Rapid response to:

Analysis

Put to the test: use of rapid testing technologies for covid-19

BMJ 2021; 372 doi: https://doi.org/10.1136/bmj.n208 (Published 03 February 2021) Cite this as: BMJ 2021;372:n208

Rapid Response:

Positive results from UK single gene PCR testing for SARS-COV-2 may be inconclusive, negative or detecting past infections

Dear Editor,

The efficacy of mass population testing for SARS-COV-2 virus is critically dependent on the reliability of the test applied, whether it be a RT-PCR or lateral flow test. Given that many RT-PCR tests do not actually target all the genes necessary to reliably detect SARS-COV-2, the results of mass testing using RT-PCR need to be revisited and reanalysed.

The ONS publish a regular infection survey [1], [15] that includes data from two UK lighthouse laboratories, where both use the same RT-PCR test kit to detect the SARS-COV-2 virus. This survey includes data on the cycle threshold (Ct) used to detect positive samples, the percentage of positive test results arising from using RT-PCR, and the combinations of the SARS-COV-2 virus target genes tested that gave rise to positives between 21 September 2020 and 1 March 2021 across the whole of the UK.

The kit used by the Glasgow and Milton Keynes lighthouse laboratories is the ThermoFisher TaqPath RT-PCR which tests for the presence of three target genes from SARS-COV-2 [2]. Despite Corman et al [3] originating the use of PCR testing for SARS-COV-2 genes there is no agreed international standard for SARS-COV-2 testing. Instead, the World Health Organisation (WHO) leaves it up to the manufacturer to determine what genes to use and instructs end users to adhere to the manufacturer instructions for use (IFU).

The WHO's emergency use assessment (EUA) for the ThermoFisher TaqPath kit [4] includes the instruction manual and contained therein (Table 6) is an interpretation algorithm describing an unequivocal requirement that two or more target genes be detected before a positive result can be declared. The latest revision of ThermoFisher's instruction manual contains the same algorithm [14]. The WHO have been sufficiently concerned about correct use of RT-PCR kits that on 20 January 2021 they issued a notice for PCR users imploring them to review manufacturer IFUs carefully and adhere to them fully [5].

The ONS's report [1] lists SARS-COV-2 positive results for valid two and three target gene combinations and does the same in [15], for samples processed by the Glasgow and Milton Keynes lighthouse laboratories. However, it also lists single gene detections as positive results (See tables 6a and 6b). Over the period reported the maximum weekly percentage of positives on a single gene is 38% for the whole of the UK for the week of 1 February. The overall UK average was 23%. The maximum percentage reported is 65%, in East England in the week beginning 5 October. In Wales it was 50%, in Northern Ireland it is 55% and in Scotland it was 56%. The full data including averages and maxima/minima are given in [17].

Professor Alan McNally, Director of the University of Birmingham Turnkey laboratory, who helped set up the Milton Keynes lighthouse laboratory, contradicted what was stated in the ONS report in a Guardian newspaper article about the new variant. He reported that all lighthouse laboratories operated a policy that adhered to the manufacturer instructions for use: requiring two-or-more genes for positive detection [6] (this policy is also documented in [16], which defines the standard operating procedure reported in [7]).

In correspondence with Mr Nicholas Lewis about single gene testing, in February 2021, the ONS confirmed that they do indeed call single gene targets as positives in their Covid-19 Infection Survey and also confirmed that the samples are processed by UK lighthouse laboratories [8], [9].

As early as April 2020, the UK lighthouse laboratories were testing for single genes and discounted the S gene as early as mid-May [10], months before the discovery of the new variant B1.1.7. Indeed, in Table 1 of [10] 18% of tests were positive on one gene only. Furthermore, in a Public Health England report on variants [2] published 8 January 2021, it states the goal of using one gene was explicitly to approximate the growth of the new B1.1.7 variant:

"There has recently been an increase in the percentage of positive cases where only the ORF1ab- and N-genes were found and a decrease in the percentage of cases with all three genes. We can use this information to approximate the growth of the new variant."

Section 10 of this ONS Covid-19 Infection Survey report [11] on 8 January 2021 confirms that one gene is sufficient for a positive result.

Obviously, there is a higher risk of encountering false positives when testing for single genes alone, because of the possibility of cross-reactivity with other human coronaviruses (HCOVs) as well as prevalent bacteria or reagent contamination. The potential for cross reactivity has already been confirmed by the German Instand laboratory report from April 2020 [12] (note that Prof. Drosten, co-author of Corman et al [2] is a cooperating partner listed in this report). The report describes the systematic blind testing of positive and negative samples anonymously sent to 463 laboratories from 36 countries and evaluated for the presence of a variety of genes associated with SARS-COV-2. They reported significant cross reactivity and resultant false positives for OC43, and HCoV 229E (a common cold virus) as well as for SARS-COV-2 negative samples, not containing any competing pathogen. Likewise, 70 Dutch laboratories were surveyed in November 2020, by the National Institute for Public Health and the Environment [13], with 76 diagnostic workflows reported as using only one target gene to diagnose the presence of SARS-COV-2 (46% of all workflows).

Without diagnostic validation it is not clear what can be concluded from a positive PCR test resulting from a single target gene call, especially if there was no confirmatory testing. Many of the reported positive results may be inconclusive, negative or from people who suffered past infection for SARS-COV-2. Even with diagnostic validation of the single target gene call, the UK lighthouse laboratories appear not to be in strict conformance with the WHO emergency use assessment and the manufacturer instructions for use. Given this it is clear the ONS and the UK lighthouse laboratories needs to publicly clarify their use of, and justify the reasons for, deviating from these standards.

References

[1] Steel K. and Fordham E. Office for National Statistics. Coronavirus (Covid-19) Infection Survey. 5 December 2020 (See tables 6a and 6b).
https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/...
[2] Public Health England "Investigation of novel SARS-COV-2 variant. Variant of concern.", 202012/01.
https://assets.publishing.service.gov.uk/government/uploads/system/uploa...
[3] Corman V., Landt O. et al "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR" Euro Surveillance. 2020 Jan;25(3):2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
[4] WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs. PUBLIC REPORT. Product: TaqPath COVID 19 CE IVD RT PCR Kit. EUL Number: EUL-0525-156-00. Page 60
https://www.who.int/diagnostics_laboratory/eual/200921_final_pqpr_eul_05...
[5] WHO Information Notice for IVD Users 2020/05. Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-CoV-2. 20 January 2021
https://www.who.int/news/item/20-01-2021-who-information-notice-for-ivd-...
[6] Alan McNally. "It's vital we act now to suppress the new coronavirus variant" Opinion section the Guardian Newspaper, 22 Dec 2020.
https://amp.theguardian.com/commentisfree/2020/dec/22/new-coronavirus-va...
[7] Richter, A., Plant, T., Kidd, M. et al. How to establish an academic SARS-CoV-2 testing laboratory. Nat Microbiol 5, 1452–1454 (2020).
https://doi.org/10.1038/s41564-020-00818-3
[8] Dr John Allen, ONS. Email correspondence to information request from Mr Nicholas Lewis, "Your ad hoc Covid-19 PCR gene detection analysis for the ONS", 22 February 2021.
[9] Zoe (?), ONS. Email correspondence to information request from Mr Nicholas Lewis, ONS, email correspondence to information request from Mr Nicholas Lewis, "ONS ad hoc Covid-19 PCR gene detection analysis", 25 February 2021.
[10] Walker S. Pritchard E et al. Viral load in community SARS-CoV-2 cases varies widely and temporally.
https://www.medrxiv.org/content/10.1101/2020.10.25.20219048v1
[11] ONS Coronavirus (COVID-19) Infection Survey, UK: 8 January 2021.
ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/coronaviruscovid19infectionsurveypilot/8january2021#the-percentage-of-those-testing-positive-who-are-compatible-for-the-new-uk-variant
[12] Zeichhardt H., and Kammel M. "Comment on the Extra ring test Group 340 SARS-Cov-2" Herausgegeben von: INSTAND Gesellschaft zur Förderung der Qualitätssicherung in medizinischen Laboratorien e.V. (INSTAND Society for the Promotion of Quality Assurance in Medical Laboratories e.V.) 3rd June 2020.
https://www.instand-ev.de/System/rv-files/340%20DE%20SARS-CoV-2%20Genom%...
[13] External Quality Assessment of laboratories Performing SARS-CoV-2 Diagnostics for the Dutch Population. National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport., November 2020.
https://www.rivm.nl/sites/default/files/2021-02/EQA%2520of%2520Laborator...
[14] TaqPath COVID-19 Combo Kit and TaqPath COVID 19 Combo Kit Advanced INSTRUCTIONS FOR USE. Revision J.0, 22 February 2021. (See Table 25, page 107). https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0019181_TaqPat...
[15] Walker, S. 21 December 2020. Covid-19 infection Survey: Ct values analysis (Glasgow and Milton Keynes identified in Table 4a)
https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/...
[16] Clinical Immunology Service, University of Birmingham. 'Competency Assessment: Reporting, Interpretation and Authorisation of Results in Turnkey Birmingham'. CIS/TK44, v1.0. September 2020.
[17] Neil M. Positive results from UK single gene testing for SARS-COV-2 may be inconclusive, negative or detecting past infections.
https://arxiv.org/abs/2102.11612

Competing interests: No competing interests

11 March 2021
Martin Neil
Professor of Computer Science and Statistics
Queen Mary University of London
Mile End Road, London

@MartinNeil9