RT-PCR Test Amplification Cycle Threshold [C t] Information

This brief article aims to provide further technical information, specifically regarding the RT-PCR test "Amplification Cycle Threshold" [C t] value. The following information has been received by BGB on this subject following a request for information directed to Public Health Wales [PHW] and Public Health England [PHE].

Information received from Public Health Wales [PHW]
Confirmation was received in August 2020 that commercial assays used in Wales for the clinical diagnosis of SARS-CoV-2 infection are all CE marked. The number of amplification cycles in tests can vary with different platforms used. Most platforms use threshold cycles that range from 27 to 43. The threshold cycle is determined by the platform used and is not something the laboratory service has control over. Confirmation was also received from PHW in October 2020 that samples from Royal Glamorgan Hospital for Covid testing may be tested in the laboratory in Royal Glamorgan Hospital or laboratories in the Public Health Wales network. The real-time PCR assays in use in Wales for Covid 19 diagnostics all run for **45 cycles**; however, the cycle number where the sample is defined as "RNA not detected" varies by platform and target gene detected by the system. This is defined by the manufacturer. One platform (Hologic) is isothermal, this means it does not cycle through temperature changes in the same way as the real-time PCR systems, therefore CT values are not reported by this system.

Information received from Public Health England [PHE]
Confirmation received 13 November 2020 that PHE does not hold information on testing kits used by non-PHE laboratories. These laboratories have a statutory duty to report "positive cases" to PHE but they are not obliged to advise PHE which tests they are using.

Comment

It is important to note that amplification cycles increase exponentially as set out on the table below.

C t	Amplification	Amplification
50	1125,899,906,842,624	1125 Trillion
45	35,184,372,088,832	35,184 Billion
40	1099,511,627,776	1099 Billion
40	1099,511,627,776	1099 Billion
35	34,359,738,368	34 Billion
30	1,073,741,824	1 Billion
20	1,048,576	1 Million
15	32,768	32 Thousand
10	1,024	1 Thousand
5	32	32
2	4	4

A C t threshold value of 45 (recommended rate for of cycles originally recommended by the W.H.O.) amplifies the fragment of RNA by **35,184 billion times**. The test actually measures the presence of partial RNA sequences present in the intact virus, which could be a piece of dead virus which cannot make the subject sick, and cannot be transmitted, and cannot make anyone else sick. A true positive test result does not necessarily indicate the presence of viable virus. In limited studies to date, many researchers have shown that some subjects remain PCR-positive long after the ability to culture virus from swabs has disappeared. They term this a 'cold positive' (to distinguish it from a 'hot positive', someone actually infected with intact virus). The key point about 'cold positives' is that they are not ill, not symptomatic, not going to become symptomatic and, furthermore, are unable to infect others.

The information received from PHW and PHE regarding C t values supports the findings of the external peer review, that the RT-PCR test is not fit for purpose. The Portuguese appeal court, on 11 November 2020, arrived at a similar conclusion. Confirmation from PHW and PHE that kit manufacturers have control over the threshold cycles used in the tests rather than the client; Public Health Authorities, the National Health Service, Welsh Government and UK Government, is a matter of grave concern. It suggests a total lack of control by the client.

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