Extrapolation of United Kingdom Pillar 2 Care home Covid-19 test data to ascertain effectiveness of lateral flow testing in low prevalence settings

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NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Abstract
Lateral flow devices are quickly being implemented for use in large scale population surveillance programs for SARS-CoV-2 infection in the United Kingdom. These programs have been piloted in city wide screening in the city of Liverpool, and are now being rolled out to support care home visits and the return home of University students for the Christmas break. Very little data exists comparing the performance of the UK lateral flow tests with gold standard PCR diagnostics, especially against comparable test populations such as the national Pillar 2 testing program in the United Kingdom. Here we utilise thousands of pillar 2 test data from our University of Birmingham test lab, and by extrapolation against the validate limit-of-detection of the lateral flow assay, provide a potential sensitivity for the test in a comparable low prevalence population captured in the pillar 2 program. Our data suggests the lateral flow assay should successfully capture around 85% of all PCR positive tests performed in our pillar 2 laboratory, and that a fully designed comparative study of lateral flow versus PCR testing is merited in a real life testing environment.
Introduction

In November 2020 the United Kingdom government announced a plan to introduce mass scale population testing for SARS-CoV-2 infection using Lateral Flow Devices (LFD). Principal of these is an LFD manufactured and marketed by Innova Medical group, a subsidiary of Xiamen Biotime Biotechnology company. The LFD is a rapid lateral flow device based on colloidal gold immunochromatography designed to detect the presence of SARS-CoV-2 nucleocapsid antigens in nasopharyngeal swabs (1). The test can provide a result within 30 minutes allowing rapid testing on a mass scale.

The Innova LFD has very quickly been put into implementation by the Department for Health and Social Care (DHSC) and was employed in the city of Liverpool to deliver an ambitious mass-scale surveillance project of the city over a 2 week period (2). Data from the city council (3) shows that 71,684 LFD tests were performed alongside 51,855 gold-standard PCR tests (a total of 119,054 residents tested) with 439 people testing positive (0.37% positivity rate). The LFD tests are now being used in a pilot project to support people visiting relatives in Care Homes, and are being rolled out to support testing of University students before leaving campus to return to their family homes for the Christmas break.

In order to support this growing planned use of the Innova LFD test, the University of Oxford and Public Health England performed a series of validation trials of the LFD, benchmarking their performance against RT-PCR using swabs from a number of research trials in the United Kingdom (4). These included comparative testing on samples taken for the FALCON study evaluating diagnostic platforms, and bespoke trials including PHE, hospital, military staff and schools. The key headline findings of the validation report were that the LFD had a Limit of Detection (LoD) of around 100 plaque forming units/ml or 100,000 RNA copies/ml. In the report it is not made clear which RT-PCR assay is used in the comparison, but the Ct
value given of 25.5 equating to 100 pfu/ml suggests it is not the ThermoFisher Covid-19 TaqPath assay (5) employed in the majority of Pillar 2 testing labs in the United Kingdom. As such the validation report may not fully indicate the potential of the performance of the LFD against the vast majority of Covid PCR testing done in the UK through Pillar 2

Methods

University of Birmingham is home to a national pillar 2 testing laboratory, termed Turnkey lab, which conducts SARS-CoV-2 PCR diagnostics on behalf of DHSC (6). The laboratory uses the ThermoFisher Covid-19 taqPath assay used routinely in the Lighthouse laboratory testing network and tests a range of samples from mobile and stationary test sites (6). As part of pillar 2 testing our laboratory also conducts PCR testing as part of the national Care Home surveillance plan implemented by DHSC to test all are home staff and residents to assist in control of Covid-19 transmission in UK care homes (7).

Between October 25th and November 5th the Birmingham Turnkey laboratory processed a total of 19,176 PCR tests on home test and care home samples from across the United Kingdom. Of these 641 samples tested positive for SARS-CoV-2 using the cut off of two of three gene targets amplifying at a Ct value of 35 or under (6). This gives a positivity rate of 3.3%, around the rate that might be reasonably be expected in a large random surveillance of the UK population at that moment in time.

The Turnkey laboratory employs a number of controls during the PCR process, including the addition of an inactivated SARS-CoV-2 from the Qnostics PCR control panel (8). The Qnostics panel allows the lab to determine the Ct value for a given range of viral titres in human samples and benchmark our RT-PCR data.
Results

We collated the RT-PR raw data from three technical replicates of assays performed on the Qnostics SARS-CoV-2 analytical Q-panel – 01 and generated average Ct values for each of the known viral titres provided in the panel (Fig 1).

| QNostic Sample ID | Copies/ml | Log10 Copies | Orf1ab | S Gene | N Gene |
|-------------------|------------|--------------|--------|--------|--------|
| SCV2AQ01-S01      | 1000000    | 6            | 15.3 (100%) | 16.5 (100%) | 15.8 (100%) |
| SCV2AQ01-S02      | 1000000    | 5            | 18.3 (100%) | 20.5 (100%) | 17.4 (100%) |
| SCV2AQ01-S03      | 10000      | 4            | 22.8 (100%) | 24.9 (100%) | 23.6 (100%) |
| SCV2AQ01-S04      | 5000       | 3.7          | 19.5 (100%) | 24.8 (100%) | 24.1 (100%) |
| SCV2AQ01-S05      | 1000       | 3            | 25.9 (100%) | 28.6 (100%) | 26.6 (100%) |
| SCV2AQ01-S06      | 500        | 2.7          | 25.8 (100%) | 29.1 (100%) | 25.8 (100%) |
| SCV2AQ01-S07      | 100        | 2            | 27.7 (33%) | 30.3 (66%) | 30.8 (100%) |
| SCV2AQ01-S08      | 50         | 1.7          | 29.3 (17%) | 31.1 (27%) | 29.1 (55%) |
| SCV2AQ01-S09      | Negative   | ----         | Negative | Negative | Negative |

Figure 1: Analytical sensitivity and specificity of the Birmingham Turnkey lab RT-PCR pipeline. This was assessed against the commercial Qnostics SARS-CoV-2 analytical Q-panel – 01. Ct values are a median of 5 independent technical replicate, and figures in parentheses indicate the percentage of replicates returning a PCR positive for that given gene target (Ct < 35).

Using this data we determined that at 100 viral copies per ml (the designated LoD for the Innova LFD – 3) the equivalent Ct values for the pillar 2 PCR assay would be a Ct of 30.8 based on the N gene target, requiring a second gene to also amplify at a Ct below 35. We collated the raw RT-PCR data for all 641 of our positive samples as of November 5th, and ranked them according the N gene Ct value (Supplementary table). We then plotted the distribution of Ct values for our 641 positive samples (Fig 2).
Figure 2: Graph plotting raw Ct values (Y-axis) for all 641 positive samples in the Birmingham Turnkey lab (Y-axis). Ct values for each of the targets (Orf1, N, S) are plotted, with a sample only called positive if at least 2 of the three targets have a Ct < 35. The red line indicates the N gene Ct value equating to 100 viral copies/ml, the previously determined LoD for the Innova LFD.

Using the LoD for the LFD reported in the Oxford University/PHE report of 100 pfu/ml, we determined that this would correlate with an N gene Ct value of 30.8 plus one other gene target amplifying at a Ct < 35. By applying this theoretical level of performance to the LFD we determine that 99 of our positive samples would not be able to be detected by the Innova LFD given that the Ct value of N gene is above 30.8. This equates to 15.44% of our
true positive RT-PCR samples being missed by the LFD. This means that the theoretical sensitivity of the Innova LFD when compared to Pillar 2 samples from low-prevalence, asymptomatic population screening similar to student and care home surveillance, the Innova LFD has a sensitivity of 84.56%.

Limitations

It should be noted that the level of performance above is a theoretical level of performance based on extrapolation of published limit-of-detection data of the Innova LFD. No direct comparison of the LFD and the pillar 2 RT-PCR has been performed on equivalent samples. Additionally the Oxford/PHE report outlines differences in sensitivity as a result of how the swab is taken, and this is not factored in our analyses. However our data does suggest an improved level of theoretical sensitivity of the Innova LFD when comparison is made to the RT-PCR assay used within Pillar 2 testing laboratories, and on sample types taken from equivalent population types on the basis of viral prevalence.

References

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