

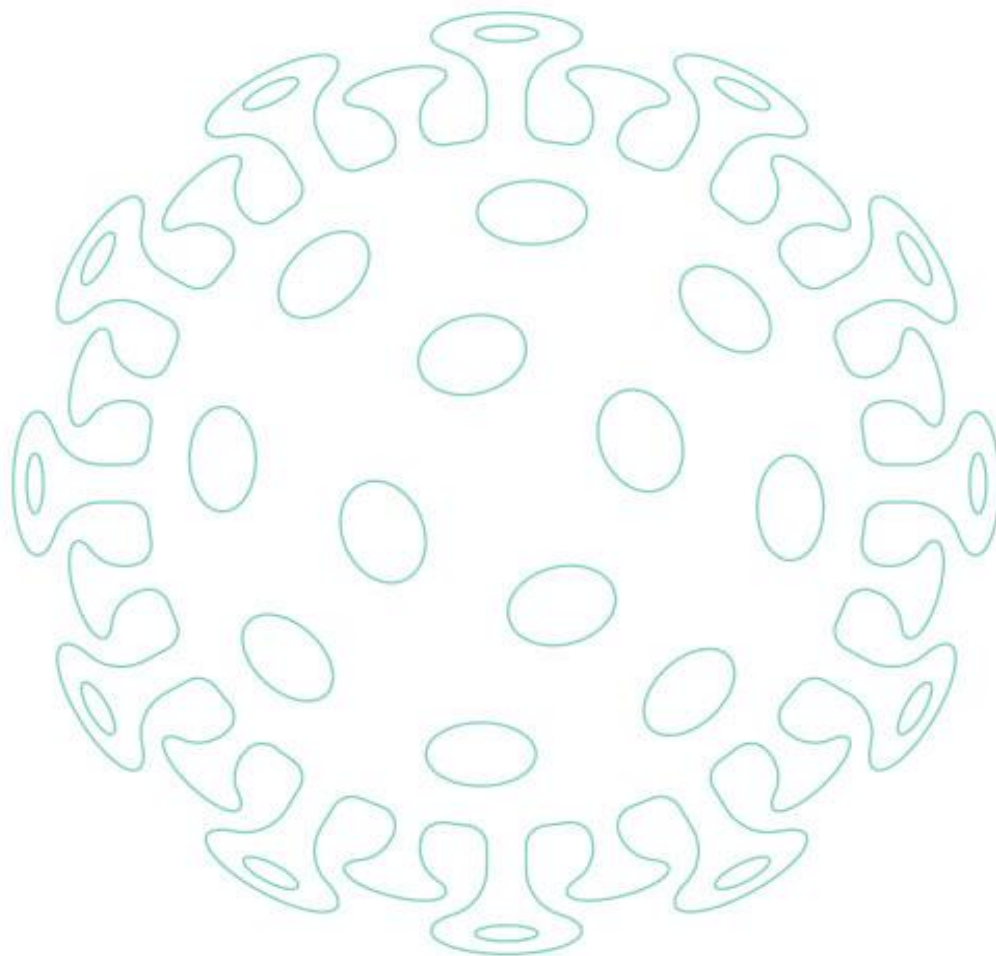


Public Health
England

Protecting and improving the nation's health

Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR

A guide for health protection teams



Contents

Important messages	3
What is RT-PCR?	4
How is RT-PCR performed?	4
RT-PCR assays	5
Summary	10
References	11

Important messages

Cycle threshold (Ct) is a semi-quantitative value that can broadly categorise the concentration of viral genetic material in a patient sample following testing by RT PCR as low, medium or high – that is, it tells us approximately how much viral genetic material is in the sample.

A **low Ct** indicates a high concentration of viral genetic material, which is typically associated with high risk of infectivity.

A **high Ct** indicates a low concentration of viral genetic material which is typically associated with a lower risk of infectivity. In the context of an upper respiratory tract sample a high Ct may also represent scenarios where a higher risk of infection remains – for example, early infection, inadequately collected or degraded sample.

A single Ct value in the absence of clinical context cannot be relied upon for decision making about a person's infectivity.

Ct values cannot be directly compared between assays of different types – not all laboratories use the same assay, and some may use more than one.

What is RT-PCR?

Reverse transcription polymerase chain reaction (RT-PCR) is an established laboratory technique that can be used to identify the presence of specific genetic material through a biochemical process of amplification using enzymes and is based on specific target recognition. Genetic material includes DNA and RNA, but in the context of RT-PCR it is RNA that is detected. SARS-CoV-2 has an RNA genome. The major benefits of RT-PCR are in the ability to detect extremely small amounts of pathogen RNA in a very short time. RT-PCR has therefore revolutionised the speed and sensitivity of clinical diagnostics and can be adapted to test at large throughput using automation with reduced need for technical expertise. Modern applications of RT-PCR allow the reaction to be monitored during each stage, known as real time RT-PCR.

How is RT-PCR performed?

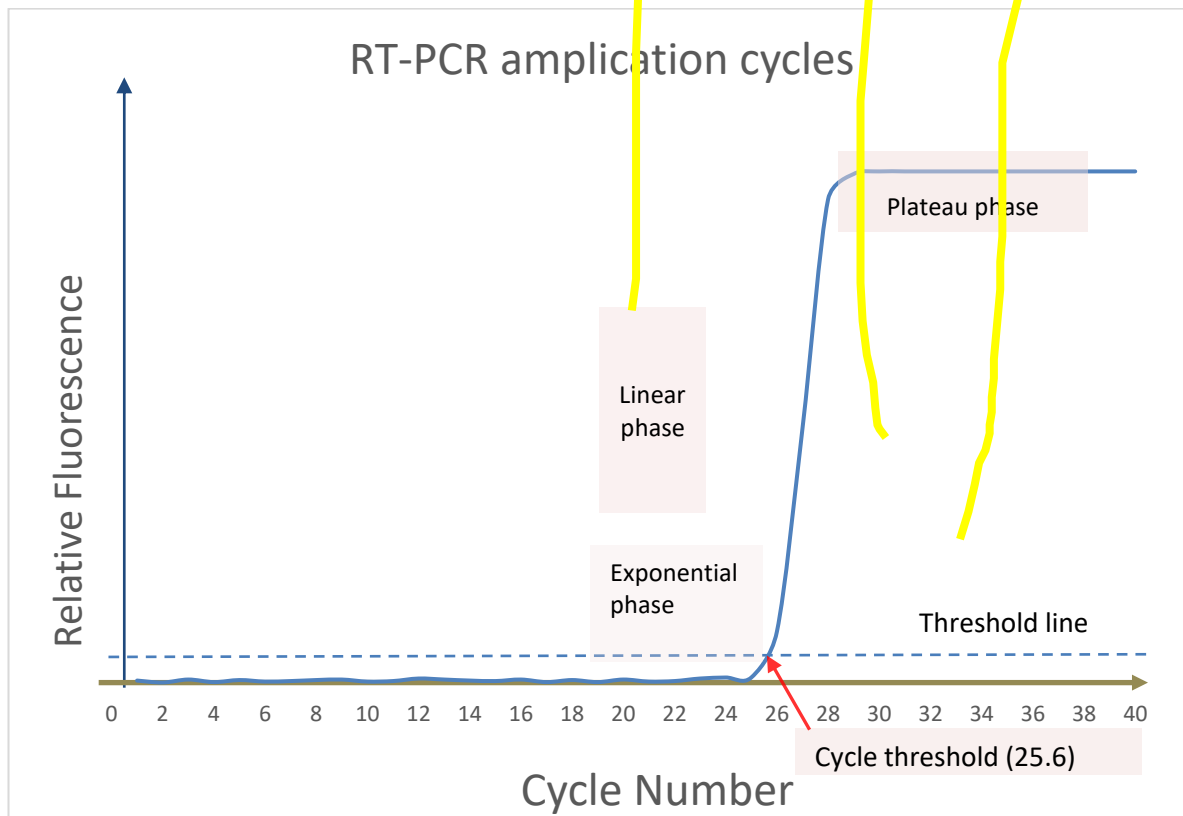
Often heralded as one of the most important scientific advances in molecular biology, PCR revolutionised the study of DNA to such an extent that its creator, Kary B. Mullis, was awarded the Nobel Prize for Chemistry in 1993. The first step in SARS-CoV-2 RT-PCR is to extract the viral RNA from the sample to purify, stabilise and concentrate it, to increase detection of samples containing low quantity of virus. The purified extract is added to a biochemical reaction mixture that includes:

- primers – short stretches of nucleic acid that match parts of the target organism genome
- nucleotide bases (the building blocks of nucleic acids)
- enzymes to initiate and complete the reaction
- fluorescently labelled probes – short stretches of nucleic acid that recognise and stick to the reaction product (the reaction indicator)

Primers attach to target regions of the viral nucleic acid, allowing the enzyme to add nucleotides to elongate a complementary DNA (cDNA) strand. The sample reaction mixture is subjected to repeated thermal cycles so that copies of the viral target are doubled per cycle leading to exponential rise. Labelled probes emit a fluorescent signal in the presence of newly synthesised target. The earlier that exponential increase occurs, the higher the quantity of virus in the sample.

Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR

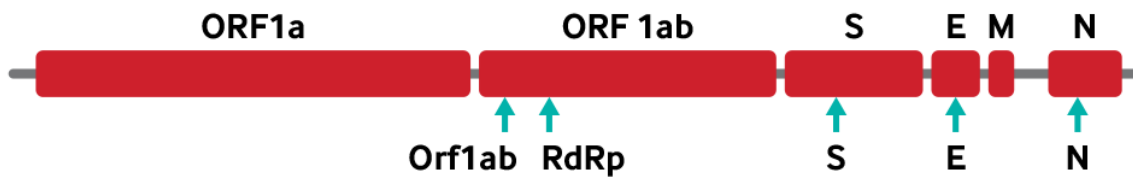
Figure 1 demonstrates the stages for RT-PCR post run analysis.



RT-PCR assays

There are many different SARS-CoV-2 RT-PCR assays/platforms in use across the UK. Each assay will have a slightly different limit of detection (LoD) – the lowest concentration of virus that can be reliably and consistently detected by the assay, and will be configured according to local arrangements. Some RT-PCRs are designed to identify a single gene target and others will detect multiple targets. Those detecting multiple targets can give greater certainty when interpreting results. Discrepancies in results between gene targets can lead to uncertainty on interpretation, particularly where a commercial assay is in use and the raw data not accessible. Highly complex assays with multiple targets may be prone to non-specific detection that can be incorrectly reported as positive.

Figure 2. Genome of SARS-CoV-2 with the most common RT-PCR targets highlighted



RT-PCR detects presence of viral genetic material in a sample but is **not able to distinguish whether infectious virus is present**. The quantity of intact virus in upper respiratory swabs will be affected by factors that are endogenous and exogenous to laboratory methods.

Laboratory exogenous factors

1. The adequacy of sample collection.
2. The quantity of virus at the collection site.
3. The presence of inhibitors.

Laboratory endogenous factors

1. The total volume of sample collection buffer/medium.
2. The sample preparation method (heat, lysis methods).
3. The laboratory reagent volumes used in each step of the RT-PCR process.
4. The RT-PCR assay of choice.

What is a Ct value?

The cycle threshold (Ct) can be defined as the thermal cycle number at which the fluorescent signal exceeds that of the background and thus passes the threshold for positivity (Figure 1, page 5).

A typical RT-PCR assay will have a maximum of 40 thermal cycles. The lower the Ct value the higher the quantity of viral genetic material in the sample (as an approximate proxy for viral load). Ct values obtained in this way are semi-quantitative and are able to distinguish between high and low viral load. A 3-point increase in Ct value is roughly equivalent to a 10-fold decrease in the quantity of viral genetic material.

In some circumstances, Ct values can be used as a more quantitative technique to accurately measure the number of viral copies per cell in the original sample – however, this requires that the sample is tested alongside verified standard dilutions and there is fixed sample input alongside quantification of cellular content of a swab sample(1,2). Most diagnostic laboratories do not routinely perform this quantification for respiratory viral swabs – quantitative PCR is more common in measuring blood-borne viral load.

Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR

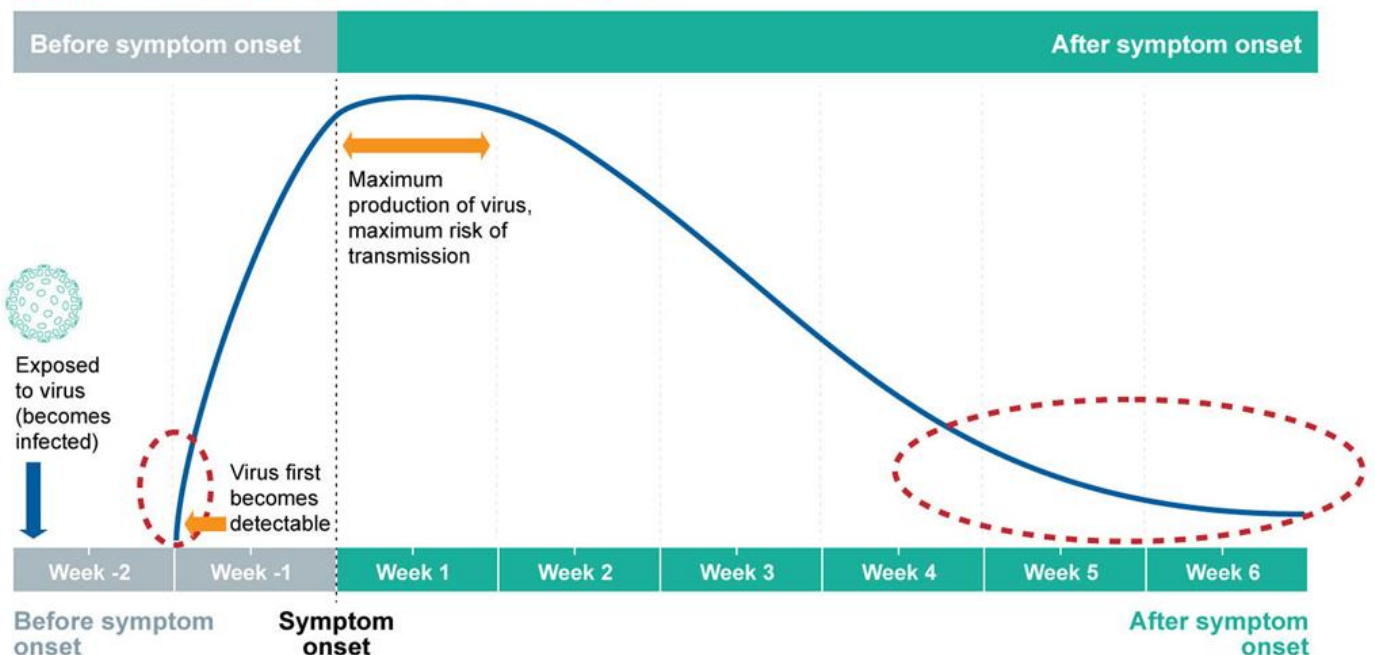
Ct values cannot be directly compared between assays of different types due to variation in the sensitivity (limit of detection), chemistry of reagents, gene targets, cycle parameters, analytical interpretive methods, sample preparation and extraction techniques. Additionally, Ct values are not provided for all SARS-CoV-2 molecular detection methods. Some commercial RT-PCR techniques are closed 'black box' systems whereby the operator cannot observe the reaction in real-time and the result is interpreted by software into a qualitative non-interrogatable positive or negative result.

Interpreting Ct values from an upper respiratory tract swab

The schematic diagram below (adapted from <https://new-learning.bmj.com/course/10065426>) illustrates the detection of SARS-CoV-2 RNA (shown by the blue line). Timings of symptom onset and detection of viral genetic material in relation to infection will vary from person to person but broadly fit within this representation.

Figure 3. Timeline of detection of SARS-CoV-2 RNA in infection

COVID-19 symptom onset schematic diagram



The clinical significance of positive results with high Ct are difficult to interpret in the absence of clinical history and context. Positive results with low viral load (high Ct) 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 (periods indicated by the dotted red line). Thresholds (Ct cutoffs) are established to ensure specific results, but in some assays an indeterminate zone is established which may require a follow up sample to determine the significance of the result. This will be

Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR

the case in laboratories that do not have clinical information to assist guidance on interpretation, and when repeat analyses may not be possible

Whilst high Ct values may be associated with reduced infectivity, a swab taken at a single point in time does not provide information about the trajectory or subsequent course of illness.

Ct values have been observed to be similar in persons who never develop symptoms (asymptomatic) compared with those who are symptomatic (3). In asymptomatic persons, it is often not known when the person became infected and what stage of illness they are in, and therefore their infectivity risk. Live and potentially infectious virus has been isolated in laboratory cell culture from samples exhibiting high Ct (>36) - to what extent this indicates a potential transmission risk from person-to-person is not fully understood.

Examples of the wide range of scenarios where high Ct may feature are detailed below:

Asymptomatic infection with unknown infectivity risk (it is often not known when the person became infected and what stage of illness they are in).

Pre-symptomatic infection that subsequently develops into symptomatic infection with high viral load and infectivity.

Inadequately collected or degraded samples (sub-optimal sample storage and handling techniques) during acute COVID-19 with high risk of infectivity. Symptomatic persons should be assumed to be potentially infectious within the first 10 days after illness onset and asymptomatic persons for 10 days after the positive swab result – guidance on self-isolation for community cases of COVID-19 can be found in [6].

Recovery phase of infection with diminishing viral load. Prolonged detection of viral genetic material that is likely to be non-infectious has been observed for SARS-CoV-2. Guidance on interpreting PCR positive results in persons in health and social care settings who have been retested after an initial illness can be found in [7].

Immunocompromised and hospitalised individuals with more severe illness that are more likely to exhibit longer shedding of potentially infectious virus(4).

Understanding cycle threshold (Ct) in SARS-CoV-2 RT-PCR

Guidance for stepdown of infection control precautions and discharge in hospital settings can be found in [8]. In complex cases, the presence of detectable neutralising antibody might support a reduced risk of infectiousness.

Lower respiratory tract severe COVID-19 disease (studies demonstrate lower viral load in the upper respiratory tract compared to the lower respiratory during severe acute disease(5)).

Summary

Ct is a semi-quantitative indicator of the concentration of viral genetic material in a patient sample.

From a laboratory perspective, Ct values should only be reported and applied for clinical interpretation and action where the linearity, limit of detection and standard quantification curves are assured.

Ct values are not directly comparable between assays and may not be reported by some RT-PCR platforms in use. Interpreting single positive Ct values for staging infectious course, prognosis, infectivity or as an indicator of recovery must be done with context about the clinical history.

Low Ct values (high viral load) more likely indicate acute disease and high infectivity.

High Ct values (low viral load) can be attributed to several clinical scenarios whereby the risk of infectivity may be reduced but interpretation requires clinical context.

Serial Ct values have greater utility for interpretation but are generally only undertaken in hospital settings for the purpose of clinical management rather than infection control purposes.

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