

8 April 2021

Our ref: FOI/24124

Barbara Jones
Email: request-735882-2b09dafb@whatdotheyknow.com

Dear Ms Jones,

Re: Limits on Amplification on the PCR test in Northern Ireland

I refer to your Freedom of Information (FOI) request received on 12 March 2021. Outlined below is Belfast Trust's response, which I hope you find useful.

Question:

What limits on amplification on the PCR test does N Ireland use and/or recommend?

Response:

Each test kit comes with the manufacturer's instructions, which state the total number of amplification cycles that must be done for the specific assay. This is not altered by Belfast Trust's laboratory.

The numerous PCR based assays for SARS-CoV2 used by the Regional Virus Laboratory in Belfast Trust employ Taqman technology. Most Taqman based PCR assays run for 40 cycles - some for 45 cycles. The manufacturer of the kit determines the total number of reaction cycles of a PCR test that should be done, along with mastermix volume, template volume, annealing temperature and PCR cycling conditions, dye wavelength channels and background fluorescence normalisation.

However, your question may also be referring to cut off (Ct) thresholds for test positivity.

A threshold Ct value is determined specifically for each kit and platform used for testing; a universal directive is not applicable, available or appropriate for determining cut off Ct values, given the substantial number of testing options not only for SARS-CoV2 but also for any virus, bacterial or fungal molecular diagnostic test.

There are many SARS-CoV2 testing work streams in Northern Ireland, each with their own Ct threshold. All laboratory tests undergo extensive validation to assess performance. The results of any laboratory diagnostic test, be it PCR or other laboratory methods, is one element of the diagnosis of an individual. All laboratory results must be considered within the clinical context and presentation of the individual. Timing and quality of samples influence the likely detection of virus. From a laboratory perspective, the Ct (cycle threshold) value is only one of a number of criteria that are considered when interpreting test results to assess positivity. Before a result is issued, variables considered include reactivity in multiple SARS-CoV2 gene targets, assessment of fluorescence levels, sigmoidal shape of amplification curves and confirmatory testing using assays with alternative specificity profiles, as appropriate. This is in line with good laboratory practice and World Health Organisation papers on PCR for SARS-CoV2 detection.

Question:

Please send me any directive or letter authorising this.

Response:

Belfast Trust do not have specific authorising letters or directives, as this is not the way laboratory diagnostic testing works.

There are many useful documents publically available from Public Health England (PHE) and the World Health Organisation (WHO) to aid in SARS-CoV2 diagnostics relevant to cycle thresholds - please see the links below for examples:

[Understanding cycle threshold \(Ct\) in SARS-CoV-2 RT-PCR \(publishing.service.gov.uk\)](https://www.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/644444/Understanding-cycle-threshold-Ct-in-SARS-CoV-2-RT-PCR.pdf)

[Assurance of SARS-CoV-2 RNA positive results during periods of low prevalence - GOV.UK \(www.gov.uk\)](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/644444/Assurance-of-SARS-CoV-2-RNA-positive-results-during-periods-of-low-prevalence-GOV.UK.pdf)

You may wish to peruse the PHE and WHO websites, should you require further documentation that may be helpful.

<https://www.gov.uk/government/organisations/public-health-england>

<https://www.who.int/>

If you are dissatisfied with the way in which we have handled your Freedom of Information (FOI) request, you may wish to complain. You should write to: Public Liaison Service, Belfast Health & Social Care Trust, Nore Villa, Knockbracken Health Care Park, Saintfield Road, Belfast BT8 8BH or alternatively e-mail: publicliaison@belfasttrust.hscni.net within two months of the date of this response and your complaint will be considered and a response provided, usually within 20 working days of receipt.

Should you still be dissatisfied with the outcome, you can proceed to the second review stage by contacting the Information Commissioner, The Information Commissioner's Office, Wycliffe House, Water Lane, Wilmslow, SK9 5AF who will undertake an independent review. It is important to note that if you refer any matter to the Information Commissioner, you will need to show evidence of having gone through the Trust's review process to try to resolve the matter with the Trust in the first instance.

If you require any further information or wish to discuss, please do not hesitate to contact me.

Yours sincerely



Public Liaison Officer