COVID-19 | SCREENING LIMITATIONS AND BACK TO WORK

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NOTES	'Back to Work' advice revised in line with PHE and other guidance on retesting post-infection.

Headlines

Nucleic acid amplification tests (NAATS) such as PCR and LAMP for COVID-19 do not pick up the virus itself, they pick up traces of its genetic material.

People are no longer infectious 10 days after symptom onset (provided clinical symptoms have improved and no fever for 48 hours), but traces of genetic material can still be detected – and prompt a positive test – up to *12 weeks* [1] after symptoms have ceased and the person is no longer infectious. This raises important questions:

- **When can people return to work after illness or a positive test while asymptomatic, especially for close-contact work?**

  WHO and Government advice is 10 days after symptom onset (or first positive test if asymptomatic) but based on the science we advise 14 days for close contact work.

- **When can they re-enter a screening programme?**

  PHE [2] *and the CDC* [3] *advise any positive result in the 90 days after the date of symptom onset requires careful interpretation by a medic. Unless productions have robust medical/scientific support in place to make that interpretation, we advise against retesting after a confirmed infection.*

- **Can RNA tests discriminate between someone who is infectious and someone who has recovered?**

  To a point, yes, depending on the specific test - and there are PHE guidelines for this too.

The clear challenge is that many people and their close contacts may be made to self-isolate when they are not actually infectious - but have a positive RNA test.

RECAP | WHAT WE KNOW

1. Forget antibody testing. We need to identify *people who* are *potentially infectious* rather than those who have had it and might (or might not) have some immunity.
2. No test is 100% accurate. There will always be false alarms and others may slip through – so testing is not the whole answer and can never replace other measures.
3. *Time to result* and *frequency of testing* give better protection than exquisite accuracy. A LAMP test that can be processed rapidly at the 'point of care' actually offers better protection than a PCR that takes 24-48 hours to process, even if the PCR can detect lower levels of virus. [4].

Background

While PCR and LAMP tests are the best available for screening, they still have numerous pitfalls..

We already know the predictive values (what proportion of people with positive test results genuinely have active infection and what proportion of people with negative results are genuinely free from active infection) aren't just influenced by a test's sensitivity and specificity, they are also affected by the prevalence of active infection in the group being tested and a number of other factors.

Essentially all mass testing produces both false alarms and missed cases and the implications of this need to be carefully considered.

'Cases' are currently defined as those in whom PCR detects viral RNA, whether active or not. This, and open access testing for anyone who self-refers, mean that 'cases' inevitably includes:

- **People with past infections** where their immune system has cleared the virus but there's some viral genetic material kicking about.
- Those with an active infection but a **test picks them up too late** to make much difference to potential onward transmission.

This is enough of an issue in a clinical setting but when applied to a screening programme the effects of these pitfalls can be amplified dramatically. Central to this challenge is what a positive PCR test *actually* indicates. According to the NHS website [(5)]:

> *A positive result means you had coronavirus when the test was done.*

This is not the case. PCR tests do not identify live, viable, infectious virus, they identify viral RNA.

And this RNA is durable. It can stay in the body for weeks – possibly months – after all viable virus has been dealt with by the immune system, giving 'positive' results for people who are no longer infectious.

An artefact of PCR screening is that we will detect ever more people with residual RNA rather than ongoing infection. The problem is, a large proportion of the *true positive test results for viral RNA* are *false positives for infectiousness*. This is not just unhelpful; if a test decides someone is infectious then potentially they, their household and other significant contacts may have to isolate for up to 14 days.

This can have a very serious impact on a workforce and carries significant economic and personal implications.

Virus Detection, and Infectivity

Because SARSCoV-2 is a novel virus (in humans, at least) early in the pandemic there was substantial uncertainty regarding levels of virus in those infected. There were questions of ***detectability*** (presence or absence in different tissues / isolates), ***viral load*** (quantity or 'titre' of virus in a bodily fluid) and how these related to ***infectivity*** and ***disease severity***.

We still don't have a complete picture but we have a far superior understanding of the trajectory of SARS-CoV-2 and a much better grasp of the duration of infectivity. We are also a great deal further forward with the link between viral load and infectivity. A recent systematic review [(6)] looked at 113 studies conducted in 17 countries which we summarise here.

Viral Load

- The evidence from **upper respiratory tract samples** suggests that the viral load of SARS-CoV-2 peaks around symptom onset or a few days thereafter, and becomes undetectable about two weeks after symptom onset.
- Viral loads from **sputum** tend to be higher, peak later and persist for longer; eight studies reported that viral RNA from sputum samples peaked generally two weeks after symptom onset. **This has clear implications for tests relying on sputum samples rather than OP or NP swabs.**
- Data on the differences in viral load dynamics between different upper respiratory sample sites are inconsistent, with some studies reporting higher viral loads in nasal samples and others reporting higher viral loads in throat samples.
- Nine studies reported an association between higher viral loads and more severe symptoms.
- Interestingly seven studies measured viral load in presymptomatic or asymptomatic patients, and generally found **little to no difference in viral load** between pre-symptomatic, asymptomatic and symptomatic patients.

Duration of Detection

What is clear is that individuals are not infectious for the entire duration of *detection* as the presence of viral RNA is not the same as the presence of transmissible 'live' virus.

This is confused by references to 'viral load' meaning *presence of viral RNA* when using PCR or LAMP, not the presence of actual viable virus. It is possible to test for actual viable virus but this is not simple and the technique does not lend itself to rapid or mass testing, unlike PCR or LAMP tests. While no study has *definitively* measured the duration of infectivity, many pieces of the puzzle are coming together.

- The lowest viral load (Ct value) for which there was positive virus culture growth was 34.3 and it has been inferred that test subjects with Ct values ≥ 34 are no longer contagious [7] [8].
- Viable virus could not be isolated from samples collected after day eight of symptom onset, in spite of ongoing viral loads still being high at approximately 10^5 RNA copies/ml of sample [8]. And this is the crux of the issue – tests for RNA can still be positive after the virus itself has been cleared.

| REMINDER

The Ct (Cycle threshold) value in PCR is the number of amplification 'cycles' needed for there to be enough genetic material for the test to pick up. Higher Ct = less genetic material to start with. In LAMP there's no cycling so the longer the test takes to give a positive result the less material there was to start with.

So, the evidence to date suggests that the viral load in respiratory tract samples peaks around symptom onset and decreases within one to three weeks. Although the duration of detection and the size of the viral load differs between patients, viral RNA generally becomes undetectable (from upper respiratory tract specimens) about two weeks after symptom onset but this is not guaranteed.

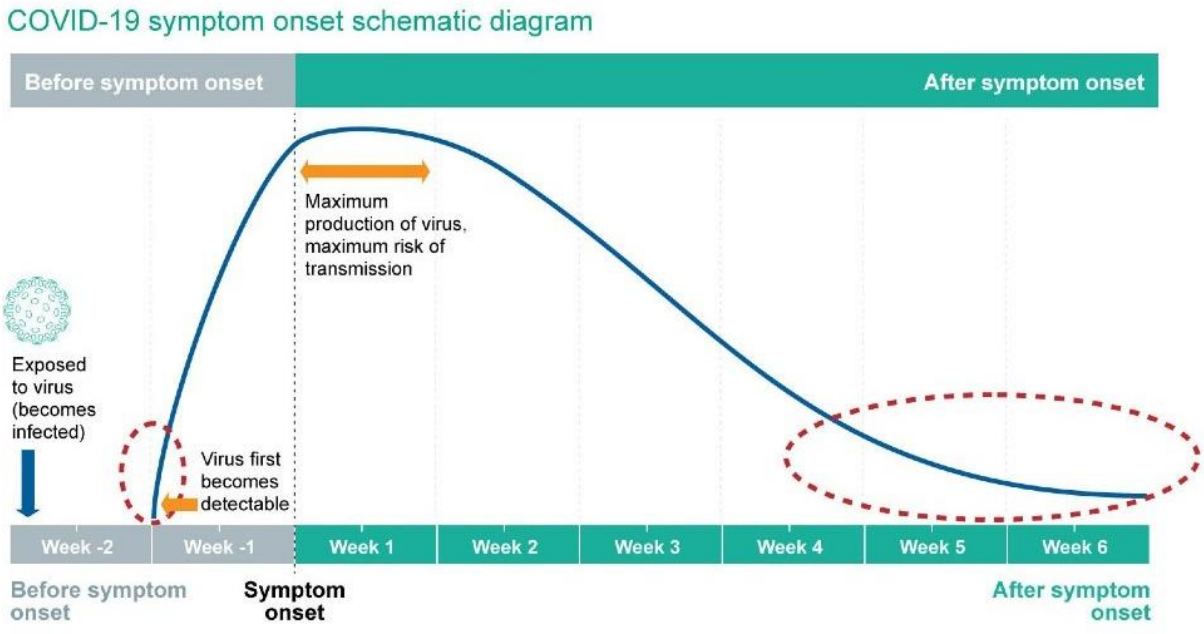
But there is a very real chance that tests – especially PCR and LAMP with low LoDs – will show those who are no longer infectious as positive.

Interpreting PCR

There is a relatively consistent trajectory of SARS-CoV-2 viral load over the course of COVID-19 from respiratory tract samples as measured by PCR. While the duration of infectivity remains uncertain it seems that high Ct values (≥ 34) are not picking up active infections and results close to the Limit of Detection (LoD) need to be treated cautiously, as was recently confirmed in guidance by Public Health England [9].

The schematic diagram below (adapted from BMJ Learning) illustrates the detection of SARS-CoV-2 RNA (shown by the blue line).

Timings of symptom onset and virus detection in relation to infection will vary from person to person, but will broadly fit within this representation. Positive results at the limit of detection can be seen in the early stages of infection (before the person becomes capable of transmission of the infection) or late in infection when the risk of transmission is low or very low (periods indicated by the dotted red line).



According to PHE [9]:

> *Positive test results at the limit of detection that occur **early in the cycle of infection** are important as these represent individuals who may go on to transmit infection.*
>
> *Positive test results at the limit of detection that occur **late in the cycle of infection** represent individuals with a low or very low risk of transmission, as a result of the decline in infectious virus production or remnants of viral RNA in respiratory secretions.*

They also discuss situations such as community or workplace testing ('Pillar 2') where clinical data is not readily available to clarify significance of the result. In these cases the Government recommends:

> *Request a repeat sample and advise self-isolation pending the results of the second sample.*
>
> *Contact tracing should only be initiated if there is a positive result from the repeat sample.*
>
> ***A positive result at the limit of detection from the repeat sample is suggestive of the late stage cycle of infection and therefore contact tracing and further self-isolation is not advised.***

In the light of this PHE guidance [9] anyone with a 'borderline' test result should isolate for 24hrs and re-test.

A second test with a low titre of viral RNA close to the LoD is not a cause for isolation or contact tracing as per the PHE advice.

Qualitative vs Quantitative

Current tests are marketed as qualitative (they give a binary, positive / negative answer) whereas in fact they can be cautiously interpreted as semi-quantitative under some circumstances. As we have seen, Ct values ≥ 34 show the test subject is no longer contagious and there will also be a correlation with the time taken to reach the LoD with LAMP tests.

It is important to note that *truly* quantitative PCR is entirely different from the qualitative RT-PCR used in COVID-19 testing and the Ct value cannot be *directly* correlated with viral load without a standard curve using reference materials.

Also Ct values can be affected by batch effects and using naive Ct values from qualitative RT-PCR as a basis for quantitation is hazardous at best – but a skilled user can still draw some broad conclusions.

That said, given the shape of the curve above – and as PHE itself says - a positive result at the limit of detection from the repeat sample is suggestive of the late stage cycle of infection.

So, with a suitable test a 'borderline' case could isolate after the first test.

- If the test subject is entering the infectious, presymptomatic phase one would expect the titre on the repeat test to be significantly higher than the first.
- If the repeat test is also borderline, under PHE guidelines this would be suggestive of the late stage cycle of infection and therefore contact tracing and further self-isolation would not be required.

It would be sensible to run more than a single replication of the test, though.

Time Between Tests

This would likely depend on the **analytical sensitivity of a particular test** and this will vary on a test-by-test basis. Each type of test from each manufacturer will be different.

Note that *analytical sensitivity* is not the same *as Limit of Detection*. LoD is the lowest detectable amount of analyte (whatever a test is looking for) that can be *reliably be distinguished from zero*.

The analytical sensitivity is about the slope of the calibration curve [10] – or *the capacity of a test to differentiate between two very close concentrations of analyte*.

There is a relationship between the two but they are not the same thing. Understanding this is key to a repeat test strategy.

A starting point of 24hrs between tests would seem sensible but would depend on the parameters above.

Back to Work | When?

The evidence is that in symptomatic patients there is a reduction in infectivity 7–10 days after symptom onset. Two virus culture studies obtained no infectious isolates from any sample taken eight days after symptom onset in spite of ongoing high viral loads.

One of these studies found that patients with Ct values ≥34 were no longer contagious. These findings appear to support early epidemiological and modelling studies, with one study suggesting that transmission may be limited to five days after symptom onset.

The World Health Organization (WHO) advice is people are considered to be no longer infectious **10 days after symptom onset** (and symptom-free for at least three days) or 10 days after first positive test if asymptomatic.

However a recent review [6] cites one paper [7] saying people **may** still shed viable virus up to 13 days after symptom onset, so our advice for those returning to close-contact work is that they do not return until **14 days after symptom onset** (and they have been symptom-free for at least three days) or 14 days from first testing positive if asymptomatic.

Back to Work | Testing

Because RNA can still be present weeks after the virus has cleared and a person is no longer infectious, care is required when admitting returnees to testing programmes that look for RNA such as PCR and LAMP. This is because fragments of inactive virus can be persistently detected by PCR in respiratory tract samples for some time following infection. On the testing after return to work post-infection question, Government advice as applied to health and social care would seem to be a sensible starting point.

> *"Staff who have previously tested positive for SARS-CoV-2 by PCR should be exempt from being retested within a period of 90 days from their initial illness onset,* ***unless they develop new possible COVID-19 symptoms****."*
>
> *"If a staff member is found to be positive for SARS-CoV-2 by PCR within 90 days from their initial illness onset,* ***depending on their symptoms and advice from an infection specialist****, they may need to self-isolate again."*

If staff are tested for SARS-CoV-2 by PCR after 90 days from their initial illness onset and are found to be positive, this should be considered as a possible new infection.

For these reasons we advise that anyone returning to work:

- Must only do so in line with line with Government guidelines regarding isolation period and end of symptoms;
- Positive test results in the 90 days since original symptom onset / first positive test if asymptomatic should be treated with caution and carefully interpreted by a doctor familiar with the particular test;
- All other transmission reduction protocols should continue to be followed as far as possible;
- A positive test after 90 days should still be treated with caution. While it is possible to be infected with COVID for a second time, this test could again be a false positive. If the test subject is asymptomatic the advice of a doctor should be sought before a decision is made regarding another self-isolation period.

  Unless productions have robust medical/scientific support in place to interpret the test results and make appropriate clinical judgements, we advise against retesting after a confirmed infection.

This is another reason we advise any testing process is conducted under the supervision of a doctor.

Different tests have different Limits of Detection, run at different Ct values or in the case of LAMP the time to result can vary widely. Understanding if a test result is close to its LoD, plus interpreting and explaining lab results and what they actually mean has many nuances.

Conclusion

Provided they have been symptom-free for three days, the evidence is people are no longer infections ten days after symptom onset. But very sensitive NAATs such as PCR and LAMP will still identify people as positive for viral RNA but who are not infectious after this time. This may lead to many people and their close contacts being told to isolate unnecessarily.

In line with WHO Guidance on the length of the infectious period and UK Government advice on self-isolation we advise that people can return to work 10 days after symptom onset (provided 3 days symptom free) but must follow normal distancing and hygiene protocols. However our further advice is that close contact work should not be undertaken for 14 days after symptom onset (provided 3 days symptom free).

Also, given what is now known about the viral load kinetics of SARS-CoV-2 it would seem that a second test after a suitable interval in borderline cases could provide sufficient reassurance of lack of infectiousness.

This is recognised in PHE guidance on the matter and needs to inform any sensible screening strategy - but careful consideration also needs to be given to testing strategies if someone who has recovered from the virus returns to work.

Rationalisation

Positive COVID Test	**Self-isolate for at least 10 days, starting from the day the test was taken.** **If you develop symptoms during this isolation period, restart your 10-day isolation from the day you developed symptoms.** You do not need to self-isolate after 10 days if you only have a cough or loss of sense of smell or taste, as these symptoms can last for several weeks after the infection has gone.	GOV.UK
Duration of Detection - Symptomatic	Of 90 studies using 2 (n=88) or 3 (n=2) consecutive negative PCRs 24hrs apart to show infection had cleared, **66 reported the duration of virus detection** from onset of symptoms using upper respiratory tract specimens. The longest duration observed was 83 days in one patient from upper respiratory tract samples. At the aggregate study-level, the median duration of virus detection from symptom onset using upper respiratory tract samples was **14.5 days** (range of study-level medians: 1–53.5 days).	Walsh et al, J Inf, 29 June 2020
Duration of Detection - Asymptomatic	Data less robust (smaller numbers) but broadly similar. Some studies showed a few days less, some a few days more.	
Epidemiological and modelling data	Different analyses show 1. Contacts were infected when first exposure occurred five days after the index case's symptom onset; 2. Infectivity declines relatively quickly within seven days of symptom onset; 3. A mean incubation period of four days and a maximum infectious period (including the incubation period) of 13 days provided the best fit of the observed data.	
Duration of Infectivity	Data less robust (smaller numbers) but it seems that **viable virus** can't be recovered from samples > about 8 days from symptom onset **despite large amounts of viral RNA still recoverable form OP/NP swabs.** For PCR Ct ≥ 34 can be ignored.	
Testing	*"Positive test results at the limit of detection that occur late in the cycle of infection represent individuals with a low or very low risk of transmission, as a result of the decline in infectious virus production or remnants of viral RNA in respiratory secretions."* *"Request a repeat sample and advise self-isolation pending the results of the second sample. Contact tracing should only be initiated if there is a positive result from the repeat sample."* *"A positive result at the limit of detection from the repeat sample is suggestive of the late stage cycle of infection and therefore contact tracing and further self-isolation is not advised."*	PHE

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