

Guidance on the management of weak positive (high Ct value) PCR results in the setting of testing individuals for SARS-CoV-2

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Version	Date	Changes from previous version
1.6	28.02.2022	Update to background material Change to text on duration of acquired immunity Content on vaccination removed as no longer directly relevant Change to recommendation for repeat testing of samples with high Ct value before reporting Extensive revision and simplification of interpretation of high Ct values in samples identified as from asymptomatic people including consideration of recent self-performed antigen tests
1.5	07/07/2021	Change in terminology and definitions on vaccine protection
1.4	22/06/2021	Updated to reflect NPHEP guidance on immunity extending to 9 months post infection People with significant vaccine protection should generally not be tested if asymptomatic
1.3	14/04/2021	Updated introduction on evidence of RNA persistence and culture of virus High Ct value and low viral load are essentially interchangeable Greater emphasis on the role of the laboratory director in determining cut off values and interpreting results Replacement of term "testing" throughout when "sample" or "sampling" is more accurate Updated to reflect NPHEP recommendation to extend period of presumptive immunity to six months Reference to virus variants and their relevance to acquired immunity and asymptomatic testing Statement that laboratories should provide Ct values on request in the context of expert interpretation Simplification to high Ct value/low viral load with removal of very high Ct value Reference to management of people with high Ct value results in hospital or residential care setting Reference to the absolute change in Ct value in the context of a fall in Ct value between samples Resequencing to place unintended testing after intended testing
1.2	22/12/2020	Expanded scope to encompass symptomatic people Revision of the title to reflect wider scope Indication that this approach may not be readily applicable in all settings
1.1	08/10/2020	Added information about previous positive cases Isolation/transmission-based precaution duration changed from 14 days to 10 days in community settings
1.0	19/08/2020	Initial guidance

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Background

SARS-CoV-2 RNA remains detectable in upper respiratory tract samples from some patients for extended periods. In one large cohort the median duration of shedding was 19 days with an interquartile range of 12-28 days. Long-term care residents had 3-5 day longer shedding durations. A small number of patients remained positive for more than 60 days. The authors specify that their “*findings do not speak to duration of infectivity but are useful for understanding the expected duration of RT-PCR positivity and for identifying reinfection*” [Duration of SARS-CoV-2 shedding: A population-based, Canadian study Susan P. Phillips and others. PLOS ONE | <https://doi.org/10.1371/journal.pone.0252217> June 17, 2021]

The experience of laboratories in Ireland is consistent with this. There are accounts of detectable RNA in repeated samples on people at, and beyond 12 weeks, and at least one instance of a result reported as detected at 19 weeks. However, studies of recovery of culturable virus indicate that virus is generally not cultured after 9 to 10 days but may be recovered up to day 12 in people with moderately severe disease, and has been reported up to day 20 in those with severe disease.

The immune response, including duration of immunity to SARS-CoV-2 infection, is not fully understood. The frequency with which reinfection with SARS-CoV-2 can occur and the time frame of recurrence is also subject to ongoing research. Prior to emergence of the Omicron variant a review of available evidence and advice provided by the Health Information and Quality Authority (HIQA), the National Public Health Emergency Team has indicated that the period of presumptive immunity should be considered as nine months following natural primary infection. However, during the recent surge in infection, the experience was that many people recovered from infection with previous variants were infected with Omicron much less than 9 months after the previous infection. This may be relevant also in the event of future variants that represent a significant level of immune escape. Therefore, presumed duration of acquired immunity is very dependent on changes in antigenic composition of circulating variants.

Testing, in particular testing of asymptomatic people, can result in the identification of people with positive tests for SARS-CoV-2 RNA which can be difficult to interpret. Specifically, interpretation is difficult when a person, often a healthcare worker (HCW) or a patient scheduled for a procedure or admission to a hospital, with no symptoms, tests positive for viral RNA at a low level. Testing of asymptomatic people should be performed within the parameters of a clearly defined public health policy regarding the testing of asymptomatic individuals or on the basis of advice from a Public Health specialist or IPC practitioner. The Consultant Microbiologist who is director of the laboratory performing the analysis has a critical role

to play in guiding the interpretation of results as they have detailed knowledge of the platforms in use and their performance characteristics.

Scope

This guidance is intended to support practitioners in avoiding testing for SARS-CoV-2 where this is unlikely to be useful, and to interpret certain difficult to interpret results.

It may not be practical and is not essential that the complexity of interpretation outlined here is applied in all settings. The detailed approach to interpretation outlined here may be particularly applicable in the context of an acute hospital setting where a multidisciplinary team is available and has capacity to critically interpret individual results and ensure appropriate communication and follow up.

When reporting confirmed results as SARS-CoV-2 RNA detected in settings where case-by-case evaluation is not practical it is appropriate, whenever possible, to include a comment differentiating a detected result qualified as a high Ct value/low viral load from a detected result without qualification. At a minimum, a laboratory should have a process whereby a clinician can obtain a Ct value, where available, from the laboratory on request when this is essential to interpretation.

Testing for SARS-CoV-2 antigens is not within the scope of this paper.

When it is not practical to implement the process outlined here and no differentiation between a positive result (unqualified) and a positive result qualified as high Ct value /low viral load is accessible it is generally necessary to proceed on the basis that a positive test is evidence that a person is infectious.

Terms

Cycle threshold

Ct (cycle threshold) values represent the number of cycles of amplification elapsed before the test system signals detection of the target. In general terms, the higher the Ct value the lower the quantity of virus target (viral load) present in the sample. Precise definition of what constitutes a high Ct value is difficult because a Ct value is not comparable to the quantitative output from a calibrated assay. The Ct value for

a given sample will be different in different laboratories depending on the test platform and other factors (see Carroll and McNamara at <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2021.26.6.2002079>).

In general terms for this report a Ct value of 30 or greater is taken as indicative of a high Ct value but it is appropriate for the director of each laboratory to make their own determination as to what constitutes a high Ct value based on their experience with the platform they are using.

Some laboratories may estimate viral load and /or prefer to use the terminology of low level of virus detected in the sample in preference to reference to high Ct values. Both high Ct value and low viral load convey essentially the same information for clinical purposes and either terminology may be used.

A SARS-CoV-2 detected test result

For the purposes of this paper a “detected” result means that the test result meets appropriate criteria to be reported as detected. In general terms, a sample with detection of RNA at a high Ct value/low viral load should be reported as SARS-CoV-2 RNA equivocal or as not confirmed as appropriate in the context of the laboratories experience. If reported as not confirmed, no further action is required and the result should not be notified to public health. If the laboratory reports the result as equivocal, it is generally appropriate to request a repeat sample. If reported as equivocal the result should not be notified to public health as a laboratory confirmed case.

Guidance

The application of this guidance should take account of the epidemiological situation (time and place) in which the sample is taken and any available clinical information. It should also take account of results for testing any other respiratory virus performed at the same time.

In general terms, a high Ct value/low viral load result in an asymptomatic person is more likely to represent residual RNA detection of no public health or infection prevention and control (IPC) significance in a situation in which the incidence of infection in the population is low and falling. Such a result is more likely to represent an early pre-symptomatic RNA detection that is of public health and IPC significance in a situation in which the incidence of infection is high and increasing. **Such a result is more likely to represent an early pre-symptomatic positive in the context of a multiple tests reported as not-detected in the preceding days, as for example in sequential testing of hospitalised patients.**

Interpretation of results is dependent on the availability of Ct values/viral load. Laboratories may not report Ct values routinely **but should provide the result in the context of expert interpretation on request**. If Ct values/viral loads are not available for any reason (for example some platforms do not display Ct values) the default is to assume a positive result represents a significant result and that the person is infectious. It may be appropriate however to retest the sample on a platform that does display Ct values.

Confirmed detection of SARS-CoV-2 RNA at high Ct value in a person tested on the basis that they had no symptoms or other clinical features at the time of sampling may represent:

1. Pre-symptomatic infection in a person who subsequently will develop symptoms or other clinical features. [Likely to be infectious];
2. Symptomatic infection in a person who has symptoms or other clinical features not noted prior to or at the time of sampling. [Likely to be infectious];
3. True asymptomatic infection. [Likely to be infectious];
4. A person with residual RNA detectable after the period of infectivity has expired. [Unlikely to be infectious];
5. A true false positive.

Guidance on the management of weak positive (high Ct value) PCR results in asymptomatic individuals

1. When a positive result with a high Ct value/low viral load is obtained on a person understood at the time of sampling to be asymptomatic, a history of relevant symptoms either in the recent past, or of symptoms that have developed since the sample was taken can guide interpretation. If the person is a COVID-19 Contact this is also relevant to interpretation. This information may not be available to the laboratory but should be available to the doctor directly responsible for care of the patient;

2. If they have developed relevant symptoms since the sample was taken they should generally be regarded as a recent onset infectious case;

For person who is not in a congregated healthcare setting

3. If they report relevant symptoms with a date of onset in the 7 days prior to sample collection, they should generally be regarded as a recent onset and currently infectious case, repeat testing is not required;
4. If they report a new positive self-performed antigen test in the 7 days prior to sample collection they should generally be regarded a recent onset and currently infectious cases, repeat testing is not required
5. In the absence of symptoms or a positive self-performed antigen test in the 7 days prior to sample collection
 - (a) if they report a positive test (laboratory or self-performed) in the previous 3 months the result may be assumed to represent residual RNA and they need not self-isolate and do not require a repeat test
 - (b) if they do not report a positive test (laboratory or self-performed) in the previous 3 months the result should be regarded as a recent onset and currently infectious cases. However, if it is practical to organise a repeat test that shows no change in Ct value or antigen test that is not detected this should be considered as part of the assessment.

For person in a congregated healthcare setting (hospital or LTRCF for older people)

6. If they report relevant symptoms with a date of onset in the 10 days prior to sample collection, they should generally be regarded as a recent onset and currently infectious case. Transmission-based precautions should apply and repeat testing is not required as a routine but clinical judgement applies;
7. If they report a new positive self-performed antigen test in the 10 days prior to sample collection they should generally be regarded a recent onset and currently infectious case. Transmission-based precautions should apply and repeat testing is not required as a routine but clinical judgement applies;
8. In the absence of symptoms or a positive self-performed antigen test in the 10 days prior to sample collection

- (a) if they report a positive test (laboratory or self-performed) in the previous 3 months the result may be assumed to represent residual RNA. Transmission-based precautions and repeat testing are not required as a routine but clinical judgement applies;
- (b) if they do not report a positive test (laboratory or self-performed) in the previous 3 months a repeat test in 24 to 48 hours should be taken if practical. While waiting for the result on the repeat sample transmission-based precautions should apply.
9. If the result of the repeat test is a similar or higher Ct value this may be considered residual RNA and transmission-based precautions can generally be discontinued in the absence of any special considerations
 10. If the repeat test shows a significantly lower Ct value they should be considered as infectious with date of onset from date of first detected sample or if they subsequently develop symptoms then from the date of onset of symptoms. A “significantly lower” Ct value is likely to be a reduction of at least 2 in most cases but this will be determined by the laboratory director based on experience with the platform they use.

Appendix 1 – Notes on the Utility & Limitations of PCR

1. PCR is primarily a method for amplifying DNA and (by extension) RNA;
2. PCR as a diagnostic methodology is exquisitely sensitive, capable under conditions of optimal sample quality of detecting fewer than 10 copies of viral RNA in a clinical sample;
3. However, PCR does not distinguish between viable virus and non-infectious RNA;
4. In individuals infected with SARS-CoV-2, PCR can often detect viral RNA for many days and weeks after the resolution of the clinical features and after the person is no longer infectious;
5. PCR-based assays can yield non-specific (or ‘false positive’) results near the limit of detection of the assay: this does not mean that the test is bad;
6. A very good PCR assay with a specificity of 99.5% can still generate 5 ‘RNA detected’ results in a cohort of 1000 individuals without the infection;
7. Although there may be variation between platforms and amplification efficiency in general standard PCR assays run for 40 cycles: in the case of a commercial, CE marked PCR assay, the assay manufacturer determines for how many cycles the assay should run;
8. Under optimal PCR conditions the amount of genetic material present doubles with each cycle, and increases by a factor of 10 every 3.3 cycles;

9. PCR results can be reported with cycle threshold (Ct) values: in general terms the lower the Ct value, the more viral RNA that is present in the clinical specimen. Note: The same sample tested on different assays/platforms can give different Ct values reflecting differences in the targets detected and the chemistry of the test used. When considering trends in Ct values it is preferable to test samples with the same assay/platform each time;
10. There are very few reports of viable SARS-CoV-2 virus being retrieved in culture from clinical specimens with a Ct value of >34;
11. Some PCR assays will try to detect more than one target (piece of viral RNA) in a clinical specimen. In that case it is important to consider if one or both targets are detected and the Ct values. Detection of only one of two intended targets at a high Ct value should generally not be reported as a positive test without qualification

ENDS