

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
1.2	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

It is increasingly apparent that SARS-CoV-2 RNA remains detectable in upper respiratory tract samples from some patients for many weeks. The published literature on this is limited but there is experience from multiple Irish laboratories of detectable RNA in repeated tests on people at and beyond 12 weeks, and at least one instance of a positive result at 19 weeks.

The immune response including duration of immunity to SARS-CoV-2 infection is not well understood. The frequency with which reinfection with SARS-CoV-2 can occur and the time frame of recurrence is also unclear at this point. However, there are now a number of reports of a second infection confirmed as a distinct infection by differences in sequence between the virus initially detected and the virus subsequently detected. Based on current evidence summarised by the Health Information and Quality Authority (HIQA) the current consensus of the Pandemic Incident Control Team (PICT) is that immunity should not be assumed beyond 12 weeks from the time of primary infection and that reinfection is therefore more likely to occur after that time.

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 workers (HCW), with no symptoms tests positive for viral RNA at a low level. Testing of asymptomatic people should only 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.

Scope

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

It is not practical and is not essential that the complexity of interpretation outlined here is applied in all settings. It may be particularly applicable in the context of a complex outbreak where multidisciplinary team are managing the situation closely and there are appropriate resources

and capacity to interpret individual results carefully and ensure appropriate communication and follow up.

When it is not necessary or practical to implement the process outlined here one should proceed on the basis that a positive test is evidence that a person is infectious.

Clarification of terms

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 present in the sample. Precise definition of what constitutes a high or very 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. In general terms for this report **a Ct value of 30 or greater is considered a high Ct value and a value of 35 or greater is considered a very high Ct value.** It is appropriate for laboratories to adjust these thresholds based on their experience with the platform they are using.

For the purposes of this paper a “positive test” means that the test result meets appropriate criteria to be reported as positive. In general terms, in an assay detecting multiple targets, detection of a single target at a high or a very high Ct value should be: (a) reported as either equivocal or not detected based on the reporting policy of the laboratory and their experience with the platform and assay in use or; (b) retested on the same or a second platform before reporting. In an assay detecting a single target, detection of that target at a high or very high Ct value should result retesting on the same or a second platform before reporting. Samples with high or very high Ct values that are not reproducible on re-testing should generally be reported to the effect that SARS-CoV-2 RNA was either not confirmed, or SARS-CoV-2 RNA was not detected. If reported as not confirmed or not detected, no further action is required and the result should not be notified to public health. If the laboratory chooses to report the result as equivocal, it is appropriate to request a repeat sample if clinically appropriate. If reported as equivocal the result should not be notified to public health.

Guidance

In general, someone who has had a previous positive test should not be retested within 12 weeks unless they develop symptoms. This statement encompasses people who are identified as close contacts of COVID-19 cases but who are noted to have tested positive in the previous 12 weeks.

The application of this guidance should take account of the epidemiological situation (time and place) in which the sample is taken. In general terms, a high Ct value PCR 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.

Interpretation of results is dependent on the availability of Ct values. Laboratories may not report Ct values routinely but may be able to provide them on request. If Ct values are not available the default is to assume a positive result represents a significant result and that the person is infectious.

When reporting confirmed positive results with high or very high Ct values in settings where case by case evaluation is not practical it is appropriate, where possible, to include an interpretive comment indicating in general terms that the result may not reflect current infectious COVID-19.

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

1. Pre-symptomatic infection in a person who subsequently will develop symptoms or other clinical features.
2. Symptomatic infection in a person who has symptoms or other clinical features not noted prior to or at the time of testing.
3. True asymptomatic infection.
4. A person who has recovered from infection and has residual RNA detectable.

Unintended testing of a person within 12 weeks of a previous diagnosis of infection

In the event that a person is tested unintentionally within 12 weeks of a diagnosis of COVID-19 and the Ct value is high or very high, it can generally be assumed to represent residual viral nucleic acid unless they are symptomatic. Under these circumstances asymptomatic people do not need to restrict their movements or take any specific measures other than those that apply to everyone, and the person should not be notified to the Department of Public Health as a new case.

If the person has symptoms or other clinical features consistent with a diagnosis of COVID19 at high or very high Ct value the default is to assume that the result is clinically significant.

Variation from this assumption should be based on a written assessment by a senior medical practitioner with relevant expertise taking account of all available information including testing for other infections as appropriate.

If the sample has a Ct value of less than 30, even if asymptomatic, the person should generally be managed as a confirmed infectious case unless an individual assessment is made by an appropriate specialist that the result can be considered to be consistent with residual viral nucleic acid. If a sample from their original test is available, consideration may be given to sequencing the viral nucleic acid in both samples, although it is unlikely that this result will be available within a time frame that supports the management of the individual person

Guidance on the management of weak positive (high Ct value) PCR results in asymptomatic individuals who have not had a diagnosis of COVID-19 in the previous 12 weeks

The evidence base on which to address this complex issue is limited. However, from a pragmatic clinical point of view there is a need to define an approach to managing this as outlined below.

If the person has symptoms or other clinical features consistent with a diagnosis of COVID19 the default is to assume that a positive result at any Ct value is clinically significant. Variation from this assumption should be based on a written assessment by a senior medical practitioner

with relevant expertise taking account of all available information including testing for other infections as appropriate.

With respect to asymptomatic people who have a diagnosis of COVID-19 more than 12, but less than 24 weeks previously, it is appropriate, where practical to do so, to make case by case decisions with respect to the categorisation of people with positive tests as infectious cases or non-infectious residual RNA positive cases. This categorisation may be made by a Consultant Microbiologist, a Consultant Infectious Disease Physician, or a Public Health specialist based on assessment of the person and all available test results. Previous test results for COVID-19 and results of tests for other respiratory viruses including influenza virus may be helpful in making that determination. If case by case assessment is not practical the person should be managed as for as for people with not previous diagnosis of COVID-19.

With respect to asymptomatic people who have a diagnosis of COVID-19 more than 24 weeks previously they should be managed as for people with not previous diagnosis of COVID-19.

With respect to people asymptomatic people with no previous diagnosis of COVID-19 he following outlines a general approach that may be adopted in most cases:

1. When a positive result is obtained on a person understood at the time of testing to be asymptomatic, it is important to establish if they had relevant symptoms either in the recent past, or if they have developed symptoms since the test was taken.
2. If they have developed relevant symptoms since the test was taken they should generally be regarded as a recent onset infectious case.
3. If they report relevant symptoms with a date of onset in the 10 days prior to testing, they should generally be regarded as a recent onset infectious cases.
4. If they report relevant symptoms with a date of onset of more than 10 days prior to the test **OR** if they report no symptoms at any time, the following approach is appropriate:
 - a. If the Ct value is high, but not very high they should be provisionally managed as an infectious case (with self-isolation, notification and contact tracing) pending further evaluation.
 - b. If the Ct value is very high they should be advised to self-isolate, but notification and initiation of contact tracing may await the outcome of further evaluation or change in the clinical condition.
5. Further evaluation must include a repeat test on the second day after the initial test (for example, if the initial test was taken on Monday the repeat test should be taken on

Wednesday). Ideally the second test should be performed on the same platform as the initial test.

- a. If the Ct value remains in the high or very high range on second day after the initial test the person may generally be considered as a remotely acquired infection and non-infectious at the time of testing. If contact tracing was initiated (high Ct value) it can be stood down and the person need no longer self-isolate
 - b. If the Ct value has fallen below the high /very high range on repeat testing they should be regarded as a recent onset infectious case.
 - c. In the event of a major change in Ct value within the high to very high range (for example Ct value changes from 39 to 31 it may be appropriate to take a further test 2 days later.
6. The person should also be asked to contact their doctor (or a dedicated phone number in the service where they work in the context of a healthcare worker) immediately if they develop new relevant symptoms at any time during the period of further evaluation:
- a. If the person develops relevant symptoms at any time during the period of further evaluation they should be treated as a recent onset infectious case.

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 syndrome.
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. In the diagnostic setting, a Ct value of 30 in a very efficient PCR assay represents a viral load of around 1000 (3 log) copies: a Ct value > 34 represents a viral load of fewer than 100 copies; a Ct value of >37 represents a viral load of fewer than 10 copies.
11. There are very few reports of viable SARS-CoV-2 virus being retrieved in culture from clinical specimens with a Ct value of >34.
12. Some PCR assays will try to detect more than one target (piece of viral RNA) in a clinical specimen.
13. If the assay sees all targets (usually 2 or 3) as present, then RNA is detected; if no targets are present, then RNA is not detected; if only some of the targets are present,

then the test should be repeated and if the result is reproducible the result may be reported as indeterminate.

- a. Individuals whose samples yield indeterminate results should be recalled for repeat sampling if this is appropriate in the clinical context.
- b. If the second sample also yields an indeterminate result, the individual should generally be considered as confirmed SARS CoV2 infection and the result notified to public health.

Approved by the Pandemic Incident Control Team in December 2020.

ENDS