UK Health Security Agency

Key points of testing for SARS-CoV-2

UK Health Security Agency Joint Modelling Team

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Preface

The purpose of this paper is to describe the nature of the SARS-CoV-2 virus, the types of infection it can cause, and how testing for the virus can help at both a clinical and population level.

During the recent pandemic, several different testing strategies were employed to ensure morbidity and mortality from COVID were kept to a minimum. These included ‘testing to find’, (finding people who are positive, getting them to self-isolate thereby reducing community transmission), ‘testing to mitigate risk’ (for example testing the contacts of positive cases), and ‘testing to ensure –ve', (for example people were tested to ensure they were –ve before visiting a vulnerable person). These different testing strategies were used at different stages of the pandemic, depending on what outcome needed to be achieved. Modelling techniques were used to predict the outcome from the proposed testing regime, in order to inform policy decisions. There is no retrospective assessment as to whether these strategies were right or wrong – they were correct at the time, given the nature of the scientific, policy and political considerations.

The outcomes from the modelling work described in this paper, provide a very helpful insight into how policy decisions can be taken after careful consideration of the science behind the virus and a understanding of the various testing regimes. Much can be learned from this process, and used as the basis for consideration in any future pandemic where testing strategies are deemed to be necessary.

The paper was originally authored by Nick Gent, Thomas Finnie, Hannah Williams, Declan Bays, Duncan Kerrod and Jane Shallcross from the UKHSA Joint Modelling Team in October 2021.

The original paper was updated to include this preface in June 2023 and adapted for publishing by the Public Health and Clinical Oversight (PHCO) team which included Chris Kenny, Tom Fowler, Andy Dodgson, Nick Germanacos, Raghavendran Kulasegaran and Edward Blandford with inputs from Emma Bennett in the Data, Analytics and Surveillance (DAS) team.

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The course of SARS-CoV-21 infection

An infectious dose of the SARS-CoV-2 virus enters the body, binds with suitable cells and takes control of them. The virus then forces these cells to create copies of it. Generally, the virus enters the body through the nose or mouth and replicates in the nose or throat. Eventually the cells burst and the multiple copies of the virus are released into the body at large. Please see Appendix 1 for detailed timelines of SARS-CoV-2 infection and detection windows (see Key point below).

Main points

The virus is essentially an RNA molecule with a protective protein coat. These proteins are termed *antigens* and trigger an immune response in the body, which leads to the production of antibodies.[2]

The infected individual may or may not experience symptoms as the body reacts to the increasing numbers of antigens; even when symptoms are experienced, they often only appear after the individual has become infectious (sheds live virus). This makes it particularly important to try and detect infected individuals before they become symptomatic, as well as infected individuals who are asymptomatic (see 'Serial testing'). The amount of virus that an individual sheds can be quantified as the viral load.

The average time for symptoms to appear, the incubation period, is around 5 days but may take up to 14 days.

The detection window of a test is the time period during the course of an infection when the test is able to detect the infection's presence in an infected individual.

As an infection spreads through the population, it becomes important for testing purposes (among others) to know its prevalence; that is, the total number of infected people as a fraction of the population. Prevalence is a function of incidence (how many people become infected per unit time) and how long they stay infected (that is, the time until they recover or die).

The tests

Tests exist for various aspects of viral infection. The ones used routinely are: PCR tests to detect the presence of viral RNA, LFD antigen tests to detect the presence of viral proteins, and serology tests to detect the existence of antibodies (whether made in response to infection or vaccination).

[1] Though often used interchangeably in casual speech, COVID-19 is the medical condition and SARS-CoV-2 is the virus that causes it.
[2] Antibodies can also be produced in response to vaccination.

Polymerase chain reaction (PCR) tests

These use PCR technology and the Taq DNA polymerase enzyme to amplify greatly the quantity of specific genetic material found in a sample by making millions of copies of it through a series of *cycles* described below. This produces enough genetic material to be detected. Samples are usually taken from the nose and throat with a swab.

PCR tests use genetic material in the form of DNA, a double-stranded molecule. Viruses, however, contain RNA, a single-stranded molecule. Through a process called *reverse transcription*, strands of RNA are converted to double- stranded DNA, which can then undergo the PCR amplification process. Each cycle of the PCR process doubles the number of copies of specific DNA. When testing for COVID-19, PCR tests are often called RT-PCR (reverse transcription polymerase chain reaction) tests to reflect this extra step.

Main points

The number of PCR cycles needed to produce sufficient viral genetic material to be detected in a sample is the cycle threshold (Ct). A low Ct indicates that a high concentration of viral genetic material was present in the sample (it had a high viral load), while a high Ct indicates the opposite.[3]

Lateral flow device (LFD) tests

These work on the same principle as pregnancy test kits but use swabbing and solution to create the fluid for analysis. A swab from the nose or throat is immersed in a solution, which is then added to an absorbent pad (the device). Capillary action in the pad draws the solution along its length until it reaches a strip containing antibodies designed to react with the SARS-CoV-2 virus antigens. If there are any viral proteins carried in the solution, the SARS-CoV-2 specific strip (usually labelled T) will change colour to give a positive result.[4]

Main points

These are simpler, cheaper and faster than PCR tests.

They do not require laboratory processing and can therefore be used in the field or at point-of-care sites (bedside testing in contrast to laboratory testing) and are now frequently used for asymptomatic testing in non-care settings, for example, schools, homes and so on. This was particularly useful earlier in the pandemic before point of care PCR and other nucleic acid amplifications methods were available.

[3] More information on how to interpret Ct values at Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR
[4] More detailed information on the use of LFD tests at Understanding lateral flow testing for people without symptoms.

Serology tests

These test for COVID-19 antibodies in the blood. Antibodies to different parts of the virus can be detected, with the spike protein and nucleocapsid protein being the ones usually tested for. As vaccinations are based around producing antibodies to the spike protein, if a vaccinated person is later tested and found to have antibodies to both proteins, it suggests that they have been infected with SARS-CoV-2 at some time in the past.

Main points

The presence of antibodies tells you that the person tested has had the disease at some stage in the past (historic infection) or has received a vaccine, but not whether they are currently infected or immune to future reinfection.

Serology testing is useful:

- to identify people who have been infected with SARS-CoV-2 but did not present symptoms, or who did present symptoms but did not get tested, or who did get tested but received a negative result
- for estimating how many people have already had COVID-19 (and developed antibodies against it); this data ia usually known as seroprevalence and can be used to track past infections when studying how the virus is spreading through a population, as is done in the UK's REACT 2 programme and others
- when trying to establish the duration of antibody responses (see Appendix 2)

Test performance[5]

Test performance parameters

The usual analytical outcomes for a screening test are:

	Test positive	Test negative
Infection present	True positive (TP)	False negative (FN)
Infection absent	False positive (FP)	True negative (TN)

Sensitivity[6] is the proportion of positives that are correctly identified and is given by the formula:

$$\frac{TP}{TP+FN}$$

Or: TP divided by TP plus FN.

[5] Testing to find evidence of possible current infection, not evidence of past infection.
[6] Statistics Notes: Diagnostic tests 1: sensitivity and specificity, DG Altman, JM Bland. British Medical Journal 1994: volume 308, page 1,552

Main points

- a test with a 100% sensitivity will always produce a positive result for a sample that is positive (though if the test has less than 100% specificity, it may also produce a positive result for a sample that is negative, that is, a false positive
- sensitivity is inversely proportional to the number of false negatives: high sensitivity means fewer false negatives (important for purposive testing, 'Purposive testing')

Specificity[6] is the proportion of negatives that are correctly identified and is given by the formula:

$$\frac{TN}{TN+FP}$$

Or: TN divided by TN plus FP.

Main points

A test with a 100% specificity will always produce a negative result for a sample that is negative (though if the test has less than 100% sensitivity, it may also produce a negative result for a sample that is positive, that is, a false negative).

Specificity is inversely proportional to the number of false positives: high specificity means fewer false positives (important for serial testing, see 'Serial testing').

Test performance alone may not decide the reliability of testing outcomes. In COVID-19 testing especially, the competence of the sample taker can play a large role, particularly in the case of self-administered sampling. Diagnostic performance is an overall test evaluation which considers the performances of sample taking and laboratory testing together.

Main points

The sensitivity of self-administered sampling has been shown to differ from that of trained testers in some studies[7] (that is, incorrectly performed swabbing fails to reach target areas at the back of the nose or throat, leading to higher false negatives and thus lower sensitivity values); saliva-based testing may be a way to overcome this drop in performance; all this, of course, assumes a willingness to self-administer in the first place on the part of the subject.

The effect of a false positive is limited mainly to the individual receiving the result, but the effect of a false negative has much more potential to impact the surrounding population at large.

[7] For example:
Christopher P Seaman and others.'Self-collected compared with professional-collected swabbing in the diagnosis of influenza in symptomatic individuals: a meta-analysis and assessment of validity' Journal of Clinical Virolology 2019
McCulloch DJ, Kim AE, Wilcox NC and others. Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection JAMA Network Open 2020
DHSC (2020) Evidence summary for lateral flow devices (LFD) in relation to care homes

Positive Predictive Value (PPV) is the probability that an individual, given a positive test result, really does have the disease. For specified values of prevalence it is given by the formula:

$$\frac{TP}{TP+FP}$$

Or: TP divided by TP plus FP.

Negative Predictive Value (NPV) **is** the probability that an individual, given a negative test result, really does not have the disease. For specified values of prevalence it is given by the formula:

$$\frac{TN}{TN+FN}$$

Or: TN divided by TN plus FN.

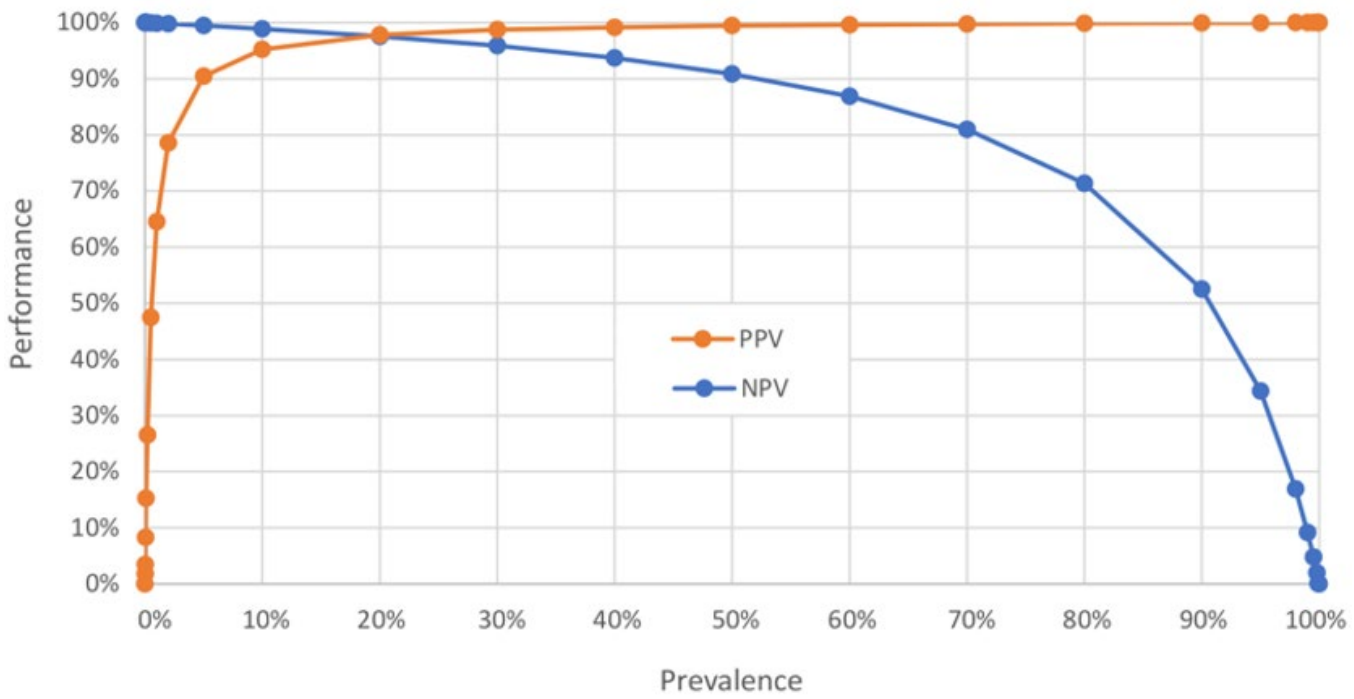
Main points

The exact values of PPV and NPV for a test depend on its intrinsic specificity and sensitivity, the performance of the sampling stage, and the existing prevalence of infection.

PPV, NPV and prevalence

PCR testing

Figure 1. Percentage values of PPV and NPV plotted against increasing levels of prevalence (sensitibity 90% and specificity 99.5%)



Main points

Current PCR tests are believed to have a test sensitivity very close to 100%, a diagnostic sensitivity of 90% and a diagnostic specificity between 98% and 99.5% for swab-based,

laboratory testing systems; most PCR systems detect 2 viral gene targets to reduce the rate of false positives; further to this, guidance exists to address low prevalence.[8]

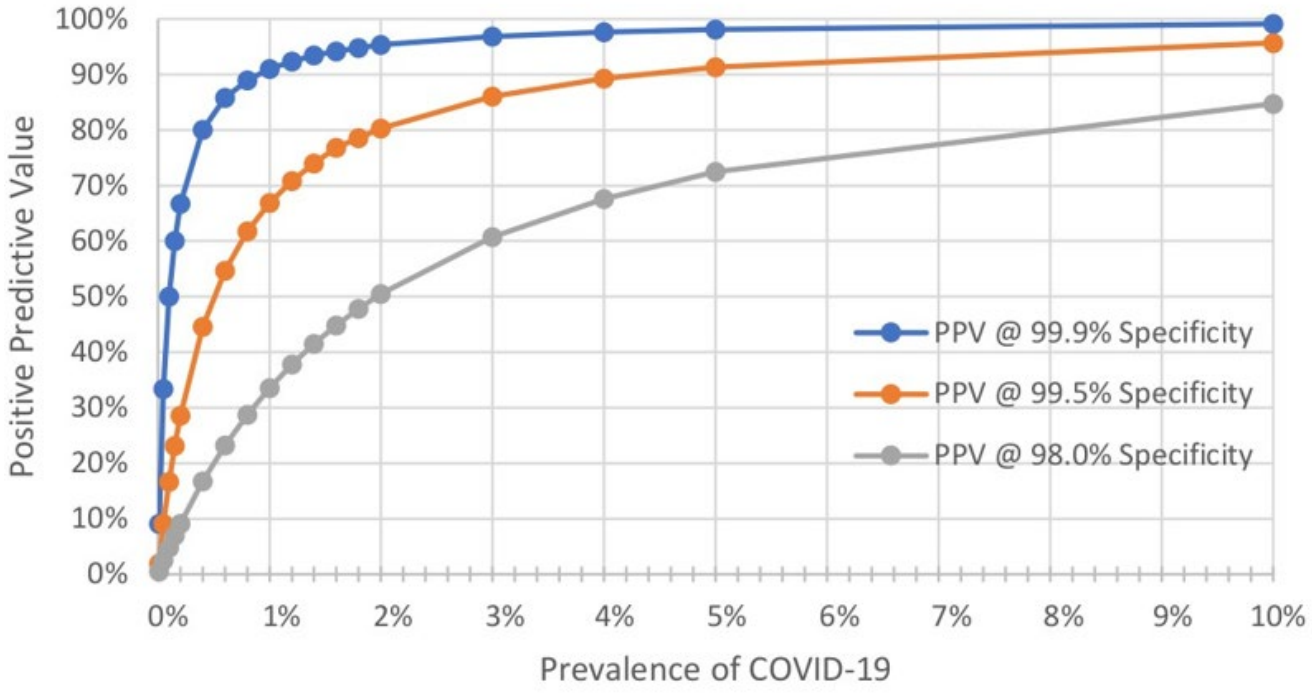
Surveys reliant on PCR testing may be over-estimating prevalence. PCR tests detect several phases of illness and a positive test cannot distinguish between late incubation-period illness, where viral shedding is potentially infectious to others, and the later recovery period where virus may be detectable but is not viable or in such small numbers as not to be infectious. So if prevalence is defined as the numbers of infectious cases in a population, then PCR based surveys may well over-estimate these.

At low prevalence, PPV is also low (as shown in the graph above). In other words, when only a small percentage of the population is infected, there is less chance of obtaining a true positive. Even with tests that have high specificity – here 99.5% – at low levels of population infection, the results will be dominated by false positives.

Note that in the 0 to 20% prevalence area of the graph where PPV is lowest, NPV is highest. At low prevalence, NPV is very high, almost 100%. This means that when the numbers of infected people are very small, the chances of a true negative are very high; as prevalence increases, however, NPV declines so that when the large majority of a population has the disease, the chances of a false negative are large.

Figure 2 and Table 1 (both below) show how PPV changes with test specificity and prevalence. (Please note that in this graph prevalence values (x-axis) have a range of only 0 to 10%)

Figure 2. Change in PPV of PCR testing by prevalence



[8] For Public Health England's recommended actions for testing during times of low prevalence, see: Assurance of SARS-CoV-2 RNA positive results during periods of low prevalence.

Example

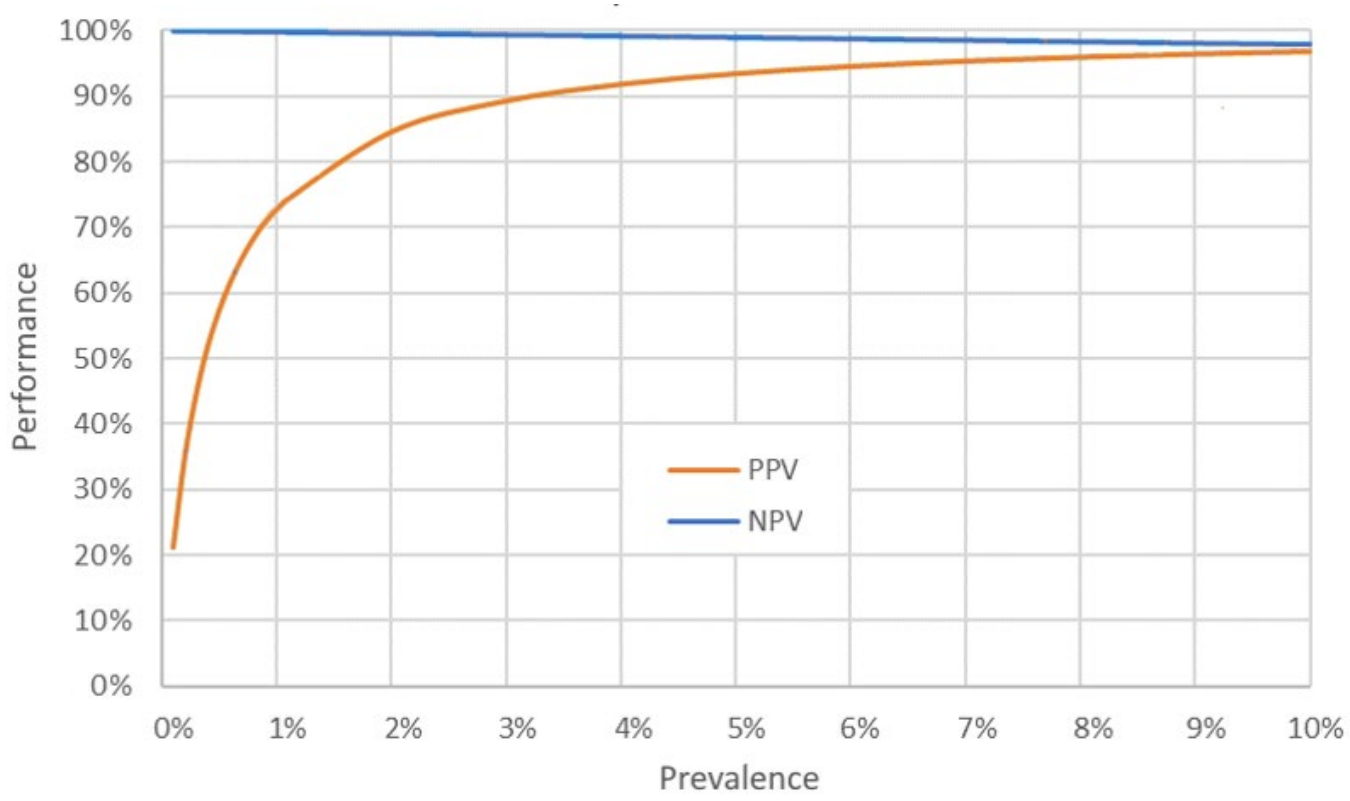
If we use COVID-19 prevalence estimates for England of mid-December of 2020 – which ranged from 1.13% in the North West to 2.89% in the East Midlands – and the real-world PCR specificities mentioned above, the resulting PPVs are:

Table 1

	Prevalence	
Specificity	Lowest: 1.13%	Highest: 2.89%
99.5%	69.6% PPV	85.6% PPV
98.0%	36.4% PPV	59.8% PPV

LFD testing

Figure 3. Percentage values of PPV and NPV plotted against prevalence
(Note that in this graph prevalence values (x-axis) have a range of only 0 to 10%)



Main points

Currently, LFD tests are believed to have a test sensitivity of 90% or more[9] compared to the infectious state and a specificity (test and diagnostic) of 99.5% or more; the diagnostic

[9] Public Health England (2020) Rapid evaluation of lateral flow viral antigen detection devices (LFDs) for mass community testing

sensitivity of LFD tests has been shown to be around 80% in professional settings but lower for untrained swabbers.[8,10] These values are unchanged for identified variant strains.

The lower sensitivities of LFD tests compared to PCR tests are a reflection of the different technologies employed and substances targeted, that is, protein antigen versus amplified nucleic acid. The lack of an amplification stage for LFD tests may lead to more false negative results and thus lower sensitivities.

LFD positivity correlates with an infectious state and high viral loads; this means that at the beginning of an infection and at the end, an LFD test may give a (false) negative result. It is recommended that a positive LFD result be followed by a PCR test to reduce the number of people isolating unnecessarily.

Using the values above, the LFD test performance by prevalence in the ranges of 0.1% to 10% may be expected to be, for every 1,000 tests undertaken:

Table 2. Performance of LFD tests over different values of prevalence
Note how PPV values decrease as prevalence decreases (and the proportion of false positives increases).

Prevalence	PPV	NPV	True +ve tests	False -ve tests	Number of infections
10.0%	96.74%	97.82%	80	20	100
5.0%	93.35%	98.96%	40	10	50
1.0%	72.93%	99.80%	8	2	10
0.5%	57.27%	99.90%	4	1	5
0.1%	21.07%	99.98%	1	0	1

In addition to the test outcomes above, the number of false positive results also needs to be considered. For example, at 1% prevalence with a PPV of 72.9% and 8 true positive results, the total number of positive results received will be 11, because there are 3 false positives in addition. This is because at this prevalence value false positive results make up approximately 27% of the positive results.[11]

If 100 people had received positive results under the above conditions (that is, prevalence of 1%; PPV of 72.9%; test parameters: sensitivity of 80% and specificity of 99.5%), 27 people would have isolated despite having no infection and 18 infected people would have been missed after receiving false negative results.[10]

[10] 'Performance of the Innova SARS-CoV-2 antigen rapid lateral flow test in the Liverpool asymptomatic testing pilot: population based cohort study' (British Medical Journal, 2021)
[11] See Appendix 3 for detailed explanations.

Comparing performance of a single LFD test with a single PCR test

The following table compares PPV and NPV by changing prevalence for these tests.

Table 3. The changing values of PPV and NPV across different levels of prevalence for PCR and LFD tests

Prevalence	PCR: PPV	PCR: NPV	LFD: PPV	LFD: NPV
10.00%	97.09%	98.90%	96.74%	97.82%
5.00%	94.04%	99.47%	93.35%	98.96%
1.00%	75.19%	99.90%	72.93%	99.80%
0.50%	60.12%	99.95%	57.27%	99.90%
0.10%	23.09%	99.99%	21.07%	99.98%

Main points

PCR and LFD tests have similarly high diagnostic specificities for viral loads likely to correlate with infectiousness. This is because they are designed respectively to detect specific viral nucleic acid and viral proteins. However, the diagnostic sensitivity of LFDs is more likely to be around 80%, while around 90% for a PCR test.

As the 2 tests have similar specificities, they can be expected to return similar proportions of false positives when prevalent infections in the community are few.

In the range of prevalence values 1% or more the performance of LFDs and PCRs are so similar that they may be expected to be indistinguishable in field use.

The shorter (and slightly delayed) detection window of LFD tests isn't necessarily a disadvantage and could be considered a useful complement to the PCR test's wider (and arguably less discriminate) detection window.

As LFD positivity correlates with high viral loads – when infected individuals are generally at their most infectious – a positive result indicates a high probability that they really are infected and need to isolate.

A PCR test on the other hand may return a positive result for individuals in the late recovery phase as they undergo viral clearance and are no longer infectious (which could be considered a false positive) but are required to enter a period of isolation nonetheless.

However, the wider detection window of the PCR test is an advantage when testing to identify people in the early stages of infection who have yet to present symptoms and would not receive a positive LFD result.

LFD testing offers a significant advantage in allowing for more rapid isolation of infectious people. The more rapid isolation of infected individuals afforded by an LFD test has the potential to remove infected people more quickly from generating community contacts in comparison to laboratory-based PCR tests.

Similar to the point above, the more frequent sampling that is possible using LFD tests mitigates to some degree the slightly lower predictive values of an LFD test result.

In practice, it is important to ensure that LFD performance is consistent across the various devices produced by different manufacturers.

Testing strategies

Testing to support clinical evaluation

Ordinarily a test is used to support an existing diagnosis based upon clinical history, signs and symptoms, and in the case of infectious diseases, evidence of exposure. Any course of action embarked upon after the test, is done so with these other supporting steps taken into account: the test result informs the clinical response but does not constitute its sole basis. This is in contrast to much of the testing being undertaken in response to COVID-19.

Main points

The results of mass asymptomatic testing and screening programmes, for example, are not supported by the other steps for clinical evaluation, and this can affect the validity of decisions based only on these results, since diagnostic tests alone are never perfect.

Serial testing

Serial testing is performed on a regular basis on the whole at-risk population to identify and remove (by isolation) infected individuals from the surrounding population. Optimisation of serial testing requires the test interval (time between successive tests) to be shorter than the serial interval (time between the onset of symptoms in one person and the onset of symptoms in the next person in a chain of transmission) and not longer than the test's detection window (the period during which the test is able to detect the presence of a disease in an infected individual). For more details on the detection windows of different tests, please see Appendix 1.

Main points

In order to ensure as far as possible that uninfected individuals are not put into isolation unnecessarily, only tests of high specificity and high PPV should be used, and if a positive result is returned, an appropriate retest pathway – scaled to match the error rate of the primary test regimen – should be put into effect.

It is advisable to retest as soon as possible all positive test results in low-prevalence populations to keep false positives to a minimum, ideally with an LFD for reasons of time and convenience.

However, as prevalence increases, the proportion of false positives (and people isolating unnecessarily) decreases.

Repeat testing for the purposes of excluding asymptomatic infected individuals from a population should be performed on a schedule commensurate with the detection window of the disease (currently understood as 6 to 11 days for COVID-19).

While a test interval greater than the detection window substantially reduces the per test effectiveness of a serial testing programme, more frequent testing does not significantly improve the time taken to detect the cases that are detected, nor provide good protection against infected individuals remaining undetected.

Situation-specific testing

Modelling reverse-cohorting scenarios in prisons

Reverse cohorting is the policy of quarantining new prison arrivals for a certain period and testing them prior to their introduction to the wider prison community in order to reduce the probability of COVID-19 infections being imported into the prison and new infections being seeded. Several scenarios and a range of time periods were modelled.

Main points

At low detection rates reverse cohorting has little effect, though the effect does become more noticeable as detection rate improves.

The longer a prisoner spends in isolation, the higher probability there is that they will be detected; this effect becomes more pronounced as detection rates improve.

‘Double testing’ travellers incoming to the UK for signs of COVID-19 infection

The requirement that all incoming travellers undergo 2 rounds of PCR-type testing, together with a period of isolation, was modelled to investigate the success rate of detecting individuals who had been infected with SARS- CoV-2 at an unknown time before travel. Multiple scenarios were considered, combining flight time, timing of second test, and length of isolation period. A baseline of performing PCR testing only at arrival (with no further measures for negative results) was also provided.

Main points

Requiring new arrivals to self-isolate before release significantly increases the amount the amount of time in which infected travellers can incubate prior to release, so there is a sizeable rise in the total proportion of infected travellers across all considered scenarios.

Requiring incoming travellers to self-isolate on arrival to the UK, and take a second PCR test at some stage during the isolation period, increases the detection rate of infected travellers compared to the baseline case.

The longer that incoming travellers are required to self-isolate, the higher the detection rate of infected travellers.

There is a slight correlation between longer flight time and detection rate. However, this effect becomes less pronounced as the period spent self-isolating increases. It's also worth noting that longer flight times result in more travellers being detected on arrival, but fewer after isolation (though an increase overall).

Test and Trace: modelling periods of self isolation and combinations of testing regimes within those isolation period and the risks of onwards transmissions

An investigation into transmission risks and their mitigation was performed by modelling different testing scenarios (irrespective of compliance or implications of isolation periods regarding time off work and so on). For symptomatic individuals, transmission risks exist during their pre-symptomatic infectious period (outside of isolation). For asymptomatic individuals, transmission risks exist during their whole infectious period (outside of isolation).

Isolation periods with a maximum of 14 days were combined with none, one or 2 tests during this period. The test type was the same for both tests when a 2-test regime was considered. The detection window for PCR tests was modelled as same as the infectious period; for LFD tests, they were modelled to begin at infection onset and end 5 days later.

Main points

When no test is used, a longer isolation period is more protective.

Symptomatic presentation is similar between 10 and 14 days, but the longer isolation period also enables the reduction of the infectious period of asymptomatic infections spent outside of isolation.

For the same isolation period, a testing regime lowers the transmission risk when compared to an isolation period without testing. When using a PCR test, the isolation period can be reduced to achieve similar results to a no-testing regime. This is not necessarily the case for LFD tests, because of the shorter detection window.

When considering the transmission risk after isolation, the number of PCR tests (one or 2) does not make a difference as long as the test is performed at the latest possible time during isolation. However, when 2 PCR tests are used, additional individuals are detected while in isolation.

The use of 2 LFD tests yields more favourable results when compared to one LFD test because of their shorter detection window.

If an LFD test is to be administered at the end of the isolation period, there will be a trade-off between the test's narrower detection window and the protective effect of isolation.

Example

An isolation period of 8 days results in a lower transmission risk than an isolation period of 9 days, but it is also equal to that of 10 days. This is because fewer people will be identified by a later test, but there is more time for an individual to display symptoms.

If 2 LFD tests are to be used, the timing is important because of the test's narrower detection window. For 10 and 14-day isolation periods, when the first test is taken closer to the second, fewer detections occur, which increases the transmission risk of the regime.

When a single test is administered at the longest possible time period after the start of isolation (for LFD tests, the day of release of the individual; for PCR tests, 2 days before owing to turnaround time), the PCR test outperforms the LFD test because of its longer detection window. The discrepancy between these will reduce as the timing of the PCR test gets closer to the start of isolation.

Mass asymptomatic testing programmes

As previously mentioned, infected people can become infectious before symptoms appear, or are sometimes entirely without symptoms, so for this reason it is important to try and detect infectious people while they are asymptomatic and thus is the rationale for mass asymptomatic testing programmes.

However, there are several aspects of these kinds of mass asymptomatic testing that still need addressing. One is the fact that it is not done within the wider medical context of observable symptoms and clinical history; another is the issue of false positives, which is of particular concern at low levels of prevalence.[12]

Main points

Mass, large-scale testing for disease will produce results where disease is indicated but none exists, that is, false positive results (see 'Test performance').

Where population prevalence levels are low, additional steps (such as whole genome sequencing) are required to give the true disease status (so that people and their contacts are not required to isolate and so on).

When prevalence is sufficiently low and testing volumes sufficiently large, the interpretation of surveillance data – based upon PCR testing with less than perfect specificity - will introduce inflated estimates of disease occurrence.

[12] For Public Health England's recommended actions for testing during times of low prevalence, see Assurance of SARS-CoV-2 RNA positive results during periods of low prevalence.

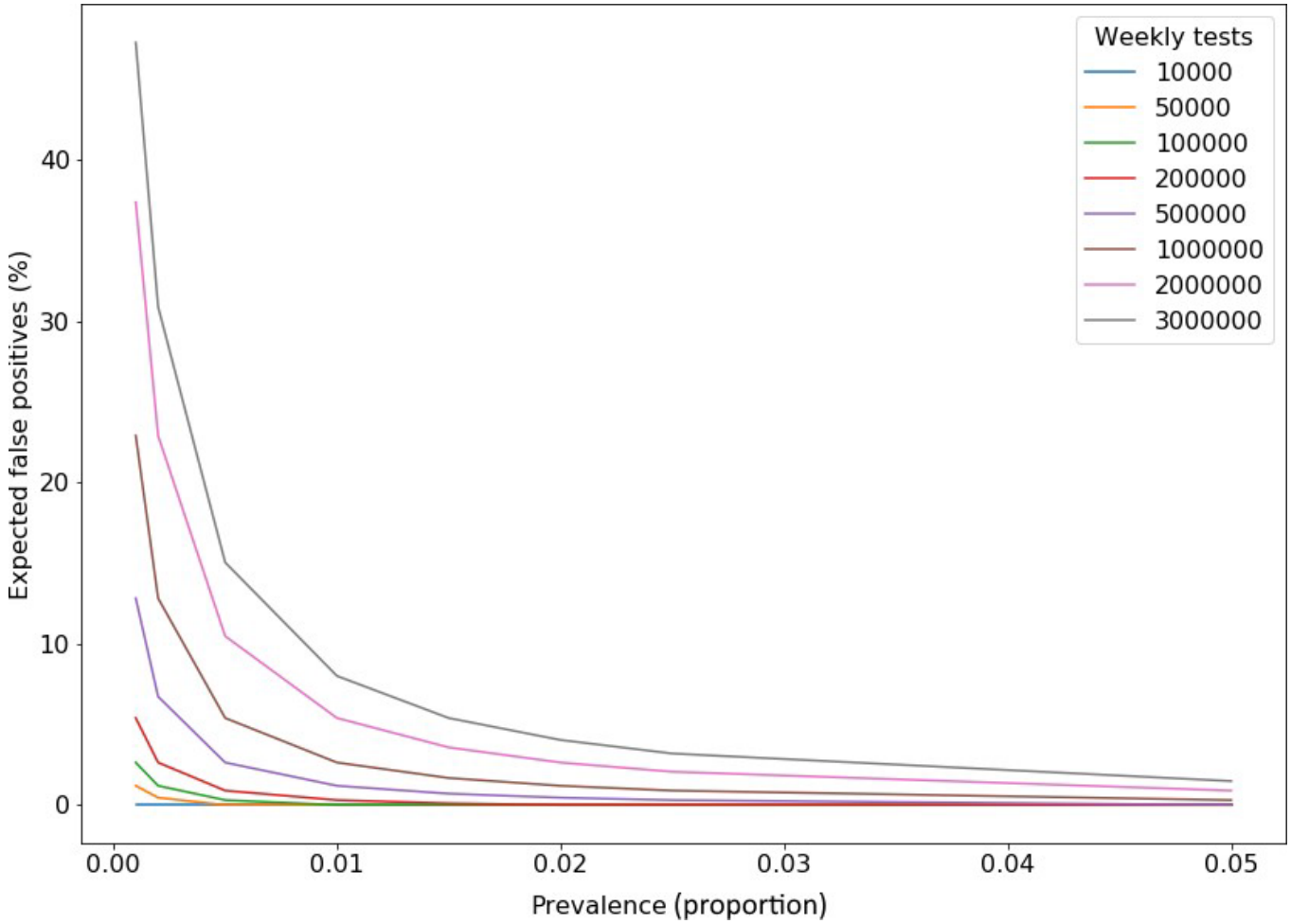
A proportion of asymptomatic cases may be false positives. The degree to which this may be the case can be investigated by reviewing the specificity of current testing systems, by reference to other validating techniques such as sequencing.

Pillar 2[13] diagnoses, approximating most closely with the general population prevalence of infections, are likely to contain higher levels of false positive results compared to populations exhibiting clinical symptoms.

There are still authoritative voices[14,15] who call into question the concept of mass asymptomatic testing itself, while others[16] have claimed recent mass testing programmes to have been a success.

The effect of false positivity rates on estimating prevalence when mass testing

Figure 4. Number of tests that would be expected to be false positives should excessive numbers of the population undergo testing at varying rates of true prevalence (assuming a false positivity rate of 6%, Lancet 2020)



[13] Testing data from swab tests performed in the community, rather than in hospitals or laboratories.
[14] 'COVID-19 mass testing programmes' The British Medical Journal 2020: volume 370, page m3262
15 'Mass screening for asymptomatic SARS-CoV-2 infection' The British Medical Journal 2021: volume 373, page n1,058
[16] Mass-testing reduced Liverpool COVID-19 cases by a fifth, study finds (Reuters 2021)

Taking selected values from Figure 4, we can focus in on the relation between prevalence and false positives in the table below:

At a prevalence of 0.1% – that is, on average one person in a thousand has the disease – 10,000 weekly tests can be performed without any false positives being generated. If the weekly test number is increased to 500,000, however, then almost 75% of positive results will be false.

At a prevalence of 2.0%, 10,000 weekly tests can be performed without producing any false positives, the same as for the previous prevalence value. If the weekly test number is increased to 500,000, though, 8.3% of your positives (much lower than the previous value) will be false.

Lastly, at 5.0% prevalence, the number of false positives expected is 0 (to one decimal place) at either number of weekly tests.

Table 4. A summary of data from the graph above used to review how the proportion of false positives rises as prevalence declines and testing numbers increase

Prevalence	Number of tests 10,000	Number of tests 500,000
0.1%	0%	75%
2.0%	0%	8.3%
5.0%	0%	0%

Main points

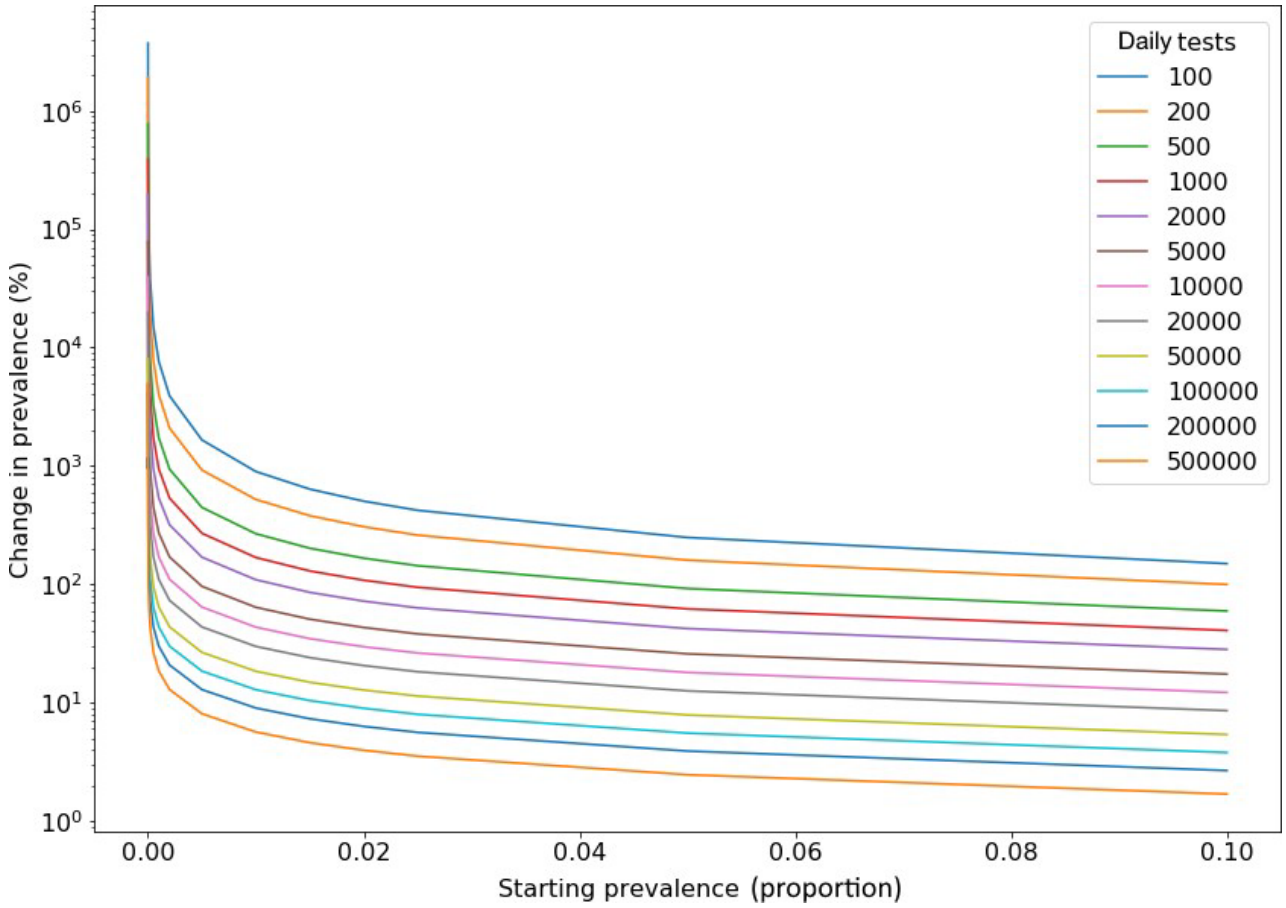
The higher the proportion of people being unnecessarily tested, the higher the number of people being falsely identified as COVID-19 positive.

Changes in prevalence of COVID-19 detectable by given rates of daily testing

What are the percentage changes in disease prevalence that could be confidently detected (that is, not mistaken for statistical noise) given the number of tests being performed each day?

These values vary according to the number of tests being administered each day and the prior level of prevalence that are compared against:

Figure 5. Graph showing the percentile increases in prevalence of COVID-19 among the population that the given rate of testing would be able to confidently detect, given the number of tests being performed each day and the rate of positivity for the preceding day



Main points

The higher the testing level, the smaller the percentile change in prevalence that can be detected at a given confidence level. This is counter-intuitive since more testing is usually considered better than less.

The lower the starting prevalence, the greater the percentile change in prevalence that can be detected for a given number of tests and confidence level.

Purposive testing

Purposive testing is most appropriate in situations where high levels of close contact are unavoidable.

Main points

A reliable negative test (that is, no or minimal false negative results) needs to be used close to the risk-event to assure that risks are minimised. All currently validated laboratory-based tests have high NPVs and high sensitivity and are suitable for these purposes.

Many of the point-of-care tests in development are likely to have good enough sensitivity to support NPV based decisions (Note: LFDs have similar NPV values to PCRs but approximately 10% lower sensitivities).

The protective effect of a negative test

When an LFD test (or any test with a similarly short turnaround period) returns a negative result, it informs us that at that time an individual does not have detectable levels of the virus. This may be because that individual is not infected, or that they are too early within their disease course to shed virus.

If the individual falls into the latter category, there is a risk that they may become infectious whilst participating in the activity they have been granted entry to. Hence there is a need to quantify the protective effect of a negative test and ensure that potential onward transmission is prevented, so that pro-sports and other events can be allowed to resume. This question of how much 'safe' time a negative test result confers was investigated by a modelling simulation with the results:

Table 5. Percentage of infected individuals who returned a negative test but would then become infectious at given intervals after that test

Event length (hours)	Lower 2.5 quantile	Median	Upper 2.5 quantile
3	2.273	2.296	2.319
6	4.557	4.588	4.621
9	6.84	6.875	6.911
12	9.113	9.154	9.197
24	18.082	18.135	18.189
48	34.72	34.809	34.882

Main points

Tests need to be carried out as close to the time of close-contact activity as possible in order to give a reliable protective effect, which is one of the benefits that point-of-care testing brings.

The current turnaround for a PCR test prior to medical procedures is approximately 72 hours. During this time at least 50% of infected individuals who returned a negative test would become infectious.

Pooled testing

For small communities and groups with little-to-no change in membership and who coexist in the same defined space such as care homes, prisons (and possibly hotels if travellers are all quarantining together), pooled testing can often be the best choice.

Main points

Samples are taken from all members of the group (the pool), combined and then tested as a single clinical specimen: this increases the efficiency of testing, minimises reagent and laboratory costs, and also saves laboratory capacity.

It is most suitable to use when prevalence is low and a good high-specificity test (that is, no/minimal false positives) with multiple targets is used. Any positive test from a pool implies that one or more contributors to the pool is positive and the whole pool then need to be individually re-tested to identify the infected contributors.

Pooled testing can be effective for testing groups of up to 32 people, but in practice the loss of efficiency through re-testing such large groups should a positive test be reported, suggests that smaller pools should be used as determined by the practical needs of the situation under consideration.

Similarly, the time taken for multiple re-tests, in the event that a positive result is reported, means that pooled testing is not particularly well suited to purposive testing, where the time between testing and high- risk activity is intentionally kept as short as possible.

Protocols for pooled testing have been developed for estimations of prevalence in populations, but these are not necessarily directly applicable to implementing efficient testing strategies for screening, or identification of individual infections for control purposes.
To find the most effective use of testing capacity when trying to detect SARS-CoV-2 in institutions containing highly vulnerable populations, such as care homes, different testing regimens were modelled. This was done with situations particularly in mind where the daily testing capacity may not be sufficient to provide coverage to all care settings on a weekly basis.

Results showed that pooled testing within homes identified more homes with a present infection and more infected individuals than the other regimens investigated.

If a community has its own dedicated sewage system, then it may be possible to sample the sewage and monitor for presence and/or trend of infection in that population. If evidence of infection is found in the sewage then at least one person has the virus, after which (ideally) each member of that community can be tested. This approach can be useful for monitoring infections at a population level where adherence to testing and NPIs is variable. This method has been proven effective in prisons in the UK.

Abbreviations and glossary

Term	Meaning
Antigen	Proteins found on the external surface of a virus that trigger an immune response in the infected individual.
Ct	Cycle threshold. This is the number of PCR cycles needed for the virus to be detected from a sample. A low Ct indicates a high concentration of viral genetic material in the sample, while a high Ct indicates a low concentration.
Cycle	One iteration of the PCR amplification process where the amount of specific genetic material in a sample is doubled.
Detection window	The detection window of a test is the time period during the course of an infection when the test is able to detect the infection's presence in an infected individual.
Diagnostic performance	An overall test evaluation which considers the performances of sample taking and laboratory testing together.
FN	False negative. A test result that fails to indicate the presence of a disease when it is present.
FP	False positive. A test result that incorrectly indicates the presence of a disease when it is absent.
Immunology testing	Immunology is the study of the immune system and its component structures and processes. Clinical immunology uses testing to investigate the complex workings of the immune system and its dysfunction.
Incidence	The occurrence of new cases of disease in a population over a specified period of time.
Incubation period	The average time for symptoms to appear after infection in an infected individual.
Infectious	Shedding or emitting from the body live virus.
Infectious dose	The amount of virus required to establish an infection in an individual.
LFD	Lateral flow device. A simple device intended to detect the presence of an antigen in a liquid sample without the need for specialized equipment.
Neutralisation	The process whereby a body's immune system defends its cells from a pathogen or infectious particle by rendering it harmless, thus neutralising any effect it has biologically.
NPV	Negative predictive value. The probability that subjects with a negative test result truly don't have the disease.

Term	Meaning
Pillar 2	Pillar 2 data comes from swab tests performed in the community, such as care homes, rather than from places like NHS hospitals or PHE laboratories (which constitute Pillar 1).
PCR	Polymerase chain reaction. A technique used to amplify small quantities of DNA in a sample to sufficient levels that they can be tested for viral presence.
Point-of-care testing	Testing performed in a near-patient setting, such as at the patient's hospital bedside or home, but not in a laboratory and so on.
PPV	Positive predictive value. The probability that subjects with a positive screening test truly have the disease.
Positivity	The percentage of tests giving a positive result, irrespective of whether they are true positives or false positives.
Prevalence	The proportion of a population which has a particular disease at a specified point in time or over a specified period of time.
Purposive testing	Purposive testing is the testing of members of population who have been chosen through purposive sampling, a technique in which researchers rely on their own judgment when choosing who to sample.
Recovery period	A later phase of illness when the body's immune system is able to destroy the infecting virus and reduce the severity of symptoms. The infected individual may still be shedding live virus, but in increasingly smaller quantities, together with dead virus.
Resolution	The resolution of an infection occurs during the recovery period as the body's immune system neutralises the invading pathogen and the severity of the infected individual's symptoms diminishes.
Reverse cohorting	Reverse cohorting is the policy of quarantining new prison arrivals for a certain period and testing them prior to their introduction to the wider prison community.
Reverse transcription	The first stage of the COVID-19 PCR test, where strands of RNA are converted to double-stranded DNA, which can then undergo the amplification process.
RT-PCR	Reverse transcripti on polymerase chain reaction. Another name for the PCR test that references the stage involving the reverse transcriptase enzyme, which is needed when testing for viruses.
Sensitivity	The ability of a test to detect the true positive rate.
Serial interval	The time between the onset of symptoms in one person and the onset of symptoms in the next person in a chain of transmission.

Term	Meaning
Serial testing	Testing which is performed on a regular basis on the whole at-risk population to identify and remove (by isolation) infected individuals from the surrounding population.
Serology testing	Serology is the scientific study of serum and other body fluids. In practice, it usually refers to the identification of antibodies in serum through diagnostic testing. Serology can be considered a component of immunology.
Seroprevalence	The number of people in a population (usually expressed as a fraction) who test positive for antibodies to a specific pathogen in a blood test.
Serum	Serum is blood with all cells and clotting factors removed. It includes all proteins not used in blood clotting, all electrolytes, antibodies, antigens, hormones, and any exogenous substances such as drugs or microorganisms. To obtain serum, a blood sample is allowed to clot and then centrifuged: this separates the clot and blood cells from the serum, the resulting liquid component.
Specificity	The ability of a test to detect the true negative rate.
Test interval	The time between successive tests in a course of serial testing.
Taq DNA polymerase	An enzyme used in the amplification stage of PCR testing.
TN	True negative. A test result that accurately reflects the absence of infection.
TP	True positive. A test result that accurately reflects the presence of infection.
Transmission risk	The risk that an infectious individual may infect another through the shedding of live virus.
Viral clearance	This occurs during the end of the recovery period after the immune system has neutralised the invading virus and the body proceeds to eliminate what remains: dead virus and viral debris.
Viral load	The amount of viral material in a sample.

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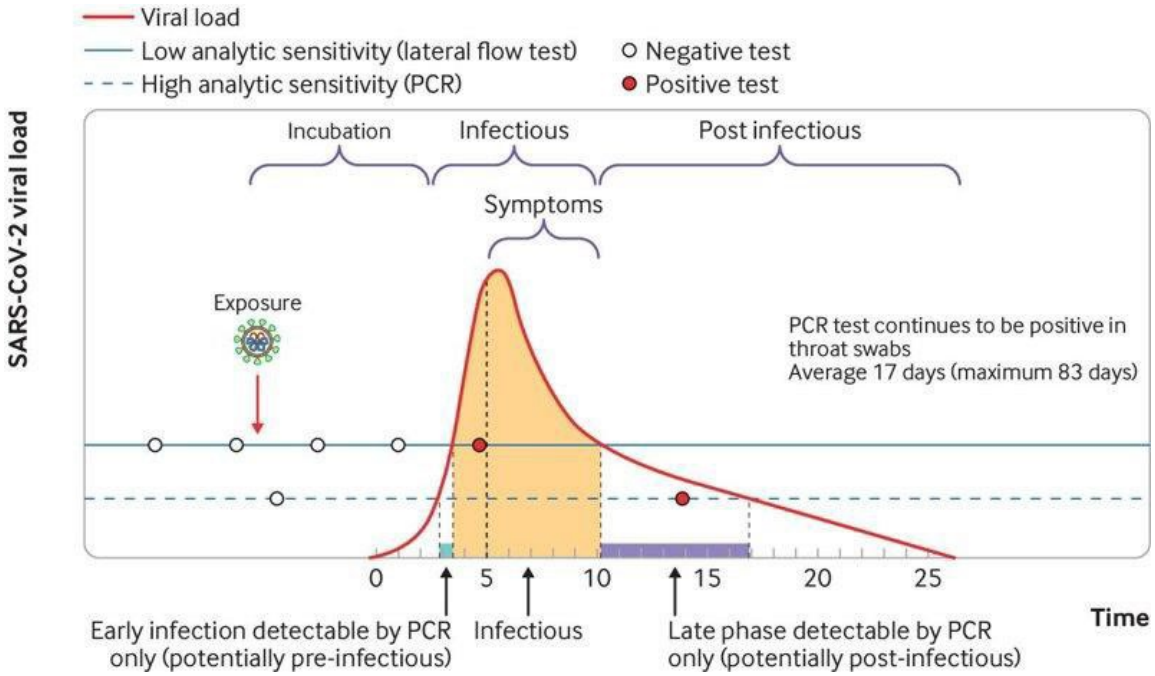
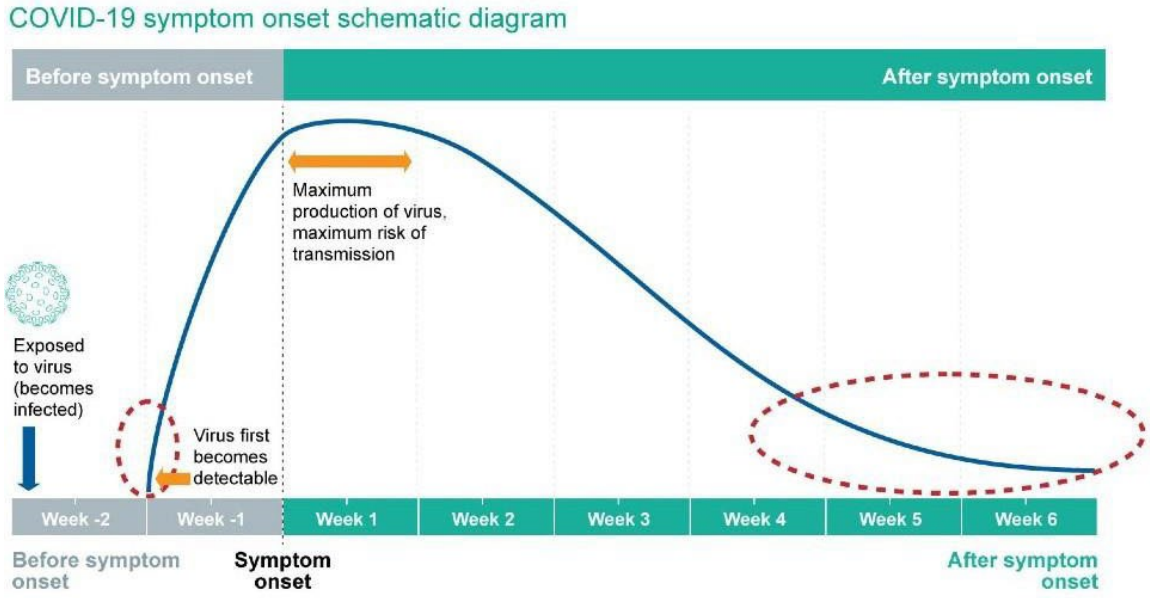
Appendix 1. A collection of timelines showing SARS-CoV-2 infection and detection windows

Graph 1



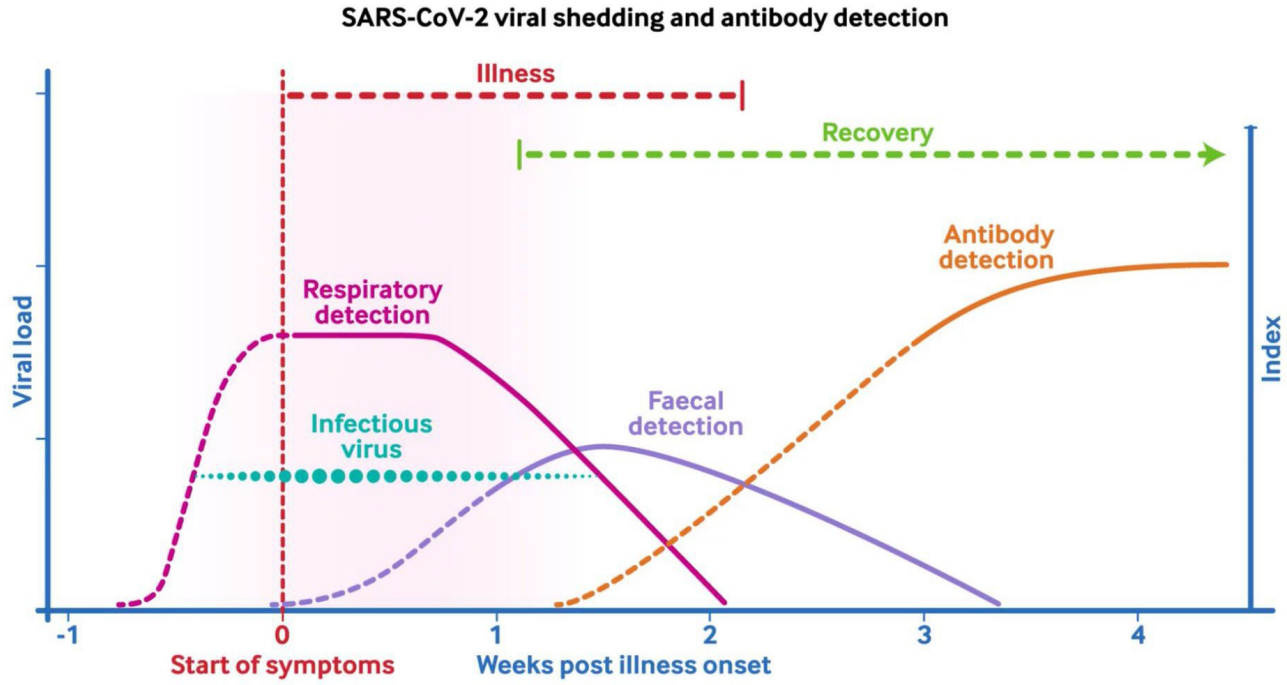
From: Alex Crozier and others. '[Put to the test: use of rapid testing technologies for COVID-19]' British Medical Journal 2021: volume 372, page n208

Graph 2



From: [Assurance of SARS-CoV-2 RNA positive results during periods of low prevalence]

Graph 3



From: [BMJ Learning: Introduction to testing for COVID-19]

Notes

Infectious virus can be identified from upper respiratory tract (nose and throat) samples for approximately one week from symptom onset for the majority of people, with viral loads being the highest around the time of symptom onset.

Virus has been detected in many other sample types including blood, urine, and faeces.

Generally, an antibody response is not detectable until at least 10 days after symptom onset (though the presence of antibodies has been recorded earlier than this). The timing of samples for antibody testing needs to take this into account.

More than 90% of individuals develop a detectable antibody response by the third week after symptom onset. Detectable antibody responses have also been recorded in asymptomatic individuals.

Estimates of the range of asymptomatic infection vary considerably, but current evidence suggests that approximately 30% of infections may be truly asymptomatic throughout the course of infection.

Studies from asymptomatic individuals have suggested that less virus may be detected from these individuals, but it should be noted that the amount of virus at any one time may be similar to a symptomatic individual.

The duration of antibody responses is not yet known, but decline in antibody titres in the months following infection has been reported.

Antibodies are likely to provide some protection from reinfection with similar viruses for at least 6 months.

Appendix 2. A note on immunity

Antibodies produced in response to SARS-CoV-19 begin to wane after the infection has been resolved and all virus (living or dead) has been removed from the body through neutralisation and viral clearance. Waning of these antibodies is not the same as waning of effective immunity because of the memory cell response (see below).

Antibody production generally peaks at about 2 to 3 weeks after infection, after which antibody levels begin to decline. In most individuals, these antibodies persist for more than 6 months after primary infection, but decline more rapidly in some patients, especially those who experienced mild symptoms[17] (though there is evidence that in more severe cases, the immune response is longer lasting).[18] This means that serology testing for antibodies can only give information about relatively recent past infections or vaccinations.

However, longer-term immunity does exist in the form of memory B cells (MBC) and memory T cells (MTC). These are produced along with antibodies during the primary infection and are believed to remain in the body at low levels for years.[19] These cells are programmed to respond to the specific antigens encountered in the primary infection (though cross-reactivity has been noted between different coronaviruses)[16, 17, 18] and can neutralise the returning virus through antibody production (MBC) and direct attack (MTC). For individuals with these memory cells in their body, the time required for an effective immune response on reinfection is much shorter than during the primary infection since the usual preliminary processes involved in a novel infection can be bypassed.

The level of cellular immunity possessed by an individual can be investigated using appropriate immunology testing.

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Appendix 3. Detailed explanations of the calculations used to determine false positives and false negatives in 'LFD testing'

Both scenarios assume a sensitivity of 80% and a specificity of 99.5% for the LFD test, a COVID-19 prevalence of 1%, and a subsequent PPV (positive predictive value) of 72.9%.

First scenario

1,000 people are tested (of whom 10 have the infection), which gives 8 true positive results and 2 false negative results. This much is given in the table. However, 3 more people will receive false positive results (so that in total 11 people will receive a positive result from the test). How can this be calculated from the existing data?

A PPV of 72.9% means that only 72.9% of test takers with a positive result do actually have COVID-19 (true positives). Here they number 8 people. It follows that 27.1% of test takers with a positive result don't actually have COVID-19 (false positives). So how many are these?

Method 1. Use the equation for PPV

$$PPV = \frac{TP}{TP + FP}$$

$$FP = TP \left(\frac{1}{PPV} - 1 \right)$$

$$= 8 \times \left(\frac{1}{0.729} - 1 \right)$$

= 2.97 (3 to the nearest whole person)

Method 2. Use ratios

$$\frac{FP}{TP} = \frac{27.1}{72.9}$$

$$FP = \frac{27.1}{72.9} \times TP$$

$$= \frac{27.1}{72.9} \times 8$$

= 2.97 (3 to the nearest whole person)

Second scenario

If 100 people had received positive results under the above conditions, (a) 27 people would have isolated despite having no infection after receiving false positive results, and (b) 18 infected people would have been missed after receiving false negative results. How were these calculated?

a) As mentioned above, a PPV of 72.9% means that 27.1% of positive tests are false positives so 27.1% x 100 = 27.1 (27 to the nearest whole person)

b) The number of true positives is 72.9% x 100 = 72.9 (73 to the nearest whole person)

Method 1: Use the equation for sensitivity

$$\text{sensitivity} = \frac{TP}{TP + FN}$$

$$FN = TP \left(\frac{1}{\text{sensitivity}} - 1 \right)$$

$$= 72.9 \times \left(\frac{1}{0.8} - 1 \right)$$

= 18.2 (18 to the nearest whole person)

Method 2: Use ratios

$$\frac{FN}{TP} = \frac{2}{8}$$

$$FN = \frac{2}{8} \times TP$$

$$= \frac{2}{8} \times 72.9$$

$$= \frac{72.9}{4} = 18.2$$ (18 to the nearest whole person)

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