

1 Ct threshold values, a proxy for viral load in community SARS-CoV-2 cases, demonstrate wide
2 variation across populations and over time

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43

44 **ABSTRACT** (151 words)

45 Information on SARS-CoV-2 in representative community surveillance is limited, particularly cycle
 46 threshold (Ct) values (a proxy for viral load). Of 3,312,159 nose and throat swabs taken 26-April-2020
 47 to 13-March-2021 in the UK's national COVID-19 Infection Survey, 27,902(0.83%) were RT-PCR-
 48 positive, 10,317(37%), 11,012(40%) and 6,550(23%) for 3, 2 or 1 of the N, S and ORF1ab genes
 49 respectively, with median Ct=29.2 (~215 copies/ml; IQR Ct=21.9-32.8, 14-56,400 copies/ml).
 50 Independent predictors of lower Cts (i.e. higher viral load) included self-reported symptoms and
 51 more genes detected, with at most small effects of sex, ethnicity and age. Single-gene positives
 52 almost invariably had Ct>30, but Cts varied widely in triple-gene positives, including without
 53 symptoms. Population-level Cts changed over time, with declining Ct preceding increasing SARS-CoV-
 54 2 positivity. Of 6,189 participants with IgG S-antibody tests post-first RT-PCR-positive, 4,808(78%)
 55 were ever antibody-positive; Cts were significantly higher in those remaining antibody-negative.
 56 Community SARS-CoV-2 Ct values could be a useful epidemiological early-warning indicator.

57
 58 **IMPACT STATEMENT**

59 Ct values from SARS-CoV-2 RT-PCR tests vary widely and over calendar time. They have the potential
 60 to be used more broadly in public testing programmes as an “early-warning” system for shifts in
 61 infectious load and hence transmission.

62 INTRODUCTION

63 After initial reductions in SARS-CoV-2 cases in mid-2020, following release of large-scale lockdowns
 64 (Flaxman et al., 2020), infection rates have undergone waves of resurgence and suppression in many
 65 countries worldwide. Proposed control strategies include new local or national lockdowns of varying
 66 intensity and mass testing, but these have major economic and practical limitations. In particular,
 67 mass testing of large numbers without symptoms (Yokota et al., 2020), and hence low pre-test
 68 probability of positivity, can mean most positives are false-positives depending on test specificity. For
 69 example, with 0.1% true prevalence, testing 100,000 individuals with a 99.9% specific test with
 70 perfect sensitivity gives 100 true-positives, but also 100 false-positives (positive predictive value
 71 (PPV) 50%), whereas specificity of 99.5% increases false-positives to 500 (PPV=17%), and of 99.0% to
 72 999 (PPV=9%), with even lower PPV with imperfect sensitivity (Adams, Ainsworth, Anand, & et al.,
 73 2020).

74
 75 Mathematical models are powerful tools for evaluating the potential effectiveness of different
 76 control strategies, but rely on population-level estimates of infectivity and other parameters.
 77 However, there are few unbiased community-based surveillance studies, including individuals both
 78 with and without symptoms. Estimates of asymptomatic infection rates vary, being 17-41% overall in
 79 recent reviews (Buitrago-Garcia et al., 2020; Byambasuren et al., 2020), but these included many
 80 studies of contacts of confirmed cases. Higher prevalence of asymptomatic infection has been
 81 reported in screening of defined populations (30% (Buitrago-Garcia et al., 2020)) and community
 82 surveillance (e.g. 42% (Lavezzo et al., 2020), 72% (Riley, Ainslie, Eales, Walters, Wang, Atchison,
 83 Fronterre, et al., 2020)). Studies have generally indicated lower rates of transmission from
 84 asymptomatic infection (Buitrago-Garcia et al., 2020; Byambasuren et al., 2020); this may be a proxy
 85 for SARS-CoV-2 viral load as a key determinant of transmission. Finally, most studies rely on
 86 “average” estimates of the asymptomatic infection percentage, independent of characteristics and

87 viral load, and have not quantified temporal variation in these key parameters for mathematical
88 models across the community.

89

90 Here we therefore characterise variation in SARS-CoV-2 positive tests in the first eleven months of
91 the UK's national COVID-19 Infection Survey. In brief (details in Methods), the survey randomly
92 selects private households to provide a representative UK sample, recruiting all consenting
93 individuals aged 2 years or older currently resident in each household to provide information on
94 demographics, symptoms, contacts and relevant behaviours and self-taken nose and throat swabs
95 for RT-PCR testing (Pouwels et al., 2020). A randomly selected subset are approached for additional
96 consent to provide blood samples for IgG S-antibody testing if aged 16 years or older. At the first
97 visit, participants can provide additional consent for longitudinal follow-up (visits every week for the
98 next month, then monthly for 12 months from enrolment). We estimate predictors of RT-PCR cycle
99 threshold (Ct) values (as a proxy for viral load), propose a classification for the strength of evidence
100 supporting positive RT-PCR test results in the community, and demonstrate how this has changed
101 over time. We also provide a preliminary assessment of seroconversion rates for community cases.

102

103 **RESULTS**

104 *Number and percentage of positive swabs*

105 From 26 April 2020 to 13 March 2021, 440,479 participants from 217,887 households in the COVID-
106 19 Infection Survey had one or more RT-PCR results from nose and throat swabs (median 8 results
107 per participant (IQR 6-9, range 1-19)). Participants were recruited between April 2020 and March
108 2021 (**Supplementary File 1**). Of 3,312,159 RT-PCR test results, 27,902 (0.84%, 95% CI 0.83-0.85%)
109 were positive, in 21,831 individuals from 16,214 households. 2,966 (14%) of these individuals were
110 positive at their first test in the study and 18,865 (86%) subsequently, after median 5 negative tests
111 (IQR 3-6, range 1-14).

112

Viral characteristics

Overall, 10,317 (37%), 11,012 (40%) and 6,550 (23%) swabs were positive for three, two or one of the three SARS-CoV-2 genes (N protein, S protein and ORF1ab) respectively (**Table 1**; 23 positives with missing Ct and gene detection excluded from this and all subsequent analysis; samples with only the S-gene detected generally not called positive, see Methods). The majority of two-gene positives (9,513 (86%)) were ORF1ab+N positive from 16 November 2020 onwards, reflecting the emergence and expansion of B.1.1.7 in the UK (Walker et al., 2021). B.1.1.7 leads to S-gene target failure (SGTF) and was estimated to account for 88% of SGTF from this time (Public Health England, 2020). Where multiple genes were detected, the Cts were highly correlated (Spearman $\rho=0.98$, $p<0.0001$). Taking the per-swab mean Ct across positive genes, the overall median Ct was 29.2 (IQR 21.9-32.8; range 9.2-38.7), reflecting the study's surveillance design testing individuals in the community at fixed timepoints regardless of symptoms. Based on calibration data (**Supplementary Figure 1**), this corresponds to a median viral load of ~215 copies/ml (IQR 14-56,400). Ct varied strongly by number of genes detected (Kruskal-Wallis $p=0.0001$), but not by their specific pattern after adjusting for number ($p=0.08$). There is no fixed Ct threshold for determining positivity (see Methods); however, only 38 (0.1%) Ct values >37 were recorded (5 positive on ORF1ab+N).

Of note, whilst single-gene positives almost invariably had Ct>30, with or without reported symptoms, triple-gene positives without reported symptoms had widely varying Ct, as did ORF1ab+N positives after 16 November 2020 (SGTF, compatible with B.1.1.7) (**Figure 1**). Ct values were slightly but significantly lower in other double-gene positives vs single-gene positives, with a small number of low Ct values in ORF1ab+N positives before 16 November likely reflecting early B.1.1.7 cases. Further, whilst the percentage reporting symptoms increased linearly as Ct values dropped from 35 (~30% reporting symptoms around the positive test) to 28 (~60% reporting symptoms), below 28 the percentages reporting symptoms increased only slightly (to ~70% at Ct=10) (**Figure 2**).

Evidence supporting positive results

Combining information on Ct values, symptoms and pre-test probability of being positive, 21,329 (77%), 4,741 (17%) and 1,809 (6%) positive tests had “higher”, “moderate” or “lower” evidence supporting genuine presence of viral RNA (**Table 2**; definitions in Methods). Even though “higher” evidence was based only on number of genes detected (two or three), “higher” evidence positives were more likely to be symptomatic than “moderate” evidence positives ($p < 0.0001$), but were similarly likely to have occupational risk factors ($p = 0.48$). “Higher” evidence positives were more likely to occur in households with other positives ($p < 0.0001$).

Predictors of Ct values

In multivariable regression models, Ct values were independently lower (i.e. viral loads higher) with more genes detected (8.2 lower in triple-gene vs single-gene positives (95% CI 7.9-8.5)), if symptoms were reported around the test (2.0 lower (1.8-2.2)), and at the first positive identified per-participant (2.2 lower than subsequent positives (2.2-2.5)), and if the positive was not the participant’s first test in the study (0.6 lower (0.2-0.9)) (all $p < 0.0001$; **Supplementary File 2A**; see Methods for details of collection of symptoms). By far the strongest effect was associated with triple-gene positives. Men had slightly lower Ct values than women (0.3 lower (0.1-0.5) $p = 0.001$), and there was marginal evidence of lower values in those reporting non-white ethnicity (0.3 lower (0-0.6) $p = 0.08$). Compared with those not reporting symptoms, Ct values were lower in those reporting cough/fever/anosmia (2.5 lower (2.3-2.8)) than other symptoms only (0.9 lower (0.7-1.2); heterogeneity $p < 0.0001$). Associations were similar for symptoms at the positive test. After adjusting for these factors, there was no evidence of independent effects of age ($p = 0.33$) or deprivation ($p = 0.67$, **Supplementary File 2A**). Even after adjusting for these factors, Ct values were 1.4 (1.2-1.6) lower in individuals where another household member was positive at any point in the study ($p < 0.0001$; other effects similar).

However, number of genes detected and symptoms are both potential mediators of effects of demographic factors (**Supplementary Figure 2**). Excluding these potential mediators (number of genes detected, symptoms), Ct values remained independently lower (i.e. viral loads higher) at the first positive identified per-participant, where the positive was not the participant's first test in the study, and in men, but were also slightly lower with increasing deprivation ($p=0.0005$; Ct 1.0 lower in the most vs least deprived (95% CI 0.6-1.5)) and in younger adults ($p=0.0001$; those aged 17-24 1.0 lower (0.3-1.7) than those under 12, and 1.4 lower (0.8-2.0) than those aged 70+) (**Supplementary File 2B**). Results were similar adjusting for date of the positive test.

Temporal changes in Ct values, evidence and symptomatic percentages

There were strong effects of calendar time on the distribution of Ct values (**Figures 3A&B**), the percentages self-reporting symptoms, or cough/fever/anosmia (**Figure 3C**), and strength of evidence supporting each positive result (**Figure 3D**; all $p<0.0001$). In particular, Ct values were markedly higher in July-August 2020 when population positivity rates were low, with correspondingly very low percentages with symptoms at/around positive tests, and more "lower" evidence positives. Decreases in Ct values in late August/early September and December 2020 coincided with increases in percentages reporting symptoms and of "higher" evidence positives, and, in England (**Figure 3B**), with initial rises in official estimates of positivity rates (Office for National Statistics, 2021) after very low rates in July/early August 2020, and with much stronger rises in December 2020 (expansion of B.1.1.7). Ct levels rose, and correspondingly percentages reporting symptoms and of "higher" evidence positives declined, as positivity peaked during November 2020 and January 2021 lockdowns.

However, even within "higher" evidence positives, median Ct varied strongly over time being higher in July/early August 2020 and after November 2020 and January 2021 lockdowns (**Figure 4A**). "Lower" evidence positives also formed a larger percentage of all tests during July/early August 2020,

despite overall positivity rates being very low (e.g. 0.022% in the 3 weeks starting 20 July 2020; **Figure 4B**). However, interestingly, from September 2020, the percentage of “lower” evidence positives increased proportionately with “moderate” and “higher” evidence positives (**Figure 4B**). The lowest non-zero observed rate of “low evidence” positives was 0.005% (both in early June and late August), providing an upper bound on the rate of false-positives as defined by identifying virus when none present.

Relationship with serostatus

One or more IgG S-antibody results were available for 6,540 (30%) participants with positive swabs. Less than 5% of antibody tests taken >30 days before the first positive swab (not necessarily the onset of infection) were positive (**Figure 5**), rising to 12% in the 30 days before the first swab positive (likely reflecting late detection of infection), 47% in the following 14 days and then 72-81% thereafter. Overall, of 6,189 participants with one or more antibody tests after their first positive swab, 4,808 (78%) were ever antibody-positive; with higher rates in those reporting symptoms around their first positive swab (2,945/3,315 (89%) vs 1,863/2,874 (65%) of those not reporting symptoms, $p < 0.0001$). Median (IQR) Ct values were also significantly lower in those ever antibody-positive to date (24.9 (18.5-31.0) vs 33.0 (29.9-34.3) in those not antibody-positive, $p < 0.0001$). Results were similar restricting to 1,477 (24%) with a negative antibody result within [-120,+21] days of their first positive swab. A small number of participants appeared to have become infected despite antecedent high anti-spike antibody titres, one case in particular which had “higher evidence” positive swab tests separated by four consecutive negative swabs with 65 days between positive swabs.

DISCUSSION

In this large community surveillance study, we found wide variation in Ct values (a proxy for viral load). Whilst Ct values were independently associated with several factors, including symptoms

at/around the test as previously reported (Edwards et al., 2020; S. Lee et al., 2020), their effects were small compared with population-level variability. Notably both triple-gene positives and S-gene target failures compatible with the B.1.1.7 variant without reported symptoms had widely varying Ct, including many with low values (**Figure 3A**), potentially explaining variation in dispersion (“k”) and super-spreading events, particularly from those without symptoms but with low Ct/high viral loads (Endo, Centre for the Mathematical Modelling of Infectious Diseases, Abbott, Kucharski, & Funk, 2020; Rasmussen & Popescu, 2021).

Compared with other single/double positives, Ct values were significantly lower in triple-gene positives and S-gene target failures compatible with the B.1.1.7 variant (after mid-November 2020). However, direct comparisons of viral load with B.1.1.7 vs other variants are not possible within this analysis, given lack of knowledge as to the true underlying variant over the included time period. We found lower Ct in those reporting cough/fever/anosmia/ageusia than other symptoms, and other symptoms vs no symptoms, supporting the importance of the “classic” symptoms for identifying the most infectious cases. Lower Ct values in the first positive per-participant likely reflects the natural history of viral load post-infection, and higher Ct values in those positive at their first test in the study over-representation of long-term shedders in this group. Lower Ct values in men and those reporting non-white ethnicity, although small, are consistent with poorer outcomes in these groups. Interestingly, small effects of age and deprivation were mediated by self-reported symptoms and number of positive genes. That is, when adjusting for these latter factors no association was observed with Ct, but without adjustment younger individuals (as shown in (Jones et al., 2020) but not (Jacot, Greub, Jaton, & Opota, 2020)) and those from more deprived areas had slightly lower Ct values, suggesting that these factors may affect the intrinsic level of virus present (**Supplementary Figure 2**). The small size of the effects mean they may variably be detected depending on study size and power.

242 Ct values varied strongly over time, as did symptoms and evidence supporting positives, suggesting
 243 changing viral burden in infection cases, with less severe infections during July/early August 2020.
 244 This strongly refutes hypotheses that declines in positivity during this period were due to declines in
 245 viral fitness. During this time, higher Ct values were also noted in the English point-prevalence
 246 surveillance study, REACT (Riley, Ainslie, Eales, Walters, Wang, Atchison, Fronterre, et al., 2020), and
 247 lower virus levels in Lausanne, Switzerland (Jacot et al., 2020). However, Ct values were higher even
 248 in “higher” evidence positives during this period, consistent with shifting viral burden (**Figure 4A**).
 249 Such a shift may also explain the preceding shift towards “moderate” evidence positives and the
 250 concurrent higher percentage of “lower” evidence positives, since the less virus present, the less
 251 likely it is to be detected on multiple genes. Whilst these findings are consistent with lower viral
 252 inoculum during this period (Gandhi, Beyrer, & Goosby, 2020), we cannot assess whether this is
 253 predominantly due to behaviour (e.g. increased time outdoors, face mask use (Gandhi & Rutherford,
 254 2020)) or other reasons (e.g. environmental/climatic factors, including relating to transport of swabs
 255 for testing). Whilst decreases in Ct values in July/early August 2020 preceded increases in positivity
 256 rates in England, later declines in Ct in early December coincided with, rather than preceded,
 257 increases in positivity due to B.1.1.7 expansion. This may potentially reflect faster transmission of
 258 B.1.1.7 but may also reflect greater sensitivity to changes in Ct distribution when case numbers are
 259 small. Subsequent increases in Ct reflected stabilising and then declining positivity in both periods.
 260
 261 We used laboratory, clinical and demographic evidence to classify our confidence in positive results.
 262 Around 70% had 2 or 3 genes detected (“higher” evidence), providing assurance in overall results,
 263 with only 0.1% of Ct values over 37. Whilst Ct values are not directly comparable between studies,
 264 REACT has also validated a Ct threshold of 37 for single-gene positives for their test performed in
 265 Germany (Riley, Ainslie, Eales, Walters, Wang, Atchison, Diggle, et al., 2020), and in the Public Health
 266 England (PHE) Schools study, only samples with Ct<37 were positive on repeat testing of the same
 267 swab at PHE laboratories (Ladhani et al., 2020). However, every diagnostic test has false-positives,

here defining a false-positive as detection of virus by RT-PCR when no virus is present in a sample, so some of our single gene “lower”, or even “moderate”, evidence positives are inevitably false. However, the false-positive rate (as defined) would generally be expected to be approximately constant over time, since it is either random or driven by external factors, although cross-contamination (which should be minimised by good laboratory practice) may theoretically be related to background prevalence/viral load. Variation in the percentage of all tests accounted for by “lower” evidence positives, and in particular the proportionate increases in “lower” evidence positives as “higher” evidence positives increased during September 2020 supports more genuinely lower-level infections occurring during the summer, and an overall false-positive rate for this test of below ~0.005% i.e. at least 99.995% specificity.

With recent expansion of antigen assays, there has been considerable debate on what “positivity” means, and hence what is a “false-positive” or a “false-negative”. First, it is clear that the detection of viral RNA is neither the same as infectiousness, although a strong relationship between Ct values and infection in contacts is observed (L. Y. W. Lee et al., 2021), nor a “disease” in its own right. However, surveillance has very distinct goals from clinical testing with its focus on isolation and contact tracing, particularly given the large percentage of asymptomatic infections. It is appropriate for surveillance to focus on detection of viral RNA, given its goal to estimate burden of current/ongoing cases that have occurred in the community. However, it is essential to recognise the difference between the RT-PCR test result (viral RNA has been detected) and the appropriate clinical action, which may legitimately differ depending on Ct value, for example if the infection is likely to have occurred sometime previously, as well as other information (e.g. preceding PCR positivity or serology). RT-PCR assays test for viral RNA presence, and hence it is more relevant to consider limits of detection, rather than “false-positives” per se. Although they were a small minority (6%), one question is whether single-gene positives with high Ct (defined as ≥ 34 in our study) solely represent long-term shedding of non-transmissible virus (Moraz et al., 2020), with, for example, infectious virus

recovered from only 8% (95% CI 3-18%) of samples with Ct>35 in a PHE study (Singanayagam et al., 2020) and studies reporting no growth of virus for Ct thresholds from >24 to >34 or higher (Jefferson, Spencer, Brassey, & Heneghan, 2020). Whilst we have not directly assessed household transmission in this analysis, it was notable that Ct values were significantly lower in positives where anyone else in the same household was ever positive, supporting a role for greater within-household transmission with lower Ct values. Ct values were 0.6 higher in positives that were a participant's first study test (where long-term shedders would be expected to be overrepresented), but these formed only 14% of the positives.

Our evaluation of serological responses is one of few in the community to our knowledge, and highlights that a significant minority (~20%) of RT-PCR-positive cases do not appear to seroconvert, particularly those with higher Ct values and not reporting symptoms. A recent systematic review estimated that 95% of adults with laboratory confirmed SARS-CoV-2 infection developed IgG antibodies (Arkhipova-Jenkins et al., 2021), peaking around 25 days. However, only 23% of included studies were in outpatient settings and 14% included only participants with asymptomatic or mild disease. Our community setting, with higher percentages not reporting symptoms and higher Ct values (both associated with seroconversion), likely explains our lower overall seroconversion estimate compared with these previous studies. We observed a small number of new swab positives in antibody-positive individuals: unfortunately whole genome sequence data were not available to confirm potential re-infections. Presumed re-infections have been reported elsewhere (Tomassini et al., 2020), including in individuals without previous functional and/or durable antibody responses (Goldman et al., 2020; To et al., 2020), and may remain relevant to virus transmission, whether they occur with or without symptoms. Our data and others (Lumley et al., 2021) suggest that these may occur in the presence of anti-spike antibodies, which correlate with neutralising antibody titres. These antibody titres are unlikely to have been false-positives, given the context, persistence, and

known diagnostic and analytical specificity of the assay (National Sars-CoV-Serology Assay Evaluation Group, 2020), or to all reflect laboratory identifier errors, and further analyses are ongoing.

A major study strength is its design, namely being a large-scale community survey recruiting randomly selected private residential households, and testing participants regardless of symptoms. However, its size and scale is also a limitation, since we were not able to collect additional data to comprehensively characterise individual positives. We may have underestimated the initial prevalence of symptoms due to originally asking about current symptoms before July 2020 (subsequently symptoms in the 7 days preceding the visit). As this was only at the earliest visits, mostly weekly, only very transient symptoms between visits would likely have been missed. Similar rates of symptom reporting in the first and last parts of the period analysed suggests that this question was likely generously interpreted in any case. We made no attempt to collect additional information on symptoms after positives were identified to minimise recall bias. This may partly explain why we observed higher rates of positive tests without reported symptoms than recent reviews (Buitrago-Garcia et al., 2020; Byambasuren et al., 2020); however, many studies in these reviews tested close contacts of index cases identified through symptoms and therefore might plausibly have higher viral loads. We compared distributions of Ct values to overall positivity rates in England, since these are the longest series of official statistics available; overall UK positivity estimates are not produced because the 4 countries making up the UK have different policies and timings regarding community restrictions including lockdowns.

Ultimately the importance of asymptomatic and low virus level infections depends on their transmissibility and their prevalence; regardless of limitations in symptom ascertainment, infection without recognition has the potential for onward transmission and unascertained infections are likely critical for avoiding resurgence after lifting lockdown (Hao et al., 2020). Our findings support the use of Ct values and genes detected more broadly in public testing programmes, predominantly testing

symptomatic individuals and case contacts, as an “early warning” system for shifts in potential infectious load and hence transmission, and hence the risks posed by individuals to others. This has recently also been proposed on the basis of theoretical work linking effective reproduction numbers to population level Ct (Hay, Kennedy-Shaffer, Kanjilal, Lipsitch, & Mina, 2020). In our study, declines in mean and median Ct values preceded or at least coincided with increases in office estimates of positivity rates (**Figure 3B**); given the far larger numbers that would be available in testing programmes, future research should investigate whether the greater power afforded by continuous outcomes could lead to significantly earlier detection of future positivity increases, particularly within small geographical areas. Ct data are widely available within laboratory management systems; providing comparisons across the wide variety of commercial assays were interpreted carefully, they could be used alongside available risk factor and symptom information to facilitate more informed and effective individual-level and public health responses to the SARS-CoV-2 pandemic.

MATERIALS AND METHODS

This study included all positive SARS-CoV-2 RT-PCR results between 26 April 2020 and 13 March 2021 from nose and throat swabs taken from participants in the Office for National Statistics (ONS) CIS (ISRCTN21086382). The survey randomly selects private households on a continuous basis from address lists and from previous surveys to provide a representative UK sample (**Supplementary File 1**). If anyone aged 2 years or older currently resident in an invited household agreed verbally to participate, a study worker visited the household to take written informed consent, which was obtained from parents/carers for those 2-15 years; those aged 10-15 years provided written assent. The study protocol is available at <https://www.ndm.ox.ac.uk/covid-19/covid-19-infection-survey/protocol-and-information-sheets>. Recruitment started 26 April 2020 in England, 29 June 2020 in Wales, 29 July 2020 in Northern Ireland and 21 September 2020 in Scotland.

370 Individuals were asked about demographics, symptoms, contacts and relevant behaviours
371 (<https://www.ndm.ox.ac.uk/covid-19/covid-19-infection-survey/case-record-forms>). To reduce
372 transmission risks, self-taken nose and throat swabs were obtained following study worker
373 instructions. Parents/carers took swabs from children under 12 years. At the first visit, participants
374 were asked for (optional) consent for follow-up visits every week for the next month, then monthly
375 for 12 months from enrolment. In a random 10-20% households, those 16 years or older were invited
376 to provide venous blood monthly for assays of anti-trimeric spike protein IgG using an immunoassay
377 developed by the University of Oxford (National Sars-CoV-Serology Assay Evaluation Group, 2020).
378 All participants in households where anyone tested positive on a swab were also invited to provide
379 blood monthly. Venous blood was not taken at any visit where any person in the household had
380 classic COVID-19 symptoms (fever, cough or anosmia). The study received ethical approval from the
381 South Central Berkshire B Research Ethics Committee (20/SC/0195).
382
383 Swabs and blood samples were collected by study workers at household visits and couriered
384 overnight to testing laboratories at ambient temperatures. They were analysed at the UK's national
385 Lighthouse Laboratories at Milton Keynes (National Biocentre) (from 26 April 2020 to 11 February
386 2021) and Glasgow (from 16 August 2020) using identical methodology, with swabs from specific
387 regions sent consistently to one laboratory. RT-PCR for three SARS-CoV-2 genes (N protein, S protein
388 and ORF1ab) used the Thermo Fisher TaqPath RT-PCR COVID-19 Kit, analysed using UgenTec Fast
389 Finder 3.300.5 (TaqMan 2019-nCoV Assay Kit V2 UK NHS ABI 7500 v2.1). The Assay Plugin contains an
390 Assay specific algorithm and decision mechanism that allows conversion of the qualitative
391 amplification Assay PCR raw data from the ABI 7500 Fast into test results with minimal manual
392 intervention. Samples are called positive in the presence of at least single N gene and/or ORF1ab but
393 may be accompanied with S gene (1, 2 or 3 gene positives). There is no specific Ct threshold for
394 determining positivity. S gene is not considered a reliable single gene positive (as of mid-May 2020).
395 Blood was analysed at the University of Oxford. Antibody titres were considered positive above 8

million units (National Sars-CoV-Serology Assay Evaluation Group, 2020) on the original fluorometric version of the assay and 42 units on the colorimetric version of the assay (used from 1 March 2021).

Twelve specific symptoms were elicited at each visit (cough, fever, myalgia, fatigue, sore throat, shortness of breath, headache, nausea, abdominal pain, diarrhoea, loss of taste, loss of smell), as was whether participants thought they had (unspecified) symptoms compatible with COVID-19. From 26 April through 22 July 2020, questions referred to current symptoms, and from 23 July 2020 to the preceding 7 days. Any positive response to any symptom question at the swab-positive visit defined the case as symptomatic “at” the test; we also separately defined any positive response at the swab-positive visit or visits either side (regardless of time between visits) as symptomatic “around” the test.

To investigate the potential increasing contribution of false-positives as population prevalence declines, from 2 August 2020 we arbitrarily classified in real-time each positive as:

- “Higher” evidence: two or three genes detected (irrespective of Ct).
- “Moderate” evidence: single-gene detected and (i) Ct below the 97.5th percentile of “higher” evidence positives (<34; supporting this threshold, whole genome sequences had been obtained from three single gene positives with Ct 30.8-33.1 by 2 August) or (ii) higher pre-test probability of infection, defined as any symptoms at/around the test or reporting working in a patient-facing healthcare or care/residential home.
- “Lower” evidence: all other positives; by definition single-gene detected at Ct≥34 in individuals not reporting symptoms/working in relevant roles.

As the Ct distribution was skewed to the left, we assessed independent predictors using median (quantile) regression. Results were broadly similar using random effects model for mean Ct with a random effect per household. We used 5 knot natural cubic splines (knots at the

422 10th/25th/50th/75th/90th percentiles of observed unique values) to assess non-linearity in the effect of
 423 calendar time, age and deprivation (index of multiple deprivation rank). Multivariable models for Ct
 424 values were constructed by first choosing the more strongly univariably predictive factor from the
 425 collinear variables (symptoms at/around the test, number of genes detected/supporting evidence for
 426 each positive) then using backwards elimination on the remaining variables. Deprivation was
 427 assessed using the index of multiple deprivation (IMD) in England, a score based on lower layer super
 428 output areas with average population of 1500 people and incorporating seven domains to produce
 429 an overall relative measure of deprivation (income, employment, education, skills and training,
 430 health and disability, crime, barriers to housing services and living environment)
 431 (<https://www.gov.uk/government/statistics/english-indices-of-deprivation-2019>) and equivalent
 432 scores in the other three countries comprising the UK. Each country's scores were converted to a
 433 within country percentile. All analyses were conducted in Stata 16.1.

434

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478 **COMPETING INTEREST**

479 DWE declares lecture fees from Gilead, outside the submitted work. No other author has a conflict of
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481

482 **DATA AVAILABILITY**

483 De-identified study data are available for access by accredited researchers in the ONS Secure
484 Research Service (SRS) for [accredited research purposes](#) under part 5, chapter 5 of the Digital
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486 Research.Support@ons.gov.uk or visit the [SRS website](#).

487

488 **CONTRIBUTIONS**

489 The study was designed and planned by ASW, JF, JB, JN, IB, ID, KBP and JVR, and is being conducted
 490 by ASW, IB and RS. This specific analysis was designed by ASW, KBP, DWE, PCM, NS and TEAP. ASW,
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605
606

607 **Table 1 Genes detected in positive swabs**

Number of genes detected	All positives (N=27,879)		First positive per participant (N=21,811)	
	n (%)	Median CT* (IQR) [range]	n (%)	Median CT* (IQR) [range]
1	6,550 (23%)	33.8 (32.9-34.7) [12.7-38.7]	5,102 (23%)	33.9 (32.9-34.7) [12.7-38.7]
2	1,145 (4%)	32.3 (30.9-33.4) [10.3-37.2]	773 (4%)	32.3 (30.7-33.4) [10.3-37.2]
2: ORF1ab+N 16 Nov 2020 onwards	9,867 (35%)	26.4 (19.4-31.1) [9.2-37.8]	8,184 (38%)	25.3 (18.6-30.7) [9.2-37.8]
3	10,317 (37%)	25.3 (19.8-29.5) [9.3-36.8]	7,752 (36%)	23.9 (18.8-28.8) [9.3-36.8]
Genes detected				
N only	4,479 (13%)	33.9 (33.0-34.8) [26.1-38.7]	3,419 (16%)	34.0 (33.1-34.8) [28.2-38.7]
ORF1ab only	2,044 (7%)	33.6 (32.6-34.5) [16.8-38.3]	1,656 (8%)	33.7 (32.7-34.6) [16.8-38.3]
S only**	27 (0.1%)	34.9 (33.5-36.1) [12.7-37.3]	27 (0.1%)	34.9 (33.5-36.1) [12.7-37.3]
N+ORF1ab: before 16 Nov 2020	731 (3%)	31.9 (30.3-32.9) [10.3-37.2]	497 (2%)	31.8 (29.7-33.0) [10.3-38.2]
N+ORF1ab: 16 Nov 2020 onwards	9,867 (35%)	26.4 (19.4-31.1) [9.2-37.8]	8,184 (38%)	23.9 (18.8-28.8) [9.3-36.8]
S+ORF1ab	190 (0.7%)	32.5 (31.2-33.5) [15.1-36.6]	138 (0.6%)	32.4 (31.0-33.6) [15.1-36.6]
N+S	224 (0.8%)	33.4 (32.5-34.2) [25.0-36.8]	138 (0.6%)	33.3 (32.4-34.3) [27.3-36.8]
N+S+ORF1ab	10,317 (37%)	25.3 (19.8-29.5) [9.3-36.8]	7,752 (36%)	25.3 (18.6-30.7) [9.2-37.8]

608 * taking the mean CT per positive swab across positive gene targets (Spearman rho=0.98 for each pair of genes where both positive, p<0.0001)

609 ** 17/27 before mid-May only: after this samples positive for the S gene only were not called positive overall by the algorithm and therefore reflect likely
610 recording errors.

611 Note: excluding 23 positive results without Ct values or genes detected available. Comparing first vs subsequent positives per participant, exact p<0.0001 for
612 both number of genes detected and specific genes detected.

613

614 **Table 2 Evidence supporting positive test results indicating presence of virus and impact on other**
615 **factors**

	Strength of evidence for true infection			p (exact)
	Higher	Moderate	Lower	
Number (col %) (N=27,879)	21,329 (77%)	4,741 (17%)	1,809 (6%)	
Factors determining classification				
Number of genes detected (row %)	3: 10,317 (48%) 2: 11,012 (52%)	1: 4,741 (100%)	1: 1,809 (100%)	
CT, median	26.2	33.4	34.8	
CT, n (row %) <34*	21,070 (98.8%)	3,613 (76%)	0 (0%)	
Symptoms around test, n (row %)	12,466 (58%)	2,243 (47%)	0 (0%)	<0.0001 (exc lower)
Occupational risk**, n (row %)	1,322 (6%)	307 (6%)	0 (0%)	0.48 (exc lower)
Other factors				
Cough, fever, anosmia, ageusia around test, n (row %)	9,345 (44%)	1,241 (26%)	0 (0%)	<0.0001 (exc lower)
First positive test n (row %) (vs subsequent positive test)	16,709 (78%)	3,508 (74%)	1,594 (88%)	<0.0001
First test in study, n (row %) (vs follow-up ie prior negative in study)	2,281 (11%)	482 (10%)	199 (11%)	0.49
Any genome sequence obtained, confirming presence of virus†	6,621/9,022 (73%)	544/2,315 (24%)	0/836 (0%)	<0.0001
Any other household member ever positive‡	11,493/18,494 (62%)	1,513/4,004 (38%)	318/1,525 (21%)	<0.0001

616 * approximate 97.5th percentile of CT in higher evidence positives through 2 August when
617 classification first applied.

618 ** reported working in a patient-facing healthcare role/care/residential home.

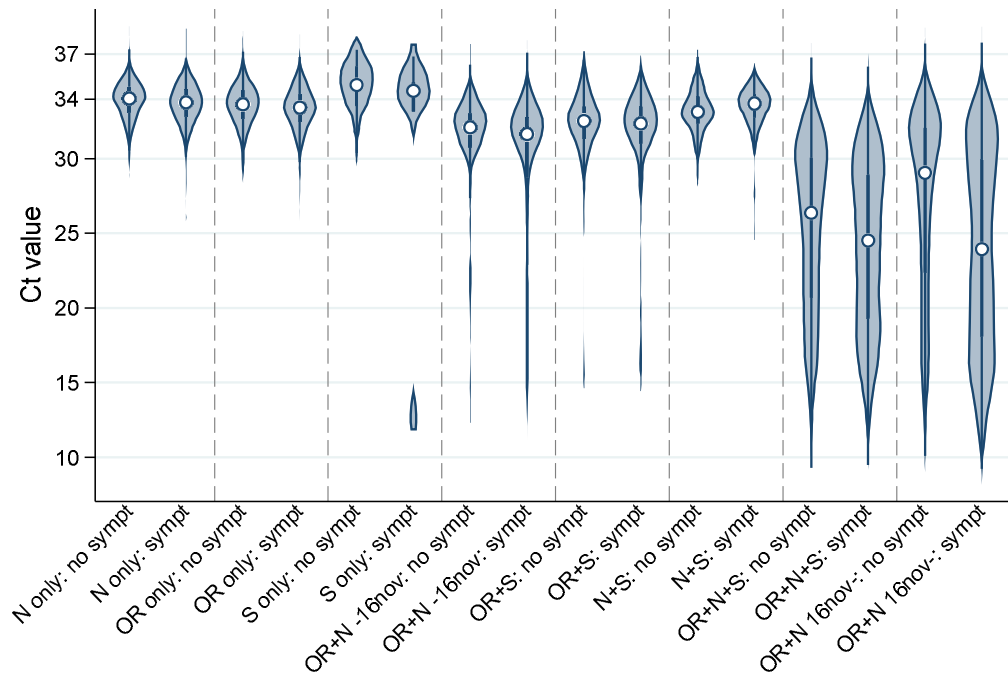
619 † any genome sequence obtained out of attempted (other positives not found or not yet attempted)

620 ‡ denominator households with 2 or more study participants.

621 Note: classification arbitrarily determined on 2 August 2020 based on the number of genes detected,
622 Ct values and pre-test probability (see Methods).

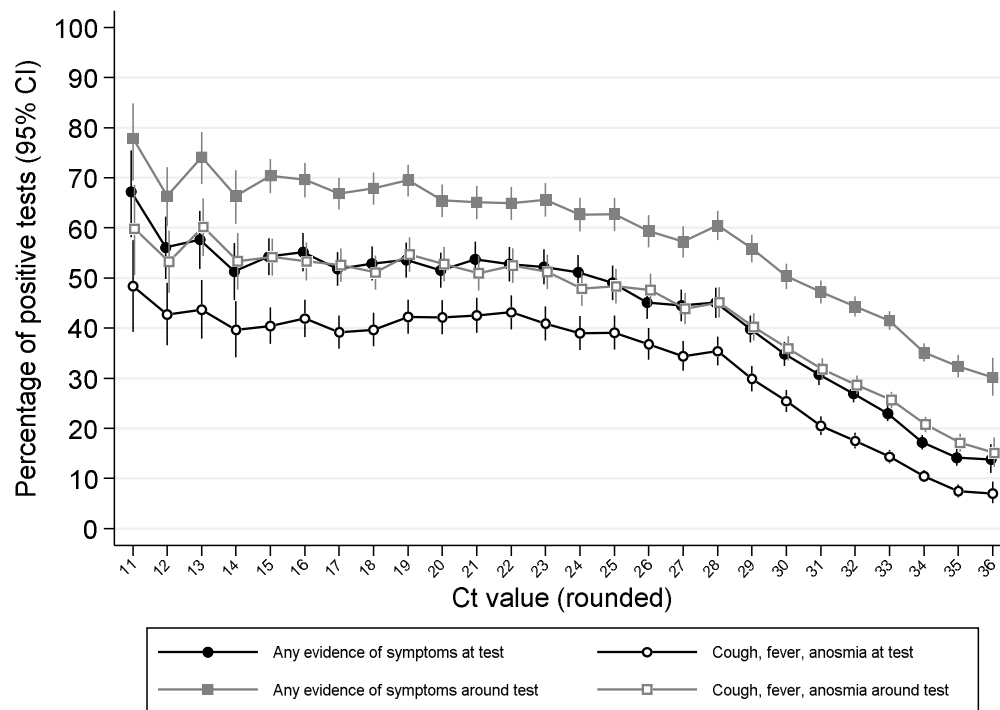
623

Figure 1 Distribution of Ct values at each positive test by genes detected and self-reported symptoms



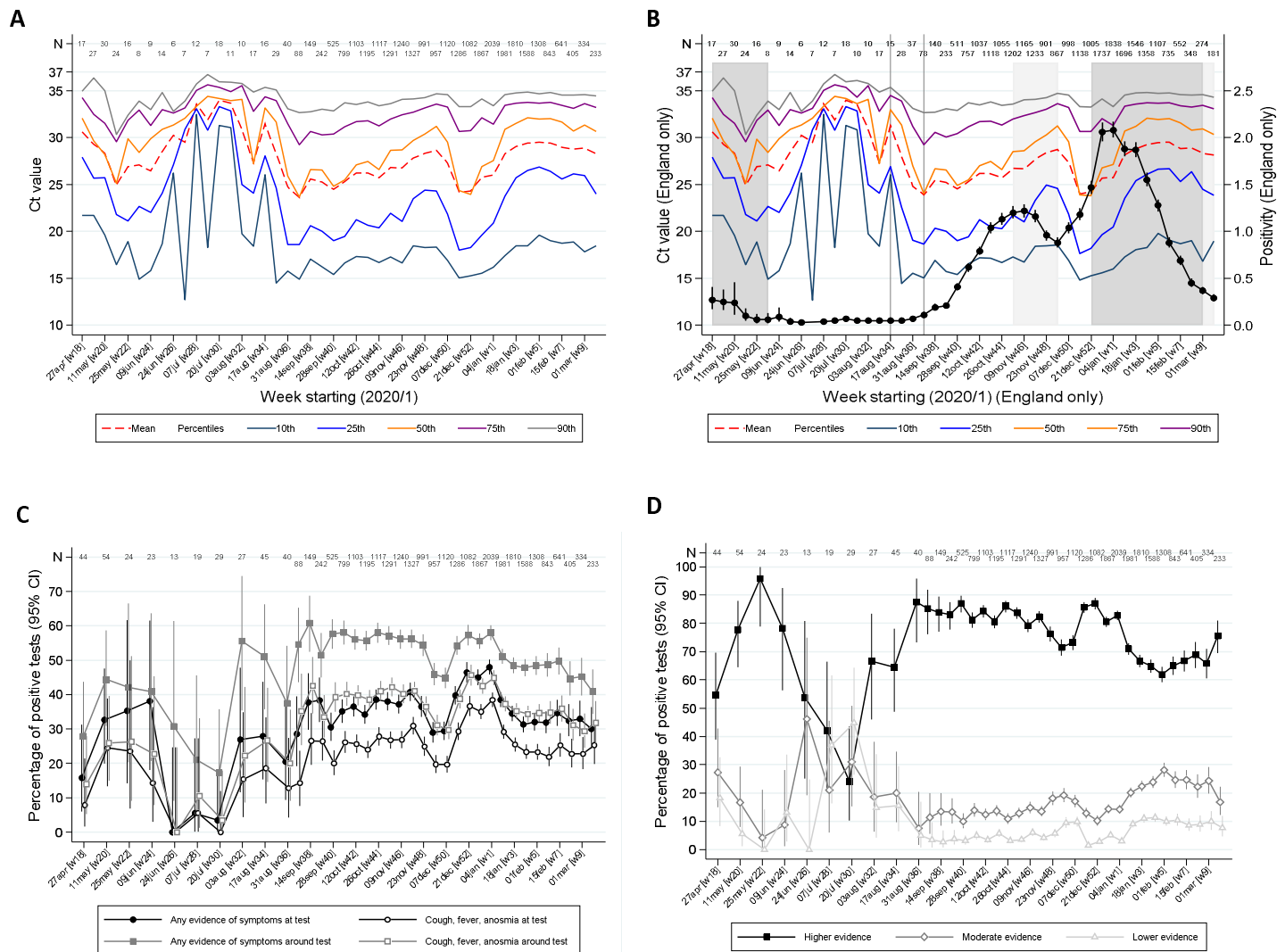
Note: points show the median and boxes the interquartile range. OR=ORF1ab.
Positives where only the ORF1ab+N genes were detected are split by whether the swab was taken before or after 16 November 2020, reflecting the expansion of B.1.1.7 (which has S-gene target failure on the assay used in the survey).

Figure 2 Percentage reporting symptoms by Ct value



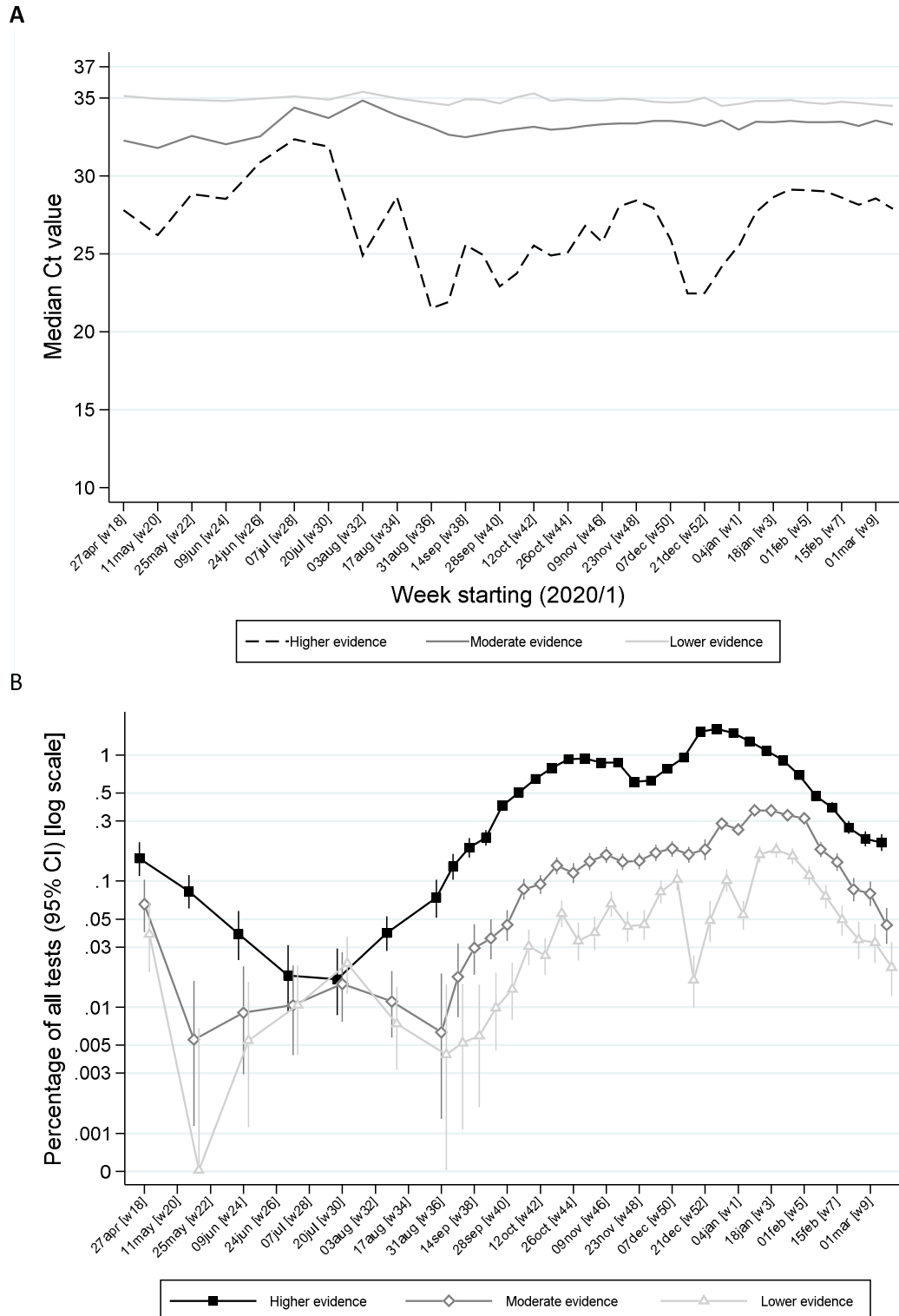
Note: Points show the percentage of positive tests with each rounded Ct value reporting any symptoms or cough, fever, anosmia/ageusia at each test or around each test (see Methods for symptoms collection and definitions). Ct values under 11 and over 36 grouped with 11 and 36 respectively.

Figure 3 Variation over calendar time in the distribution of Ct values in the UK (A) and England (B) together with percentage positivity in England (B), and in self-reported symptoms (C) and evidence supporting positives (D)



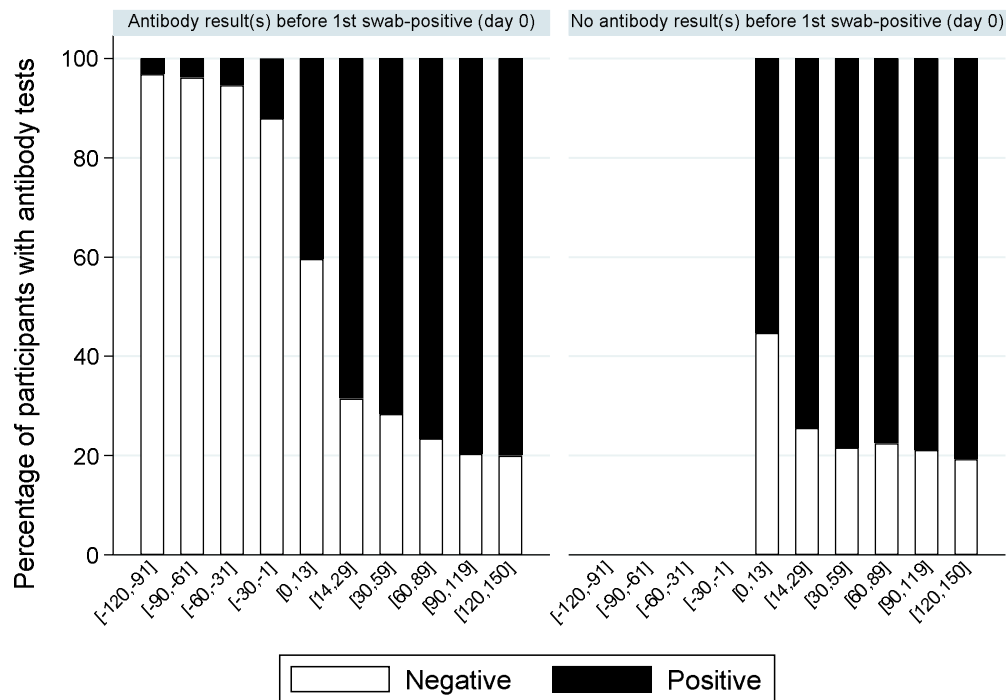
Note: panel (A) shows the distribution of Ct values each week including all positives across the UK. Panel (B) is restricted to England shown together with the official estimates of positivity as reported by the Office for National Statistics (black line) and periods of national "stay-at-home" restrictions (schools shut in dark grey, schools open in light grey). Panels (C) and (D) show the proportions reporting symptoms and with different levels of evidence supporting the positive test respectively. Variation in the width of 95% CI reflects the increase in size of the survey from mid August (**Supplementary File 1**).

Figure 4 Ct values (A) and percentage positive of all tests (B) by level of evidence and time



Note: panel (A) shows median Ct values according to level of evidence and panel (B) percentage of all swab tests positive according to level of evidence over calendar time. The early part of the study is grouped into three week periods due to lower numbers of positives.

Figure 5 Percentage of positive antibody tests over time from first positive swab



Note: showing the percentage of participants with S-antibody positive or negative tests according to days from their first positive swab, separately for those with and without any antibody results prior to their first positive swab.