

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

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Abstract

The UK Office for National Statistics (ONS) publish a regular infection survey that reports data on positive RT-PCR test results for SARS-COV-2 virus. This survey reports that a large proportion of positive test results are based on the detection of a single gene rather than on two or more genes as required in the manufacturer instructions for use, and by the WHO in their emergency use assessment. The proportion of positives called on single genes increased over time, suggesting a shift in testing policy around mid-November 2020 coincident with the reported significant increase in transmission of the new variant B1.1.7. Without diagnostic validation of the single gene call, for both the original and the B1.1.7 variant it can only be assumed that, in the absence of confirmatory testing, many of the reported positive results may in fact be inconclusive, negative or from people who suffered past infection for SARS-COV-2.

Background

The ONS publish a regular infection survey [1] that includes data from two UK lighthouse laboratories, based in Glasgow and Milton Keynes, 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, and the percentage of positive test results arising from using RT-PCR, and the combinations of the SARS-COV-2 virus genes tested that gave rise to positives between 21 September 2020 and 30 January 2021 across the whole of the UK.

ThermoFisher TaqPath kit¹ is used by the Glasgow and Milton Keynes lighthouse laboratories to test for the presence of three genes from SARS-COV-2². Despite Corman et al [2] originating the use of PCR testing for SARS-COV-2 genes³ there is no agreed international standard for SARS-COV-2 testing. Instead, the World Health Organisation (WHO) leaves it up to manufacturer to determine what genes to use and merely requires end users to adhere to manufacturer instructions for use (IFU). As a result of this we now have an opaque plethora of commercially available testing kits, that can be applied using a variety of test criteria. Other UK laboratories use different testing kit, and test for different genes.

The WHO's emergency use assessment (EUA) for the ThermoFisher TaqPath kit [3], used by the Glasgow and Milton Keynes lighthouse laboratories, includes the instruction manual and

¹ The full name for ThermoFisher TaqPath kit is TaqPath COVID-19 CE-IVD RT-PCR.

² N, S and ORF1ab genes

³ Corman et al recommended the E, N and RdRp genes

contained therein is an interpretation algorithm describing an unequivocal requirement that two-or-more genes be detected before a positive result can be declared. The WHO have been so concerned about correct use of RT-PCR kit that on 20 January 2021 they issued a notice for PCR users imploring them to review manufacturer IFUs carefully and adhere to them fully [4].

Increasing proportion of single gene “calls”

The ONS’s report [1] lists SARS-COV-2 positive results for valid two and three gene combinations⁴ from the Glasgow and Milton Keynes lighthouse laboratories. However, it also lists *inconclusive* single gene as positive results⁵. This use of single gene “calls” therefore suggests that Glasgow and Milton Keynes lighthouse laboratories may have breached WHO emergency use assessment (EUA) and may have violated the manufacturer instructions for use (IFU).

Over the period reported the average percentage of *positives on a single gene* is 35% for the whole of the UK. The maximum percentage reported is 81%, in London in the week beginning 21 December. In Wales it is 48%, in Northern Ireland it is 47% and in Scotland it is 49%. The full data including averages and maxima/minima are given in Table 1.

	UK	England	Wales	NI	Scotland	North East	North West	Yorkshire and Humber	East Midlands	West Midlands	East of England	London	South East	South West
21 September 2020	17	17	0	33	0	6	1	7	10	18	18	12	10	29
28 September 2020	13	13	0	0	0	8	9	6	10	14	21	21	33	20
5 October 2020	19	19	14	6	16	6	11	18	9	9	18	18	26	27
12 October 2020	15	15	16	14	21	1	9	8	14	9	15	21	14	30
19 October 2020	19	19	34	24	13	10	7	10	8	7	20	22	19	16
26 October 2020	13	15	4	25	5	5	7	10	14	8	20	19	14	20
2 November 2020	16	16	18	10	23	17	14	17	15	14	26	25	13	22
9 November 2020	21	20	25	40	29	12	10	13	13	16	33	27	21	25
16 November 2020	17	17	10	6	22	9	17	14	17	19	23	25	26	16
23 November 2020	24	23	44	7	34	26	19	16	17	13	39	39	44	35
30 November 2020	29	29	25	18	32	19	23	24	20	23	38	45	44	39
7 December 2020	27	27	21	13	31	32	31	21	26	24	55	61	51	40
14 December 2020	15	15	1	7	28	29	18	29	27	31	72	73	68	40
21 December 2020	13	13	12	0	32	56	40	32	47	57	74	81	70	37
28 December 2020	20	20	19	10	31	49	49	37	53	53	71	77	74	55
4 January 2021	17	15	22	14	35	50	58	40	40	79	80	79	79	71
11 January 2021	29	28	48	21	38	67	68	63	66	75	67	77	76	70
18 January 2021	33	32	39	38	49	73	74	71	68	64	73	74	76	71
25 January 2021	35	33	45	47	36	74	71	61	61	70	71	72	71	70
Average	21	20	21	18	25	29	28	26	28	32	44	46	44	39
Min	13	13	0	0	0	1	1	6	8	7	15	12	10	16
Max	35	33	48	47	49	74	74	71	68	79	80	81	79	71

Table 1: Proportion of weekly single gene positives from 21 September 2020 to 25 January 2021, including averages and maxima/minima.

Figure 1 shows the proportion of positives called on single genes increased over time, suggesting a shift in testing policy around mid-November, coincident with the reported

⁴ N+S+ORF, ORF+S, N+S and N+ORF gene combinations

⁵ N alone, ORF alone (note that the S gene is included in the ONS analysis but is never counted as a positive if it is detected in isolation)

significant increase in transmission of the new variant B1.1.7 in the UK [5]. However, given this new variant was concentrated in Kent and NE London, with limited spread into the rest of London, Anglia and Essex its presence cannot explain why single gene calls increased in other regions such as the North East and the West Midlands.

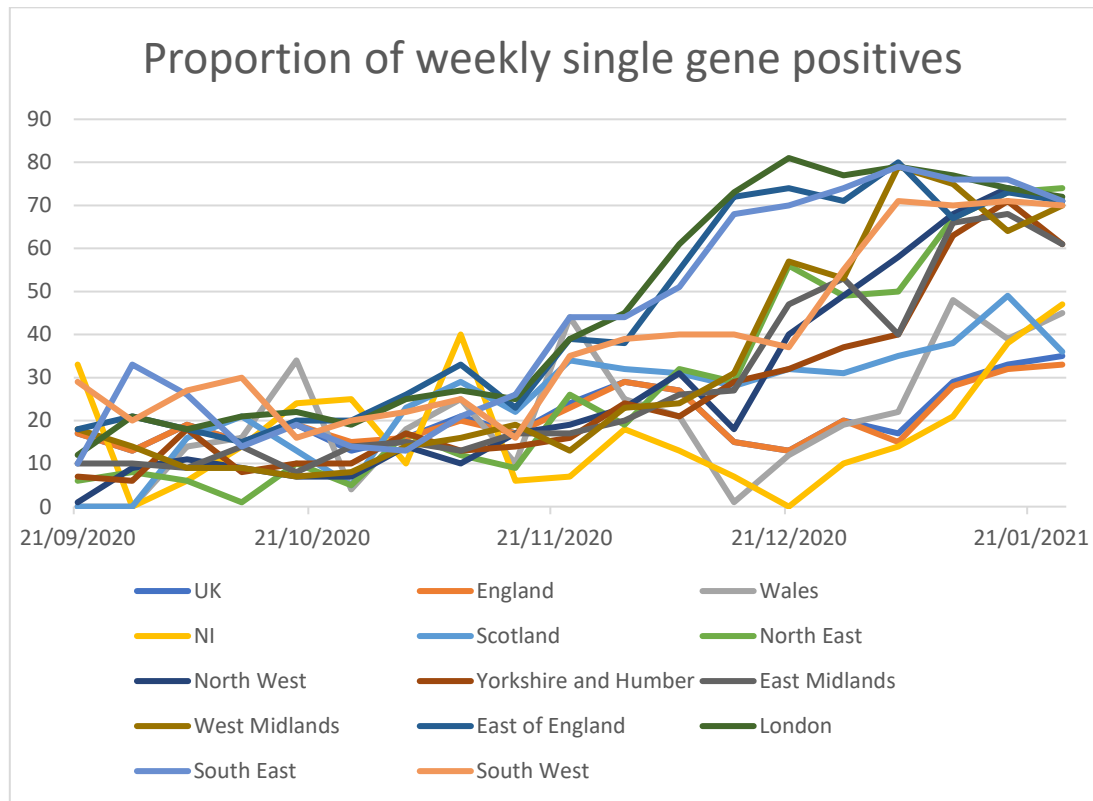


Figure 1: Proportion of weekly single gene positives from 21 September 2020 to 25 January 2021

In startling contradiction to the ONS report Professor Alan McNally, Director of the University of Birmingham Turnkey laboratory, who helped set up the Milton Keynes lighthouse laboratory, reported in the Guardian newspaper, in an article about the new variant, 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 the supplementary material provided in [7]).

Quality control and cross reactivity

Quality control problems have already been reported in UK laboratories [8, 9, 10] and there have been concerns expressed about the potential for false positives arising consequently. Recent suspicion focused on problems potentially caused by breaches in acceptable Ct thresholds ($Ct > 37$), suggesting no, or past, infection. However, this new ONS data shows that an additional potential source of false positives may actually be dominant, at least within the period covered by the ONS report, if not beyond; specifically, positives caused by potential breach of WHO end user assessment and manufacturer instructions for use.

Concerns about testing in commercial laboratories were documented by the ONS as early as May 2020 [11], when the REACT study discovered that circa 40% of positive tests from commercial laboratories were in fact false positive. A similar false positive rate (44%) was

reported in Australia [12] in April 2020. More recently Nicholas Lewis claims that, despite very low false positive rates (0.033%) from testing done by non-commercial and academic laboratories, there may be good reason to suspect the operational false positive rates from lighthouse laboratories are worse than these by some orders of magnitude [13].

Obviously, there is higher risk of encountering false positives when testing for single genes alone, because of the possibility of cross-reactivity with other HCOVs and prevalent nasopharyngeal bacteria or reagent contamination. The potential for cross reactivity when testing for SARS-COV-2 has already been confirmed by the German Instand laboratory report from April 2020 [14]. This report describes the systematic blind testing of positive and negative samples anonymously sent to many laboratories throughout Germany and evaluated for the presence of a variety of genes associated with SARS-COV-2⁶. 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 [15], by the National Institute for Public Health and the Environment, with 76 diagnostic workflows reported as using only one target gene to diagnose the presence of SARS-COV-2 (46% of all workflows).

Conclusions

Unless the UK lighthouse laboratories have performed diagnostic validation of their single gene call, for both the original and the B.1.1.7 variant, and there is no evidence of this in the public domain, it can only be assumed that, in the absence of confirmatory testing, many of the reported positive results may in fact be inconclusive, negative or from people who suffered past infection for SARS-COV-2. And even with diagnostic validation of the single gene call the UK lighthouse laboratories appear to be in breach of both the WHO emergency use assessment and, also to have potentially violated the ThermoFisher TaqPath kit instructions for use.

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