



OPEN ACCESS



Performance of the Innova SARS-CoV-2 antigen rapid lateral flow test in the Liverpool asymptomatic testing pilot: population based cohort study

Marta García-Fiñana,¹ David M Hughes,¹ Christopher P Cheyne,¹ Girvan Burnside,¹ Mark Stockbridge,² Tom A Fowler,^{2,3} Veronica L Fowler,² Mark H Wilcox,⁴ Malcolm G Semple,^{5,6} Iain Buchan⁷

¹Department of Health Data Science, Institute of Population Health, University of Liverpool, Liverpool, UK

²Department of Health and Social Care, London, UK

³William Harvey Research Institute, Queen Mary University of London, London, UK

⁴Leeds Institute of Medical Research, University of Leeds, Leeds, UK

⁵NIHR Health Protection Research Unit in Emerging and Zoonotic Infections and Institute of Translational Medicine, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, UK

⁶Respiratory Medicine, Alder Hey Children's Hospital, Liverpool, UK

⁷Institute of Population Health Sciences, University of Liverpool, Liverpool, UK

Correspondence to:

M García-Fiñana
martaf@liverpool.ac.uk
(ORCID 0000-0003-4939-0575)

Additional material is published online only. To view please visit the journal online.

Cite this as: *BMJ* 2021;374:n1637
<https://dx.doi.org/10.1136/bmj.n1637>

Accepted: 22 June 2021

ABSTRACT

OBJECTIVE

To assess the performance of the SARS-CoV-2 antigen rapid lateral flow test (LFT) versus polymerase chain reaction testing in the asymptomatic general population attending testing centres.

DESIGN

Observational cohort study.

SETTING

Community LFT pilot at covid-19 testing sites in Liverpool, UK.

PARTICIPANTS

5869 asymptomatic adults (≥18 years) voluntarily attending one of 48 testing sites during 6-29 November 2020.

INTERVENTIONS

Participants were tested using both an Innova LFT and a quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) test based on supervised self-administered swabbing at testing sites.

MAIN OUTCOME MEASURES

Sensitivity, specificity, and predictive values of LFT compared with RT-qPCR in an epidemic steady state of covid-19 among adults with no classic symptoms of the disease.

RESULTS

Of 5869 test results, 22 (0.4%) LFT results and 343 (5.8%) RT-qPCR results were void (that is, when the control line fails to appear within 30 minutes). Excluding the void results, the LFT versus RT-qPCR showed a sensitivity of 40.0% (95% confidence interval 28.5% to 52.4%; 28/70), specificity of 99.9%

(99.8% to 99.99%; 5431/5434), positive predictive value of 90.3% (74.2% to 98.0%; 28/31), and negative predictive value of 99.2% (99.0% to 99.4%; 5431/5473). When the void samples were assumed to be negative, a sensitivity was observed for LFT of 37.8% (26.8% to 49.9%; 28/74), specificity of 99.6% (99.4% to 99.8%; 5431/5452), positive predictive value of 84.8% (68.1% to 94.9%; 28/33), and negative predictive value of 93.4% (92.7% to 94.0%; 5431/5814). The sensitivity in participants with an RT-qPCR cycle threshold (Ct) of <18.3 (approximate viral loads >10⁶ RNA copies/mL) was 90.9% (58.7% to 99.8%; 10/11), a Ct of <24.4 (>10⁴ RNA copies/mL) was 69.4% (51.9% to 83.7%; 25/36), and a Ct of >24.4 (<10⁴ RNA copies/mL) was 9.7% (1.9% to 23.7%; 3/34). LFT is likely to detect at least three fifths and at most 998 in every 1000 people with a positive RT-qPCR test result with high viral load.

CONCLUSIONS

The Innova LFT can be useful for identifying infections among adults who report no symptoms of covid-19, particularly those with high viral load who are more likely to infect others. The number of asymptomatic adults with lower Ct (indicating higher viral load) missed by LFT, although small, should be considered when using single LFT in high consequence settings. Clear and accurate communication with the public about how to interpret test results is important, given the chance of missing some cases, even at high viral loads. Further research is needed to understand how infectiousness is reflected in the viral antigen shedding detected by LFT versus the viral loads approximated by RT-qPCR.

Introduction

The global SARS-CoV-2 pandemic has been responsible for many deaths and has had a profound, enduring effect on the livelihoods and life chances of people worldwide. Identifying those with covid-19 has become a key strategy for limiting the spread of the disease, with quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) testing being used as the reference standard in the diagnosis of covid-19. In a public health rather than clinical context, however, the hours or days taken to receive a RT-qPCR test result is time when infected people might be transmitting the virus to others. The cost and capacity of RT-qPCR testing also limit its value for public health uses. As a consequence, low cost, rapid lateral flow technology (LFT), such as that used in pregnancy test kits, was developed to detect the nucleocapsid protein antigen

WHAT IS ALREADY KNOWN ON THIS TOPIC

The Innova SARS-CoV-2 antigen rapid lateral flow test (LFT) was adopted for large scale UK pilots of community testing in response to covid-19, including open access testing for asymptomatic residents of Liverpool, UK

Although the evaluation of the test's accuracy in people with symptoms is well documented, limited data are available for its use in asymptomatic people

WHAT THIS STUDY ADDS

Innova LFT results were positive for most people with high viral load (likely to be infectious) who tested positive using reverse-transcriptase polymerase chain reaction (RT-qPCR), whereas results were negative for most people with viral load <10⁴ RNA copies/mL (expected to be less infectious)

Policy makers need to consider LFT utility in context sensitive ways, regarding population levels of infection, phases of epidemic curves, and the transmission risks and consequences in specific settings

of SARS-CoV-2, the virus responsible for covid-19.¹ The LFT provides results within 30 minutes and can be carried out without the need for a laboratory, offering the potential to rapidly identify people with covid-19, ideally leading to quick isolation, and breaking transmission chains.

The Innova LFT was adopted for large scale English pilots of community testing in response to covid-19, including open access testing for asymptomatic residents of Liverpool in England. An initial evaluation, undertaken as part of the test development and evaluation programme commissioned by the Department of Health and Social Care, and conducted by Public Health England Porton Down laboratory and the University of Oxford, reported test sensitivity compared with RT-qPCR of 79% when the LFT was read by laboratory scientists, 73% when read by trained healthcare workers, and 58% when read by self-trained staff members at a test and trace centre.² The study considered both people with and without symptoms and found no evidence of a difference in sensitivity. However, the study participants were part of a testing service for people with symptoms and details are lacking as to why asymptomatic people were tested. A recent review suggested that LFT can be less accurate in asymptomatic people than in those with symptoms.³

We assessed the performance of LFT to detect current SARS-CoV-2 infection in the general population without symptoms attending covid-19 testing centres. The use of RT-qPCR as the reference standard test is, however, questionable because RT-qPCR can detect

SARS-Cov-2 for an average of 17 days, and often for longer. For much of this period, the individual is likely to have stopped being infectious.⁴⁻⁶ The RT-qPCR cycle threshold (Ct) offers an indication of viral load, which is understood to be linked to the level of infectiousness of someone with covid-19. Nevertheless, the precise relation in any individual between Ct and viral load, and between viral load and infectiousness is still unclear.⁷⁻⁸ The PHE evaluation² indicated that LFT was able to identify more than 90% of people with a Ct <25, corresponding approximately to a viral load of >10⁵ RNA copies/mL, indicating that the LFT has the potential to identify most of those who are infectious. Some concerns remain about the use of LFT, however, most noticeably about the accuracy reported by self-trained or non-expert readers in the asymptomatic population.⁹

On 6 November 2020 a community testing programme, piloted in Liverpool and sponsored by the Department of Health and Social Care, started with the aim of identifying more infected people earlier in their infection cycle so they could self-isolate and break transmission chains. To assess the performance and appropriate implementation of the Innova LFT in Liverpool, we performed a quality assurance exercise. We compared the sensitivity, specificity, and predictive values of the LFT with a RT-qPCR test sampled within minutes of self-administered swabbing by the same individual at the testing site. The sensitivity of the LFT as a function of PCR Ct (viral load proxy) was also investigated.

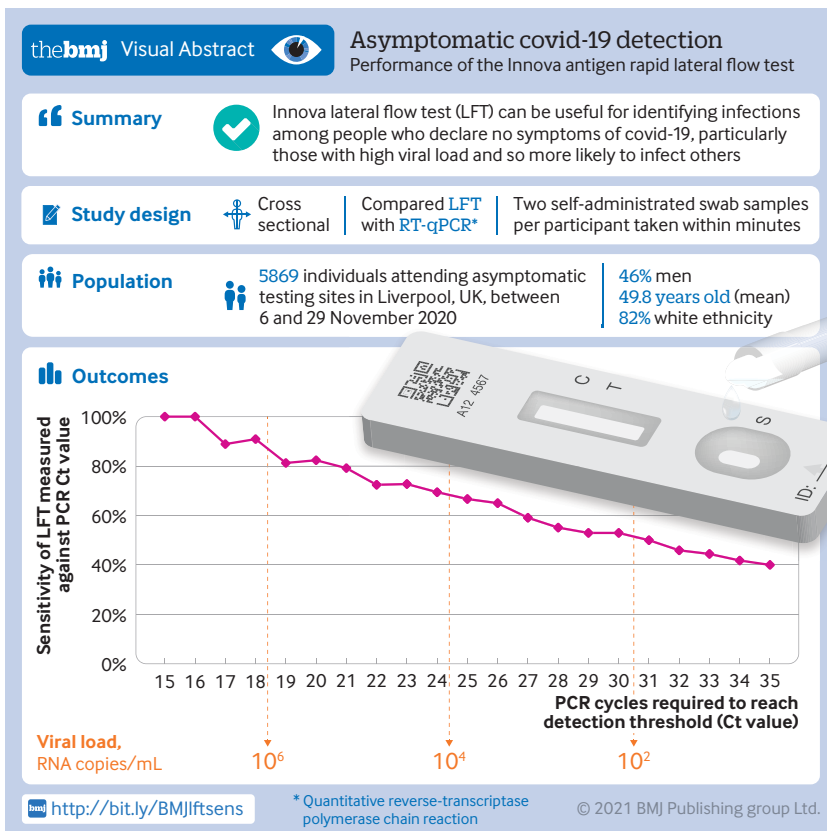
Some initial results from this Liverpool pilot have been reported previously^{10,11} and have been debated in the literature,¹²⁻¹⁹ but to date have not been published in the peer reviewed literature. We report the quality assurance findings of that study.

Methods

Study design and participants

Asymptomatic people attending testing sites in Liverpool between 8 and 29 November 2020 were invited to participate in this prospective quality assurance exercise. Eligible participants were adults (≥18 years) who did not display any of the UK government's list of symptoms for covid-19 (new continuous cough, high temperature, or loss of, or change in, normal sense of taste or smell). Participants provided verbal consent (recorded) to take part. Recruitment rotated between 48 test sites until the team had either recruited 200 participants at a site, or two days of recruitment at that site had elapsed without the target being reached. Reasons for not being invited to participate were age younger than 18 years old or the volume of people at a test centre prohibited this additional recruitment step. Otherwise all individuals consecutively attending the relevant test centres were invited to participate.

Sample size was predefined by NHS Test and Trace service at 200 in each testing site and not based on statistical calculations from the evaluation team. This balance was pragmatic to account for potential



variability across sites (eg, differences in personal and socioeconomic characteristics). Similar numbers of participants (n=200) were recruited in each site. Assuming about a 1% prevalence for covid-19, the sample size was anticipated to provide on average one or two positive test results in each test site. Participants received both an Innova LFT and an RT-qPCR test to be self-administered swabs (combined throat and nose) under supervision and taken at the same appointment within minutes. The LFT test was always taken first and graded within 30 minutes. Trained observers monitored this process to ensure that the manufacturer's instructions were followed. The site team analysed the LFT directly according to the Department of Health and Social Care standard protocol, with the result confirmed by the quality assurance team. The team were members of the armed forces who were provided with a set of standard operating instructions, detailing how to process the swab sample and how to interpret the results. The same version of the Innova LFT (product code BT1309) was used for the city-wide testing pilot. The second swab was sent for RT-qPCR analysis at one of the Lighthouse laboratories using its standard RT-qPCR SARS-CoV-2 assay (TaqPath; ThermoFisher Scientific). Neither test result was graded with knowledge of the other test result. The results were sent from NHS Test and Trace to Liverpool's integrated health and social care and public health data system (www.cipha.nhs.uk) and analysed by an independent team at the University of Liverpool. The supplementary file provides further information on the quality assurance protocol developed by the Department of Health and Social Care.

Ct values and connection with viral load

Ct is defined as the number of cycles required for the sample fluorescence in the laboratory to exceed a chosen threshold for positivity. Ct values provide an indication of the concentration of viral RNA in a sample tested by the RT-qPCR method, such that low Ct values tend to be linked to high viral loads and, conversely, high Ct values tend to be linked to low viral loads. Lack of standardisation of Ct values across different laboratories and equipment makes it challenging to compare Ct values between studies using different laboratories. The RT-qPCR test used here corresponds to the standard test used in UK Lighthouse laboratories studies, and we have used the same Ct thresholds to calculate sensitivity of LFT compared with RT-qPCR to allow easy comparison.⁸ Our study protocol had no prespecified Ct cut-offs. Ct values were converted into approximate viral loads using the calibration curve performed for the Glasgow laboratory ($\log_{10}(\text{viral load}) = 12 - 0.328 \times \text{Ct}$), since it is understood to be generalisable to other Lighthouse laboratories using similar methods.⁸

Statistical analysis

We used the Cochran-Pearson exact method to estimate the sensitivity, specificity, positive predictive value, and negative predictive values along with corresponding

95% confidence intervals. For the purposes of this analysis, we regarded RT-qPCR as the reference standard, although we acknowledge that viral shedding (reflected by LFT) and viral loads (reflected by RT-qPCR Ct) are temporally aligned processes. Estimates of sensitivity were provided for each Ct interval, which we calculated as the combined average of existing genes (such that if a particular gene had a missing value, the average was calculated over the remaining Ct scores). Void results were not included in calculation of accuracy variables for the main analysis. However, for completeness, we also assessed accuracy taking into account void results (for either test) by grouping them with the negative results.²⁰ A voided RT-qPCR test is one that is either operationally non-analysable (eg, insufficient sample for analysis) or has an analytically inconclusive fluorescence amplification curve. In some of these cases amplification might have been observed for one of the target genes, but this was determined to be based on either background signal or sample contamination. A voided LFT test result is one where the control line fails to appear within 30 minutes. Estimates of sensitivity were also generated based on the N gene (the nucleocapsid antigen that LFT is designed to detect) as well as based on the S (spike protein) gene and ORF1ab (open reading frame 1ab) for completeness.

When multiple LFT and RT-qPCR tests were identified from the same participant, we included only one pair of test results in the main analysis following predefined selection criteria (see supplementary file). Sensitivity analyses were performed to consider scenarios where agreement between LFT and RT-qPCR results was maximised and minimised based on the results from multiple tests for each individual. An additional analysis including all multiple tests (assuming independence) was also conducted. Statistical analyses were carried out in R (version 3.6.1 or later) and checked by a second statistician using SAS software (version 9.4).

Patient and public involvement

This study uses data from Liverpool's integrated health and social care and public health data system, and it is an urgent public health research study in response to a public health emergency of international concern. Patients and the public were not involved in the design, conduct, or reporting of the study.

Results

Personal characteristics of the participants were not collected as part of this quality assurance study. It was, however, possible to use the Combined Intelligence for Population Health Action platform to access results for all but 116 of the LFT tests conducted in the study to extract basic personal information. The mean age of the participants was 50 years (SD 18 years), 54% were women, and most were of white ethnicity (82%). Supplementary table S4 and figures S1-S4 provide additional information on the characteristics of the study cohort, flowchart, distribution of the number of

tests by test centre, and test results over time. Personal characteristics were similar between the study participants and those who attended the test centres on the same day as the study team (supplementary table S5).

Forty participants had taken part in the study on more than one occasion—30 had the same LFT and RT-qPCR paired results and 10 showed inconsistent results for LFT or RT-qPCR, or both (see supplementary file).

Overall, 5869 asymptomatic adults from 48 testing sites in Liverpool participated in this study. Table 1 shows the results for pairs of LFT and RT-qPCR tests for each participant. After excluding void test results, the sensitivity was found to be 40.0% (95% confidence interval 28.5% to 52.4%; 28/70), specificity 99.9% (99.8% to 99.99%; 5431/5434), positive predictive value 90.3% (74.2% to 98.0%; 28/31), and negative predictive value 99.2% (99.0% to 99.4%; 5431/5473). Positive and negative predictive values were directly estimated from the data, which showed a 1.3% prevalence; the Office for National Statistics reported a prevalence of 2.3% for West Lancashire, Liverpool, Knowsley, and Sefton on 20 November.²¹ The lower percentage of positive test results in the current study reflects that all testing was conducted in people without covid-19 symptoms.

When the void results for either test were grouped with the negative results, a sensitivity was observed for LFT of 37.8% (26.8% to 49.9%; 28/74), specificity of 99.6% (99.4% to 99.8%; 5431/5452), positive predictive value of 84.8% (68.1% to 94.9%; 28/33), and negative predictive value of 93.4% (92.7% to 94.0%; 5431/5814). Including void results in this way provides a more conservative estimate of sensitivity, given that void LFT results linked to a positive PCR result are assumed to be negative.²⁰

Figure 1 and table 2 present the results for sensitivity of the LFT against RT-qPCR by viral load. In the context of a positive RT-qPCR test result with high viral load ($>10^6$ RNA copies/mL) the LFT was able to detect 90.9% (58.7% to 99.8%; 10/11) of cases. The LFT detection rate of participants with a positive RT-qPCR result decreased substantially as viral load decreased. For example, for those with a viral load $<10^4$ RNA copies/mL the sensitivity was only 9.7% (1.9% to 23.7%; 3/34). Figure 2 shows LFT sensitivity against mean Ct value.

The LFT results assessed on site showed a high level of concordance (99.9%) with the quality assurance

team's readings, with 5845 concordant and two discordant samples (99.9% concordance, excluding voids, almost identical if voids are included; see supplementary table S2). Interobserver agreement did not differ noticeably across test sites.

Two additional analyses based on agreement or disagreement between LFT and RT-qPCR test results for those participants with multiple tests showed that the selection criteria followed had a minor effect on the accuracy variables (see supplementary table S3). Results remained similar when multiple tests were included (ie, treating multiple pairs of tests for the same individuals as if they were independent observations; supplementary table S4).

Discussion

We report a city-scale evaluation of Innova LFT versus RT-qPCR test results among the general population attending asymptomatic testing centres voluntarily in Liverpool between 8 and 29 November 2020. Participants declared that they did not have classic symptoms of covid-19 and we have drawn no conclusions about those who may have been (pre-; pauci-) symptomatic. The sample was representative of those attending asymptomatic testing centres concurrently (supplementary table S5). Contrasts with the wider population have been described elsewhere.²²

The overall sensitivity of the Innova LFT was 40%—a potentially misleading statistic if RT-qPCR detects a large proportion of people post-infection.¹⁸ RT-qPCR is exquisitely sensitive for detecting SARS-CoV-2 RNA in those with replicating virus or those whose immune systems have controlled the infection and are less likely to be infectious. So a substantial proportion (depending on the epidemic phase) of people with a positive RT-qPCR test result will no longer be infectious.^{5,7} In our study, LFT achieved 90% sensitivity compared with people found to be positive by RT-qPCR testing with a high viral load $>10^6$ RNA copies/mL, and the corresponding 95% confidence interval indicated that LFT is likely to detect at least three fifths and at most 998 in every 1000 people with a positive RT-qPCR test result with high viral load. For comparison, LFT achieved a sensitivity of 72.4% (52.8% to 87.3%) in those with an approximate viral load $>10^5$ RNA copies/mL.

Comparison with other studies

Other rapid antigen tests have been assessed for their ability to detect people with confirmed covid-19 by RT-

Table 1 | Results of rapid lateral flow test (LFT) and quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) tests in quality assurance dataset (5869 participants)

| LFT result | RT-qPCR result | | | Total (%) | Predictive values |
|-------------------------|----------------------|-------------|-----------|-------------|---------------------|
| | Positive | Negative | Void | | |
| Positive | 28 | 3 | 2 | 33 (0.6) | 90.3 (74.2 to 98.0) |
| Negative | 42 | 5431 | 341 | 5814 (99.1) | 99.2 (99.0 to 99.4) |
| Void | 4 | 18 | 0 | 22 (0.4) | |
| Total (%) | 74 (1.3) | 5452 (92.9) | 343 (5.8) | 5869 | |
| Sensitivity (%; 95% CI) | 40.0 (28.5 to 52.4) | | | | |
| Specificity (%; 95% CI) | 99.9 (99.8 to 99.99) | | | | |

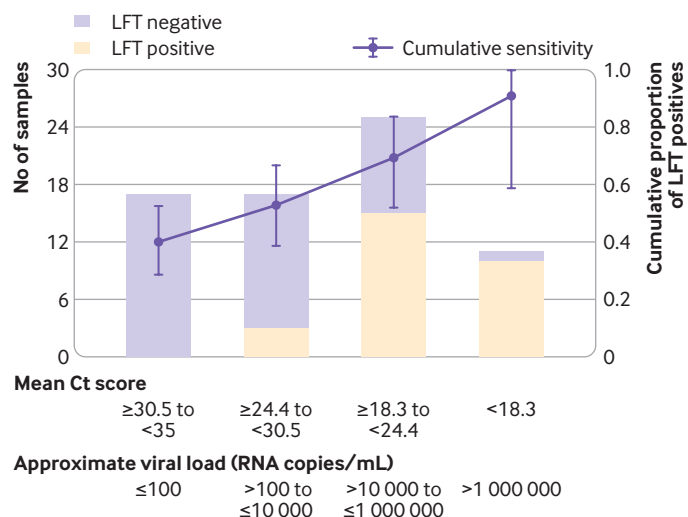


Fig 1 | Number of participants with negative and positive lateral flow test (LFT) results by quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) viral load (based on mean cycle threshold (Ct) score across three gene targets). Intervals show the 95% confidence interval for the cumulative sensitivity to detect viral loads >1 , $>10^2$, $>10^4$, and $>10^6$ RNA copies/mL

qPCR testing, reporting broadly similar sensitivity and specificity in asymptomatic people as we report for the Innova LFT in the Liverpool pilot.²³⁻²⁵ All three studies also report similar ability to detect lower Ct values with higher sensitivity. Although our study involved mainly white British people, comparable results have been recorded in other countries and ethnic groups.^{26 27}

The association between covid-19 viral load and infectiousness has not yet been fully characterised.^{8 28} Analysis of NHS Test and Trace contact data suggests that a lower Ct in the index case (ie, higher viral load) increases the likelihood of contacts testing positive for covid-19.⁸ This study traced 64% of all contacts and observed that 13% of case-contact pairs with a contact who tested positive by RT-qPCR had case viral loads $<10^4$ RNA copies/mL, 40% of case-contact pairs had case viral loads between 10^4 and 10^6 RNA copies/mL, and around 47% of case-contact pairs with contacts who tested positive by RT-qPCR had case viral loads $>10^6$ RNA copies/mL. A recent Spanish contact tracing study²⁸ identified that more than 85% of transmission events occurred in clusters linked to an index case with a viral load of $>10^5$ RNA copies/mL. These studies indicate that LFT can identify most of the those with a

Table 2 | Results of rapid lateral flow test (LFT) with a breakdown by cycle threshold (Ct) value

| | RT-qPCR test (mean across three gene targets) | | | | Void tests with Ct value | | | | | |
|-------------------------------------|---|--------------------------------|--------------------------------|-------------------------|--------------------------|-------|-----|------|---------------|----------|
| | Ct (RNA copies/mL) | | | | Positive (NA)* | 30-35 | | ≥35 | No Ct values† | Negative |
| RT-qPCR Ct | <18.3 ($>10^6$) | 18.3-24.4 ($>10^4$ - 10^6) | 24.4-30.5 ($>10^2$ - 10^4) | 30.5-35 ($\leq 10^2$) | | | | | | |
| LFT site results: | | | | | | | | | | |
| Negative | 1 | 10 | 14 | 17 | 5 | 8 | 328 | 5431 | | |
| Positive | 10 | 15 | 3 | 0 | 0 | 0 | 2 | 3 | | |
| Void | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 18 | | |
| Sensitivity (%; 95% CI) | 90.9 (58.7 to 99.8) | 60.0 (38.7 to 78.9) | 17.6 (3.8 to 43.4) | 0.0 (0.0 to 19.5) | | | | | | |
| Cumulative sensitivity‡ (%; 95% CI) | 90.9 (58.7 to 99.8) | 69.4 (51.9 to 83.7) | 52.8 (38.6 to 66.7) | 40.0 (28.5 to 52.4) | | | | | | |
| N gene | | | | | | | | | | |
| LFT site results: | | | | | | | | | | |
| Negative | 1 | 8 | 16 | 15 | 2 | 6 | 4 | 331 | 5431 | |
| Positive | 7 | 17 | 3 | 1 | 0 | 0 | 0 | 2 | 3 | |
| Void | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 18 | | |
| Sensitivity (%; 95% CI) | 87.5 (47.3 to 99.7) | 68.0 (46.5 to 85.1) | 15.8 (3.4 to 39.6) | 6.3 (0.1 to 30.2) | | | | | | |
| Cumulative sensitivity‡ (%; 95% CI) | 87.5 (47.3 to 99.7) | 72.7 (54.5 to 86.7) | 51.9 (37.6 to 66.0) | 41.2 (29.4 to 53.8) | | | | | | |
| S gene | | | | | | | | | | |
| LFT site results: | | | | | | | | | | |
| Negative | 1 | 10 | 13 | 9 | 9 | 1 | 4 | 336 | 5431 | |
| Positive | 10 | 15 | 3 | 0 | 0 | 0 | 2 | 3 | | |
| Void | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 18 | | |
| Sensitivity (%; 95% CI) | 90.9 (58.7 to 99.8) | 60.0 (38.6 to 78.9) | 18.8 (4.0 to 45.6) | 0.0 (0.0 to 33.6) | | | | | | |
| Cumulative sensitivity‡ (%; 95% CI) | 90.9 (58.7 to 99.8) | 69.4 (52.9 to 83.7) | 53.8 (39.5 to 67.8) | 45.9 (33.1 to 59.2) | | | | | | |
| ORF1ab gene | | | | | | | | | | |
| LFT site results: | | | | | | | | | | |
| Negative | 3 | 10 | 13 | 8 | 7 | 1 | 2 | 338 | 5431 | |
| Positive | 12 | 14 | 2 | 0 | 0 | 0 | 2 | 3 | | |
| Void | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 18 | | |
| Sensitivity (%; 95% CI) | 80.0 (51.9 to 95.7) | 58.3 (36.6 to 77.9) | 13.3 (1.7 to 40.5) | 0.0 (0.0 to 36.9) | | | | | | |
| Cumulative sensitivity‡ (%; 95% CI) | 80.0 (51.9 to 95.7) | 66.7 (49.8 to 80.9) | 51.9 (37.8 to 65.7) | 45.2 (32.5 to 58.3) | | | | | | |

RT-qPCR=quantitative reverse-transcriptase polymerase chain reaction; NA=not applicable (missing gene target).

*Implies PCR result was graded as positive but particular target gene was not detected for that sample.

†When void PCR results were returned, Ct value was observed for at least one target gene in a small number of participants, but this was determined to be inconclusive.

‡Defined using number of samples with Ct less than upper threshold.

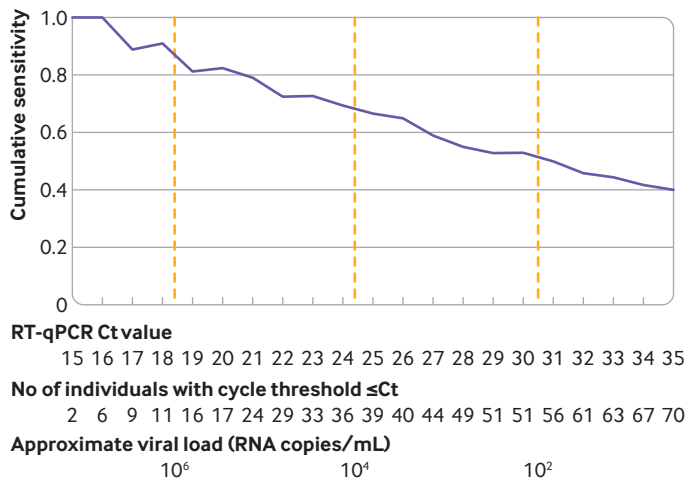


Fig 2 | Cumulative sensitivity of Innova lateral flow test (LFT) against quantitative reverse-transcriptase polymerase chain reaction (RT-qPCR) cycle threshold (Ct). Dashed orange lines show approximate viral loads (RNA copies/mL)

higher chance of infecting others, but they also show that transmission of covid-19 can occur in those with low viral load. It is nevertheless possible that some of these instances of transmission were confounded by third party transmission, or that the transmission occurred when the individual's viral load was much higher. However, the evidence gathered to date suggest that people with a low viral load (about $\leq 10^5$ RNA copies/mL) could still be infectious, although the chance of infecting others is lower than for those with a higher viral load. Although our study, conducted in an asymptomatic population, shows that LFT can detect most people with a high viral load, and therefore those at a higher risk of transmitting SARS-CoV-2, the LFT missed 10% of people with a high viral load $>10^6$ RNA copies/mL, 30% of cases with a viral load $>10^4$ RNA copies/mL, and most of the cases with a viral load $<10^4$ RNA copies/mL who might have contributed to virus transmission.⁸ This suggests that care is needed when conveying negative LFT results so as not to give false reassurance.¹⁵ Both of these contact tracing studies were in people with symptoms, who generally had higher viral loads than observed in our study. Contact tracing studies are currently the best instruments we have of transmission in whole populations. However, contact tracing systems evolve over time, and selection biases might be present related both to the recall of the original case and to the response of the contacts. In addition, these studies provide a snapshot of people at the point of PCR testing, so do not necessarily reflect the interaction between people or the progression of the virus in people before being tested. For these reasons caution is needed in using contact tracing studies to infer the proportion of infectious people detected by LFT.

Limitations of this study

A technical limitation is that RT-qPCR targeting the N gene might detect fragments of RNA and hence could overestimate the number of viral copies in a sample. The ORF1ab gene is only detected on viral genomic

RNA and might be a better estimate of the actual number of genome copies per millilitre.²⁹ In our study we found an insubstantial reduction in sensitivity for detecting high viral loads when only the ORF1ab cycle thresholds were considered (table 2).

Although RT-qPCR tests can detect small amounts of RNA, sensitivity can be affected by swabbing technique and by substantial and largely unexplained heterogeneity between studies in sensitivity to detect SARS-Cov-2 positivity in people who might have tested positive with a different swab sample. A recent systematic review identified 34 studies with RT-qPCR false negative rates (1-negative predictive value) ranging from 2% to 58%.³⁰ The sensitivity of the LFT is also substantially affected by the quality of the sample and swabbing technique.^{2 31 32} The LFT was always done first. In theory this could have depleted the source material and hence virus nucleic acid available for the subsequent swab for RT-qPCR testing, although it is also possible that the second swab benefited from a training effect. In addition, variation in LFT device build quality, lack of quality assurance certification by batch of test, and storage temperatures are additional sources of heterogeneity that are poorly recorded.¹⁰ The instructions for use of the LFT state that the tests should be stored between 2°C and 30°C and used at room temperature of between 15°C and 30°C. Given the relatively mild autumn temperatures in the UK, ambient storage conditions should have remained within these ranges and therefore we do not anticipate this had an impact on the performance of the LFTs.

Conclusions

Our study suggests that the LFT can be a useful tool among wider public health measures for the control of SARS-CoV-2 transmission and to mitigate risk of covid-19. The predictive value of testing, and in particular of LFT, varies with the phase of the epidemic curve and the population prevalence of infection,³³ which was declining at the time of this quality assurance study in Liverpool.¹⁸ Our study also shows that about one 10th of the people with higher viral load ($>10^6$ RNA copies/mL) detected by RT-qPCR are missed, which highlights the need to assess the impact that false negative test results might have in specific contexts. Some false negative LFT results occur at a time when an individual's viral load is declining, and therefore they might no longer be highly infectious. A proportion of false negative LFT results are expected to correspond to the early phase of the infectious trajectory, when viral load might increase in the next 48 hours. For such individuals, serial testing could improve sensitivity, and optimal testing regimens need further study. Although false negative results might reflect those who are no longer infectious, there is a risk of false reassurance leading to behaviours that increase transmission. Careful communication is needed around all negative test results, and symptomatic people should continue to be encouraged to attend for PCR testing, allowing LFT to be seen to be an early screening for the most infectious cases.

The Innova LFT seems, in combination with other health protection measures, to be a valuable tool in wider public health responses to covid-19 for identifying those with higher viral loads who are more likely to be infectious but do not report classic symptoms. To maximise the value of LFT, care should be taken to train test operatives, communicate the meaning of results to tested people, target testing with reference to background case rates, and avoid single LFT results for access to vulnerable settings (such as care homes) where the consequences of infection are severe (unless comprehensive additional risk reduction measures are considered). Further studies are needed to understand the relation between LFT results and infectiousness.

We thank the Combined Intelligence for Population Health Action analytics team, in particular Sarah Adams and Helen Duckworth.

Contributors: MGF and DMH contributed equally to this work as joint first authors. IB and MGS contributed equally to this work as joint last authors. IB, MGS, and MS designed the study and contributed to the protocol. TF and MS were responsible for the rollout and quality assurance data collection. MS, DMH, CPC, and GB managed the data. DMH conducted the initial analysis and CPC and GB conducted independent analyses in R and SAS, under supervision of MGF. MGF, DMH, and IB drafted the initial manuscript. VGF, MHW, IB, and MGS provided clinical interpretation and all authors contributed to the revision of the manuscript. IB is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Funding: MGF acknowledges the support of the UK Engineering and Physical Sciences Research Council (grant EP/N014499/1). DMH acknowledges the support of a Medical Research Council UK innovation fellowship (research project MR/RO24847/1). MHW is supported by the National Institute for Health Research infrastructure at Leeds. This report is independent research funded by the Department of Health and Social Care (DHSC). The views expressed in this publication are those of the authors and not necessarily those of the National Health Service or the DHSC.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: MGF reports grants from the Engineering and Physical Sciences Research Council; DMH reports grants from the Medical Research Council (MRC); IB reports grants from the National Institute for Health Research (NIHR), during the conduct of the study; personal fees and other from AstraZeneca, personal fees from Microsoft Research, outside the submitted work; MGS reports grants from the Department of Health and Social Care (DHSC) NIHR UK, grants from MRC UK, grants from NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, during the conduct of the study, and a minority shareholder in Integrum Scientific, Greensboro, NC, USA, a company that has interests in covid-19 testing but not with lateral flow technology, outside the submitted work; IEB, MGF, MGS, DMH, GB, and CPC received grant funding from the DHSC to evaluate LFT in the Liverpool pilot discussed in this article; MHW reports grants for the NIHR, during the conduct of the study. VGF, MS, and TF are employed by the DHSC who commissioned and designed the study. The views expressed in this publication are those of the authors and not necessarily those of the National Health Service or the DHSC.

Ethical approval: The University of Liverpool provided secondary data analysis as part of a UK national service evaluation with data collected by the Department of Health and Social Care (DHSC; sponsor) for quality assurance of Innova lateral flow tests in a public health service intervention. As per the National Health Service Health Research Authority guidelines, this work did not require ethical approval. This work forms part of a quality audit of a testing service and device. Participants only received the results of the LFT as the standard of testing of the Liverpool pilot of asymptomatic community testing, and participation in this quality assurance evaluation did not impact their onward treatment. No randomisation was performed. The quality assurance exercise does, however, form part of the clinical standard operating procedures signed off as part of NHS Test and Trace mass testing roll-out by the DHSC Public Health and Clinical Oversight team.

Data sharing: Data are accessible through Combined Intelligence for Population Health Action. Requests can be made to the data access

committee for extracts of the larger scale data, which cannot be released openly owing to information governance requirements. All R code is accessible from the corresponding author.

The lead authors (MGF, IB) affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Dissemination to participants and related patient and public communities: This topic has garnered interest from academia, policy makers, social commentators, and many parts of the media. We will disseminate using traditional academic publications such as this, push to traditional press through our media offices and with support of the Science Media Centre, and disseminate on contemporary social media platforms.

Provenance and peer review: Not commissioned; externally peer reviewed.

This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

- Peto TUK COVID-19 Lateral Flow Oversight Team. COVID-19: Rapid antigen detection for SARS-CoV-2 by lateral flow assay: A national systematic evaluation of sensitivity and specificity for mass-testing. *EClinicalMedicine* 2021; published online 30 May. doi:10.1016/j.eclinm.2021.100924.
- Oxford University and PHE Porton Down. University of Oxford SARS-CoV-2 test development and validation cell. Preliminary report from the Joint PHE Porton Down & University of Oxford SARS-CoV-2 test development and validation cell: Rapid evaluation of Lateral Flow Viral Antigen detection devices (LFDs) for mass community testing; 2020. www.ox.ac.uk/sites/files/oxford/media_wysiwyg/UK%20evaluation_PHE%20Porton%20Down%20%20University%20of%20Oxford_final.pdf
- Dimnes J, Deeks JJ, Berhane S, et al. Cochrane COVID-19 Diagnostic Test Accuracy Group. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst Rev* 2021;3:CD013705.
- Cevik M, Tate M, Lloyd O, et al. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microbe* 2021;2:e13-22.
- Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 test sensitivity—A strategy for containment. *N Engl J Med* 2020;383:e120. doi:10.1056/NEJMp2025631
- Tom MR, Mina MJ. To interpret the SARS-CoV-2 test, consider the cycle threshold value. *Clin Infect Dis* 2020;71:2252-4. doi:10.1093/cid/ciaa619
- Dahdouh E, Lázaro-Perona F, Romero-Gómez MP, Mingorance J, García-Rodríguez J. C_t values from SARS-CoV-2 diagnostic PCR assays should not be used as direct estimates of viral load. *J Infect* 2021;82:414-51. doi:10.1016/j.jinf.2020.10.017
- Lee YW, Rozmanowski S, Pang M, et al. SARS-CoV-2 infectivity by viral load, S gene variants and demographic factors and the utility of lateral flow devices to prevent transmission. *Clin Infect Dis* 2021; published online 11 May. doi:10.1093/cid/ciab421.
- Ferguson J, Dunn S, Best A, et al. Validation testing to determine the sensitivity of lateral flow testing for asymptomatic SARS-CoV-2 detection in low prevalence settings: Testing frequency and public health messaging is key. *PLoS Biol* 2021;19:e3001216. doi:10.1371/journal.pbio.3001216
- Buchan I. Liverpool Covid-19 Community Testing Pilot. Interim evaluation report. 2020 <https://www.liverpool.ac.uk/media/livacuk/coronavirus/Liverpool,Community,Testing,Pilot,Interim,Evaluation.pdf>
- García-Finana M, Hughes DM, Cheyne CP, et al. Innova Lateral Flow SARS-CoV-2 Antigen test accuracy in Liverpool Pilot: preliminary data, 26 November 2020. *Scientific Advisory Group for Emergencies (SAGE)* 2020 <https://www.gov.uk/government/publications/innova-lateral-flow-sars-cov-2-antigen-test-accuracy-in-liverpool-pilot-preliminary-data-26-november-2020>
- Armstrong S. Covid-19: Tests on students are highly inaccurate, early findings show. *BMJ* 2020;371:m4941.
- Crozier A, Rajan S, Buchan I, McKee M. Put to the test: use of rapid testing technologies for covid-19. *BMJ* 2021;372:n208.
- Deeks J, Raffle A, Gill M. Covid-19: government must urgently rethink lateral flow test roll out. *BMJ* 2021, published online 12 January. <https://blogs.bmj.com/bmj/2021/01/12/covid-19-government-must-urgently-rethink-lateral-flow-test-roll-out/>
- Deeks JJ, Raffle AE. Lateral flow tests cannot rule out SARS-CoV-2 infection. *BMJ* 2020;371:m4787.

- 16 Fearon E, Davis EL, Stage HB, et al. Covid-19: Controversial rapid test policy divides doctors and scientists [electronic response to Deeks J, et al. Covid-19: government must urgently rethink lateral flow test roll-out]. *BMJ* 2021; <https://www.bmj.com/content/372/bmj.n81/r>
- 17 Kmiotowicz Z. Covid-19: Controversial rapid test policy divides doctors and scientists. *BMJ* 2021;372:n81.
- 18 Mina MJ, Peto TE, García-Fiñana M, Semple MG, Buchan IE. Clarifying the evidence on SARS-CoV-2 antigen rapid tests in public health responses to COVID-19. *Lancet* 2021;397:1425-7. doi:10.1016/S0140-6736(21)00425-6
- 19 Wise J. Covid-19: Lateral flow tests miss over half of cases, Liverpool pilot data show. *BMJ* 2020;371:m4848.
- 20 Shinkins B, Thompson M, Mallett S, Perera R. Diagnostic accuracy studies: how to report and analyse inconclusive test results. *BMJ* 2013;346:f2778. doi:10.1136/bmj.f2778
- 21 Coronavirus ONS. (COVID-19) Infection Survey, UK: 20 November 2020. 2021 <https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/coronaviruscovid19infectionsurvey/pilot/20november2020#sub-regional-analysis-of-the-number-of-people-in-england-who-had-covid-19>
- 22 Green MA, García-Fiñana M, Barr B, et al. Evaluating social and spatial inequalities of large scale rapid lateral flow SARS-CoV-2 antigen testing in COVID-19 management: An observational study of Liverpool, UK (November 2020 to January 2021). *Lancet Reg Health Eur* 2021;6:100107. doi:10.1016/j.lanep.2021.100107
- 23 Jakobsen KK, Jensen JS, Todsén T, et al. Detection of SARS-CoV-2 infection by rapid antigen test in comparison with RT-PCR in a public setting. *medRxiv* 2021
- 24 Pray IW, Ford L, Cole D, et al, CDC COVID-19 Surge Laboratory Group. Performance of an antigen-based test for asymptomatic and symptomatic SARS-CoV-2 testing at two university campuses—Wisconsin, September–October 2020. *MMWR Morb Mortal Wkly Rep* 2021;69:1642-7.
- 25 Prince-Guerra JL, Almdares O, Nolen LD, et al. Evaluation of Abbott BinaxNOW rapid antigen test for SARS-CoV-2 infection at two community-based testing sites—Pima County, Arizona, November 3–17, 2020. *MMWR Morb Mortal Wkly Rep* 2021;70:100-5. doi:10.15585/mmwr.mm7003e3
- 26 Bello-Chavolla OY, Antonio-Villa NE, Fernández-Chirino L, et al. Diagnostic performance and clinical implications of rapid SARS-CoV-2 antigen testing in Mexico using real-world nationwide COVID-19 registry data. *medRxiv* 2021
- 27 Boum Y, Fai KN, Nicolay B, et al. Performance and operational feasibility of antigen and antibody rapid diagnostic tests for COVID-19 in symptomatic and asymptomatic patients in Cameroon: a clinical, prospective, diagnostic accuracy study. *Lancet Infect Dis* 2021; published online 25 March. S1473-3099(21)00132-8.
- 28 Marks M, Millat-Martinez P, Ouchi D, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. *Lancet Infect Dis* 2021;21:629-36. doi:10.1016/S1473-3099(20)30985-3
- 29 Alexandersen S, Chamings A, Bhatta TR. SARS-CoV-2 genomic and subgenomic RNAs in diagnostic samples are not an indicator of active replication. *Nat Commun* 2020;11:6059. doi:10.1038/s41467-020-19883-7
- 30 Arevalo-Rodríguez I, Buitrago-García D, Simancas-Racines D, et al. False-negative results of initial RT-PCR assays for COVID-19: A systematic review. *PLoS One* 2020;15:e0242958. doi:10.1371/journal.pone.0242958
- 31 World Health Organization. SARS-CoV-2 antigen-detecting rapid diagnostic tests: an implementation guide. 2020
- 32 Torjesen I. Covid-19: How the UK is using lateral flow tests in the pandemic. *BMJ* 2021;372:n287.
- 33 García-Fiñana M, Buchan IE. Rapid antigen testing in COVID-19 responses. *Science* 2021;372:571-2. doi:10.1126/science.abi6680

Supplementary information: additional information, tables, and figures

Supplementary information: quality assurance protocol developed by the Department of Health and Social Care