only tool, as it requires highly-specialised techniques not currently possible in routine service laboratories. **It is vital to note as set out above that the presence of viral RNA alone does NOT necessarily mean the presence of infectious virus.** We do not know the relationship between viable virus and the

ability to cause an infection, since the infectious dose of the virus is not known. At this stage, therefore, these results need to be viewed with caution. The current advice regarding the period of self isolation (10 days) and quarantine for contacts (14 days) remains unchanged, but will remain under review.

A number of small studies have attempted to define the time course of infectious virus following infection. One small study in mildly affected patients found no viable virus eight days following symptoms.[10] In a study of 90 samples positive for SARS-CoV-2 in the PCR assay from symptomatic patients, none grew viable virus beyond eight days after symptom onset.[11] Another larger study found that in hospitalised patients, it took 15 days for there to be 95% certainty that live virus had been cleared from nasopharyngeal samples.[12] In all these studies, viral PCR detection continued in some cases in excess of 20 days. A recent study analysed recovery of viable virus in symptomatic patients and found that only 6% of patients still had viable virus in combined nasopharyngeal swabs 10 days after symptom onset.[13] It is not known if the time for clearance of viable virus in asymptomatic individuals will be the same.

What does a repeat positive RT-PCR test in a patient who has recovered from severe COVID-19 indicate?

A small number of cases of reinfection with SARS-CoV-2 have been reported. While early cases involved minimal symptoms associated with reinfection,[14] later cases have been linked to more significant illness requiring hospitalisation.[15] The extent to which re-infection occurs remains uncertain.

Based on published reports, viral shedding can continue for 28 days or longer after COVID-19 infection, and this shedding of likely non-viable viral RNA may lead to what appear to be re-infections. There are reports of inconsistency in the detection of viral RNA on RT-PCR over time, as viral loads fluctuate and non-viable viral RNA leads to false positives. In two preprint studies, 14% of patients discharged from hospital had a ‘redetectable positive’ PCR result and PCR was positive as late as 50 days after symptom onset in a cohort of patients with follow-up testing.

An unpublished study performed in South Korea of patients discharged from hospital after infection with COVID-19 found 447 patients with repeat positive PCR tests for SARS-CoV-2, up to 82 days after discharge. In 108 cases viral culture was attempted and was negative. Epidemiological investigation of contacts of these retesting positive cases showed no evidence of transmission. The authors concluded that there was no evidence of infectivity of these individuals who re-tested positive after discharge.

Does a negative test mean that the person does not have COVID infection?

A negative test means that viral RNA has not been recovered from the swab site.

The result has to be interpreted in the context of the clinical condition of the person (do they have symptoms of COVID-19 or not), whether or not they have had any previous tests (why and when), if they are a contact of a known case of COVID-19 and if they are living/working in an environment where COVID-19 is currently active.

In a person with symptoms compatible with COVID-19 a negative test cannot rule out infection but must be interpreted alongside the clinical context as above. If suspicion remains high, the test should be repeated.

In a person with symptoms that are not compatible with COVID-19 a negative test result means that SAR-CoV-2 is not likely to be the cause of their symptoms.

In a person with no symptoms, a negative test result does not rule out that they are in the incubation phase of infection, ie they could become positive at any point after the test was taken. In addition, as the sensitivity of the test is not 100%, it could be a false negative as discussed above.

What does an inconclusive/void test mean?

This was a catch all term previously used by the Lighthouse laboratory to indicate that an acceptable test result was not obtained. This could have been because, for instance, the specimen has leaked, there is something in the specimen that has inhibited the PCR reaction or there was a machine failure and the specimen was lost.

The term was phased out in late October 2020 and the current wording for such circumstances is:

“We could not read your coronavirus test sample. This means it’s not possible to say if you had the virus when the test was done.”

Is it possible to grow the virus from an infected person?

Viral culture is the only way to determine if a sample actually contains infectious virus. Samples have to be inoculated onto cells grown in tissue culture to observe characteristic cytopathic effects of the virus on the cell layer.[16] Currently, viral culture can only be performed in specialist laboratories and is not generally available.

What does sequencing the virus tell us?

The genome of SARS-CoV-2 has been sequenced in its entirety. Comparison of different genome variants has revealed many important aspects of the evolution and spread of SARS-CoV-2.[17,18] Such studies have enabled tracking of different viral isolates to follow different means of transmission, such as nosocomial spread and

introductions from different countries. Viral sequencing is being used to track outbreaks in near real time and for epidemiological surveillance.

Lateral flow antigen testing

Lateral flow antigen testing detects the presence of the COVID-19 viral antigen from a swab sample. The test is administered by handheld devices producing results in 30 minutes and can be self administered, following provision of training materials. Lateral flow antigen testing has a lower sensitivity than RT-PCR. However, studies to date suggest that these lateral flow antigen tests are more sensitive at higher viral loads, hence may be more practical for detecting individuals who are infectious, rather than individuals who may have had COVID-19 in the recent past but are no longer infectious (RT-PCR will detect both).[19]

Serology

What is a serology (antibody) test?

A serology test measures the production of antibodies to SARS-CoV-2. Currently available tests either detect both Immunoglobulin M (IgM) and Immunoglobulin G (IgG) at the same time or just IgG.

How accurate is the serology test?

As with any test, the issue is of sensitivity and specificity. At the moment it is unclear how long antibody levels persist and to what degree those who are infected generate an antibody response. One recent study found that antibody persisted for up to two months, but 2–8.5% of those with proven infection did not mount a detectable antibody response, even weeks after infection.[20] Another study has examined antibody levels in symptomatic and asymptomatic patients.[21] They found that about 80% of both groups had IgG responses 3–4 weeks after infection. However, at eight weeks after discharge 40% of asymptomatic patients were seronegative. Viral-specific T-cell responses are present following infection, but again their duration and ability to prevent disease are not clear.[22]

A Cochrane review concluded: “Antibody tests one week after first symptoms only detected 30% of people who had COVID-19. Accuracy increased in week 2 with 70% detected, and was highest in week 3 (more than 90% detected). Little evidence was available after week 3. Tests gave false positive results in 2% of those without COVID-19.”[23]

Does a positive serology (antibody) test mean the person cannot pass COVID-19 to other people?

A European health technology assessment (HTA) concludes there is very limited evidence on this question and what is available does not suggest any value of antibody tests for ruling out re-infection or virus transmission in recovered COVID-19 patients.[3]

Only two studies were identified that addressed the association between IgM and IgG concentration and reinfection after recovery from COVID-19.[3] Both studies were at moderate risk of bias. In the first study (n=262) plasma antibody levels at discharge from hospital were similar in patients who later retested positive and those who retested negative. In the second study (n=74) patients who retested positive had significantly lower IgG levels within seven days of discharge from hospital compared with patients who retested negative. There were no significant differences in IgM concentration. A project to track and trace 285 patients in Korea who retested positive for COVID-19 and their 790 contacts found that 44% of those investigated re-presented with symptoms, but of 108 samples cultured, none grew virus. After investigating the 790 contacts the authors found no evidence indicating infectivity of patients who retested positive. In 23 cases where serial antibody levels were available for these patients, 96% were positive for neutralising antibodies.[3]

The HTA also sought to answer the question: what role can antibody tests have in assessing protective immunity in subjects with past SARS-CoV-2 infection? No studies were identified to address this question.[3]

Appendix – some additional scenarios and suggested responses

Asymptomatic healthcare worker (HCW) returning to work

Scenario
A HCW had a positive SARS-CoV-2 RT-PCR test after an outbreak on the ward where they worked, which prompted all staff to be screened. They had no symptoms when tested and were advised to self isolate for 10 days at home before returning to work. They remained asymptomatic during these 10 days but are nervous about returning to work and have asked for a further test? Will another SARS-CoV-2 PCR test show that they could still pass the infection to someone else?

A further test will not show if infectious virus is present and studies have shown a very low possibility of infectious virus recovery 10 days after symptom onset.[13] Current guidance specifies no further testing for a period of 90 days following a positive test result unless the individual develops symptoms consistent with COVID-19.

Severe COVID-19 illness with repeat positive RT-PCR test result

Scenario
A 72 year old man spent 4 weeks in ITU with severe SARS-CoV-2 infection confirmed by PCR test. Prior to ward stepdown he was retested and his SARS-CoV-2 PCR test was negative. 5 days later he deteriorated and became hypoxic. He was transferred back to ITU and a further SARS-CoV-2 test was taken and is positive. Has this man developed a second SARS-CoV-2 infection, are the staff at risk from aerosol-generated procedures (AGPs) in intensive care?

It is not uncommon for tests to be only intermittently positive as the infection is cleared and the presence of viral RNA does not necessarily indicate viable virus. However, given his illness pattern, it would still be important to maintain all the recommended PPE and precautions if AGPs are undertaken.

Scenario
A 55 year old woman spent 2 weeks as an inpatient. Prior to discharge when medically fit she was assessed as requiring temporary home support to aid her recovery. A repeat SARS-CoV-2 PCR test was taken. This was positive, so her discharge was delayed. The test has been repeated at weekly intervals for the last 3 weeks and is still positive. As she is medically well and wants to go home but will need home care support. Does she need to isolate on discharge?

No. There is good evidence to support the view that 15 days after infection, the chances of her shedding viable virus are extremely low.

Prehospital screening

Scenario
A 47 year old woman had a SARS-CoV-2 PCR test taken 3 days prior to elective surgery. She is currently well, but describes feeling non-specifically unwell with mild diarrhoea 5 days ago for 2-3 days. She did not have a cough or fever or altered smell/taste. Her test is positive. Should I delay her surgery?

Yes. Positive test results may be misleading in this context, but the outcomes from surgery if suffering from SARS-CoV-2 infection are very poor.

This woman's surgery was delayed and she has been retested 7 days later. She has no symptoms but her test is still positive. Should I delay her surgery again?

The interval from symptoms is now over 12 days and the chances of her having active infection very remote. An individual risk assessment should be carried out to evaluate the risks of further delaying surgery against the risk of poor outcome if surgery proceeds in the presence of SARS-CoV-2 infection.

Scenario
A 68 year old man has self isolated for 14 days prior to elective surgery. He was tested three days prior to the planned procedure. He has no symptoms, but his test is positive. What should I do?

The test may well be a false positive. The test should be repeated after 24 hours and, if negative and remaining asymptomatic, surgery could proceed. If positive, surgery should be deferred.

Inconclusive results

Scenario
A 36 year old man was advised by his employer to seek a SARS-CoV-2 test before restarting his job even though he has not had any symptoms. He booked a test online through gov.scot and stated that he had a fever in order to get the test. The result came back as inconclusive. Should he delay starting work until he has another test done?

He should take advice from his employer. If their risk assessment requires a negative test, then it should be repeated. He should follow the guidance on self isolation and quarantine for the household pending the results of a second test.

Scenario
A 44 year old woman booked a SARS-CoV-2 test online. She had a headache, itchy eyes, a running nose and felt that her temperature was elevated. Her symptoms

resolved the day after the test. The test came back inconclusive. Should she have another test?

Although unlikely, she may well have COVID-19. It is important that all cases and their contacts are identified. She should have another test and be advised to follow the guidance on self isolation and quarantine for the household pending the results of this second test.

Repeat positive tests after mild illness

Scenario
A 40 year old man was advised by his employer to seek a SARS-CoV-2 test. He booked a test online, stating he had a fever, despite being symptom free. The test came back positive. He did have 3 days of a mild 'viral illness' about 3 weeks ago that self resolved. His employer requested a further test before return to work. He again stated that he had a fever on the online form 7 days later. The test is still positive. Can he return to work, should he do another test?

It is highly likely that he is no longer infectious and he should be advised of that fact. Another test will not contribute much. The responsibility for providing advice rests with the employer's occupational health provider.

Abbreviations

AGP	aerosol-generated procedure
CI	confidence interval
COVID-19	coronavirus disease 2019
CT	computed tomography
C_t	cycle threshold
EUnetHTA	European Network for Health Technology Assessment
HCW	healthcare worker
HIS	Healthcare Improvement Scotland
HPS	Health Protection Scotland
HTA	health technology assessment
IDSA	Infectious Diseases Society of America
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ILI	influenza like illness
LAMP	loop-mediated isothermal amplification assay
LRT	lower respiratory tract
MERS	middle eastern respiratory syndrome
MT	mid-turbinate
NHS	National Health Service
NPV	negative predictive value
PHE	Public Health England
PPE	personal protective equipment
PPV	positive predictive value
RNA	ribonucleic acid
RT-PCR	real-time reverse-transcription polymerase chain reaction
SARS	severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
URT	upper respiratory tract
URTI	upper respiratory tract infection

References

1 Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. BMJ 2020;369:m1808.

2 Health Technology Wales. The clinical effectiveness of tests to detect the presence of SARS-CoV-2 virus, and antibodies to SARS-CoV-2, to inform COVID-19 diagnosis [cited Available from url: https://www.healthtechnology.wales/wp-content/uploads/2020/05/EAR025-COVID19-diagnostics-report-v2.6.pdf

3 European Network for Health Technology Assessment. Rapid collaborative review on the current role of antibody tests for novel coronavirus SARS-CoV-2 in the management of the pandemic. 2020. [cited 21 Aug 2020]. Available from url: https://eunethta.eu/wp-content/uploads/2020/06/RCR_OT_01-_Antibody-tests-for-SARS-CoV-2_23-06-2020.pdf

4 Infectious Diseases Society of America. Infectious Diseases Society of America guidelines on the diagnosis of COVID-19. [cited Available from url: https://www.idsociety.org/practice-guideline/covid-19-guideline-diagnostics/

5 Karp DG, Danh K, Seftel D, Robinson P, Tsai CT. A serological assay to detect SARS-CoV-2 antibodies in at-home collected finger-prick dried blood spots. MedRxiv : the Preprint Server for Health Sciences 2020;30:30.

6 Guest JL, Sullivan PS, Valentine-Graves M, Valencia R, Adam E, Luisi N, et al. Suitability and Sufficiency of telehealth clinician-observed participant-collected samples for SARS-CoV2 testing: the iCollect Cohort Pilot Study. JMIR Public Health and Surveillance 2020;29:29.

7 Kim JM, Kim HM, Lee EJ, Jo HJ, Yoon Y, Lee NJ, et al. Detection and Isolation of SARS-CoV-2 in Serum, Urine, and Stool Specimens of COVID-19 Patients from the Republic of Korea. Osong Public Health & Research Perspectives 2020;11(3):112-7.

8 Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X, et al. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microbes Infect 2020.

9 Kim H, Hong H, Yoon SH. Diagnostic Performance of CT and Reverse Transcriptase-Polymerase Chain Reaction for Coronavirus Disease 2019: A Meta-Analysis. Radiology 2020:201343.

10 Wolfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Muller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581(7809):465-9.

11 Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis 2020.

12 van Kampen JJA, van de Vijver DAMC, Fraaij PLA, Haagmans BL, Lamers MM, Okba N, et al. Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants. medRxiv 2020:2020.06.08.20125310.

13 Singanayagam A, Patel M, Charlett A, Lopez Bernal J, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. Euro Surveill 2020;25(32).

14 To KK-W, Hung IF-N, Ip JD, Chu AW-H, Chan W-M, Tam AR, et al. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. Clinical Infectious Diseases 2020.

15 Tillett RL, Sevinsky JR, Hartley PD, Kerwin H, Crawford N, Gorzalski A, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. https://doi.org/10.1016/S1473-3099(20)30764-7. The Lancet Infectious Diseases 2020.

16 Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel S, Murray J, et al. Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States. Emerging Infectious Disease journal 2020;26(6):1266.

17 Filipe ADS, Shepherd J, Williams T, Hughes J, Aranday-Cortes E, Asamaphan P, et al. Genomic epidemiology of SARS-CoV-2 spread in Scotland highlights the role of European travel in COVID-19 emergence. medRxiv 2020:2020.06.08.20124834.

18 Meredith LW, Hamilton WL, Warne B, Houldcroft CJ, Hosmillo M, Jahun AS, et al. Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study. Lancet Infect Dis 2020.

19 Joint PHE Porton Down & University of Oxford SARS-CoV-2 test development and validation cell. Preliminary report: Rapid evaluation of Lateral Flow Viral Antigen detection devices (LFDs) for mass community testing. 2020. [cited 08 Nov 2020]. Available from url: https://www.ox.ac.uk/sites/files/oxford/media_wysiwyg/UK%20evaluation_PHE%20Porton%20Down%20%20University%20of%20Oxford_final.pdf

20 Staines HM, Kirwan DE, Clark DJ, Adams ER, Augustin Y, Byrne RL, et al. Dynamics of IgG seroconversion and pathophysiology of COVID-19 infections. medRxiv 2020:2020.06.07.20124636.

21 Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020.

22 Ni L, Ye F, Cheng ML, Feng Y, Deng YQ, Zhao H, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. Immunity 2020;52(6):971-7 e3.

23 Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 2020;6(6):CD013652.