UK Health Security Agency

Technical report: an analysis of real-world performance of lateral flow antigen devices in detecting SARS-CoV-2 Omicron variant (B.1.1.529)

Date of reporting period: 10 November 2021 to 13 March 2022

Contents

Introduction

Background

The UK Health Security Agency (UKHSA) operates a programme of post-market surveillance to evaluate performance of lateral flow devices (LFDs) in detecting new and emerging variants of SARS-CoV-2. This comprises real-world performance evaluation, via routine monitoring of 'real-world' data (data collected from non-interventional and non-controlled settings). Real-world data (RWD) in this context refers to data captured systematically from individuals as part of the testing programme[1] , and mostly stored in databases from health electronic records (HER). This Real-World Performance Monitoring (RWPM) is essential to detect early signals of changes in actual performance of the diagnostic tests with emerging SARS-CoV-2 variants as used in the National Testing Programme (NTP).

While capture of the data within RWPM is systematic, the inherent nature of the source data means its interpretation must always be treated with caution, recognising the limitations of the specific data looked at, the testing regimes in place and taking into account other information available. It is carried out alongside the wider UKHSA Porton Down programme, where in vitro assessment using live virus cultured from clinical samples gives the ability to monitor the ability of LFD's ability to detect emerging variants. Alongside the evaluations using HER, an evaluation with primary collection of data was conducted as a services evaluation (1) to provide evidence when the information required was not routinely collected.

SARS-CoV-2 variants that are considered to have concerning epidemiological, immunological or pathogenic properties are put under investigation and designated a variant of concern (VOC)[2] by the relevant expert committee.

Omicron (B.1.1.529) VOC (VOC-21NOV-01) was first detected in the UK in November 2021 (2) and the sub-variant BA.2 in December 2021. Omicron became the dominant variant in the UK in December 2021 (3). As part of the activities of the RWPM, an enhanced variant monitoring process was triggered by the emergence of this new VOC. This process sought to track the ability of LFDs used by NHS Test and Trace (NHSTT) to detect the VOC (measured as detection rate – definition given in methodology) and identify any issues with detection rate of Omicron that needed to be escalated for further investigation. The decision to cease enhanced RWPM, that is, return to routine monitoring, is done when enhanced RWPM analysis shows that the LFD are performing within acceptable levels described in methods.

[1] For more information see MHRA guidance on the use of real-world data in clinical studies to support regulatory decisions and Analysis of routinely collected data: descriptive studies.

[2] Viral variants with changes in transmissibility, severity, or immune evasion compared to the current dominant variant, and/or a growth rate potentially compatible with the eventual replacement of the current dominant variant. See: UKHSA 'COVID-19 variants identified in the UK', last update on 6 May 2022, and UKHSA Research and analysis 'Variants: distribution of cases data'. Updated 6 May 2022.

This document describes 4 evaluations conducted between November 2021 and March 2022 when the SARS-CoV-2 viral variants predominant were Omicron B.1.1.529 and Omicron BA.2. These evaluations aimed to assess the performance of the diagnostic tests for coronavirus (COVID-19) (Table 1 below). Three evaluations were based on HER, and one was a cross-sectional service evaluation of ongoing performance.

Overview of the studies

The main characteristics of these 4 evaluations are described below and in Table 1.

Three RWPM evaluations (hereafter referred to as eval 1, eval 2, and eval 3) were based on HER, conducted as the enhanced variant monitoring process triggered by the emergence of Omicron (B.1.1.529), and aimed to gather information on the performance of LFD to detect Omicron. The main evaluation question was to assess whether the LFD ability to detect the new viral variant (Omicron) was comparable with the performance exhibited for variants that occurred before (known performance). These evaluations were undertaken in the order presented in response to emerging concerns with each subsequent analysis approach informed by the results of the previous analysis.

A diagnostic accuracy evaluation was conducted as a cross-sectional study (hereafter referred to as eval 4). The first evaluation aimed to assess the sensitivity of a specific LFD device (Acon Flowflex LFD test) for detecting the Omicron VOC (B.1.1.529), and the second evaluation aimed to assess the difference in average viral concentration obtained from 2 sequential PCR samples (during the period when Omicron BA.2 was dominant).

Table 1. Main design characteristics of the evaluations conducted to assess the performance of the diagnostic tests for COVID-19

Evaluation[1]	Study design[2]	Matching LFD and PCR results	Objective	Study period[3]	Viral variant analysed	
					Under investigation	Reference[4]
Eval 1 14 January 2022	HER	On the same day	Compare detection rates by viral variant and LFD test, and difference between detection rates	10/11/2021 to 10/01/2022	Omicron (B.1.1.529)	Delta (B.1.617.2)
Eval 2 15 February 2022	HER	Within 2 days	Further analysis to assess the distribution of viral concentration and concentration of >1 million viral copies per millilitre (mL) as reference	10/11/2021 to 06/01/2022	Omicron (B.1.1.529)	Delta (B.1.617.2)
Eval 3 21 March 2022	HER	On the same day	Compare detection rates by viral variant and LFD test, and difference between detection rates	19/01/2022 to 13/03/2022	Omicron BA.2 strain	Omicron (B.1.1.529)
Eval 4 5 May 2022	CS	N/A	Estimate the sensitivity of Acon Flowflex LFD test for Omicron using PCR as reference test	11/01/2022 to 02/02/2022	Omicron (B.1.1.529)	NA

Notes to Table 1

[1] Date of the original report.

[2] HER: cross-sectional analysis based on routinely collected health electronic records CS: cross-sectional study.

[3] For eval 1, eval 2, eval 3, this is the date that the data used in the analysis was registered in the HER databases, and for eval 4 the date of the data collection.

[4] Viral variant previously assessed and used as known performance.

Ethics

Within the context of the pandemic public health response and roll out of testing interventions, a research ethics approval by the UK Health Research Authority (HRA) was not required based on the HRA tool and after further discussions with the HRA. After an initial period, it was determined to gain Public Health England's Research Ethics and Governance Group (PHE REGG) approval for service evaluations and ongoing evaluations where additional samples/tests were requested to ensure further external scrutiny and assurance on this approach. This was reviewed and approved under REGG R and D 438.

Evaluations based on health electronic records (eval 1, 2 and 3)

Objectives

The 3 evaluations based on HER (eval 1, eval 2, eval 3) assessed the ability of lateral flow devices (LFDs) used in the national testing programme for detecting (detection rate) B.1.1.529 Omicron (VOC-21Nov-01). The LFDs used were:

1. Innova SARS-CoV-2 lateral flow antigen tests (Innova 25s) and DHSC COVID-19 self-test kits (DHSC 3&7s) together analysed as 'Biotime', since these are essentially the same kits but with different buffer bottles, and Innova 25s are for professional use where DHSC 3&7s are for self-testing.
2. ACON Flowflex SARS-CoV-2 antigen rapid test (Acon).
3. Orient Gene COVID-19 Ag Rapid Test Cassette LFD antigen tests (Orient Gene).
4. SureScreen SARS-CoV-2 Antigen Rapid Test Cassette V2 (SureScreen).

These evaluations used the LFD as the test under investigation (index test) and the PCR as the reference standard. These evaluations assessed the performance of LFDs for Omicron in comparison to the performance of LFDs for a variant with a known performance profile. The evaluations corresponded to 3 analyses:

1. Eval 1: Analysis aimed to compare detection rates for the 1) variant Omicron (B.1.1.529) BA.1 lineage with the detection rate for the 2) variant Delta (B.1.617.2) (used as the reference variant, that is, with a known performance profile).
2. Eval 2: Further analysis aimed to give more details on the comparison between detection rates for Omicron (B.1.1.529) BA.1 and Delta (B.1.617.2), in relation to the distribution of viral concentration and taking viral concentration >1 million RNA as the reference value.
3. Eval 3: Analysis aimed to compare the detection rates for the 1) variant Omicron BA.2 lineage with the detections for the 2) BA.1 lineage (used as the reference variant, that is, with a known performance profile).

Methodology

Participants

In Real-World Performance Monitoring (RWPM), the target population is the total number of individuals tested for SARS-CoV-2 infection. In the context of the eval 1, eval 2 and eval 3, the target population[3] was conceived as composed by all individuals resident in England who took their tests under Pillar 2 of the National COVID-19 Testing Programme, during the period that the data was retrieved from the HER databases in each evaluation (Table 1). The population analysed were those from the target population who had valid data on the PCR test and LFD test results in the HER databases. At the time of analysis, all individuals with a positive LFD result were instructed (via text message when registering the result and in national guidance) to undertake a confirmatory PCR. Certain other groups also undertook dual PCR-LFD testing such as Adult Social Care and NHS staff. The population analysed was considered to be likely representative of the target population.

Data source and study period

The HERs LFD and PCR results were retrieved from the National Pathology Exchange (NPEX) database with viral variants identified through the Public Health England and Wellcome Sanger Institute's Second-Generation Surveillance System (SGSS). The data were retrieved from the databases on different dates: Eval 1, 10 November 2021 to 10 January 2022; Eval 2, 10 November 2021 to 06 January 2022; Eval 3 19 January 2022 to 13 March 2022.

Method to match LFD and PCR tests

Initially, all valid LFD results (positive or negative) were matched with valid PCR results (positive or negative) belonging to the same individual within 2 days, by linking the subject ID field in the databases. Then, any LFD test that was matched to 3 or more PCRs within 3 days were eliminated from the analysis to avoid matching tests that belong to different individuals.

Eval 1 and eval 3 aimed to compare detection rates and only data from matched pairs of LFD-PCR results registered on the same day were used. In the eval 2, data from matched pairs of LFD-PCR results within 2 days were used. We assumed that if the LFD and PCR tests were conducted on the same day, or within 2 days (LFD before the PCR), the PCR was conducted to confirm a previous result from an LFD test (confirmatory PCR).

[3] Target population means to whom the results of an analysis intend to make inference to. In this evaluation, the population comprised by those who attend the NHSTT to have a COVID test.

PCR tests

The PCR tests were processed in Lighthouse Laboratories (4). The number of SARS-CoV-2 RNA copies in the samples analysed (viral concentration) is measured as the number of RNA copies per mL of the sample examined. In the PCR method (PCR), cycle threshold (Ct) is the number of cycles required to return a positive result. The viral concentration is derived from the Ct value. If a PCR test requires a high number of cycles to detect RNA copies, the concentration of viral RNA in the sample is low, and vice versa. The higher the viral concentration, the higher the risk of transmission (5), and LFD tests require approximately ≥10,000 RNA copies per mL to be positive (6).

Statistical analysis

Detection rate

Among those paired LFD-PCR samples registered on the same day, and with a positive PCR result for the variant in question, the following ratio was calculated: 1) the number of individuals with positive LFD results divided by 2) the total number of individuals with positive and negative LFD results, as the formula below:

$$\frac{\text{Number of positive LFD results among those with positive PCR for variant x}}{\text{Total number of LFD results (positives and negatives) among those with positive PCR for variant x}}$$

Text equivalent of formula
Number of positive LFD results among those with positive PCR for variant x divided by the total number of LFD results (positives and negatives) among those with positive PCR for variant x.

The detection rate was expressed as a proportion (or percentage) with 95% confidence intervals (CI) calculated using the Wald method, and stratified by viral variants, LFD kit, and viral concentration expressed as a categorical variable. Viral concentration was broken into 6 categories: above 10 million (>10 million), one to 10 million (1 to 10 million), 100,000 to one million (100,000 to 1 million), 10,000 to 100,000 (10,000 to 100,000), 1,000 to 10,000 (1,000 to 10,000), and 100 to 1,000 (100 to 1,000) RNA copies per mL.

It is worth noting that this analysis relied on the results from PCR assumed as confirmatory PCR results, that is, taken to confirm a previous LFD test result. This means that people who had a negative LFD result were less likely to have a PCR test and be included in this analysis. The assumption is made that this bias is equivalent between variants, meaning it would not differentially affect the groups being compared and, as such, is not expected to bias the results of the question under investigation. However, this artificially inflates the proportion of LFD results that are also positive PCR results (True Positive). Because of that, the calculation of detection rate is not the same as ‘sensitivity rate’ despite the fact they have similar formulae: the

proportion of reported positive LFD tests among those with a positive PCR result does not correspond to a sensitivity rate. To estimate a sensitivity rate in a typical diagnostic accuracy study, all individuals submitted to a test under investigation (in this case, LFD tests) are also submitted to the reference tests (in this case, the PCR tests).

Relative detection rate

To compare the performance of the LFD tests for a viral VOC to the performance for another viral variant taken as reference, the absolute difference in the detection rates (hereafter referred to as relative detection rate) was calculated:

For Omicron variant (B.1.1.529) in relation to Delta variant (B.1.617.2):

$$(\text{Detection rate for Omicron B.1.1.529}) - (\text{detection rate for Delta B.1.617.2})$$

Text equivalent of formula
Detection rate for Omicron B.1.1.529 minus detection rate for Delta B.1.617.2.

And for Omicron (BA.2) in relation to Omicron variant (B.1.1.529):

$$(\text{Detection rate for Omicron BA.2}) - (\text{detection rate for Omicron B.1.1.529})$$

Text equivalent of formula
Detection rate for Omicron BA.2 minus detection rate for Omicron B.1.1.529.

The relative detection rates are presented as difference in percentage points and with the respective 95% CI using the Wald method.

Normalisation

It is known that the sensitivity of the LFD tests increases as viral concentration of the sample increases (7, 8, 9). Therefore, any difference in detection rates for detecting 2 different variants could potentially be due to differences in the viral concentration in the samples analysed. To ensure that the comparison of detection rates between a VOC and a variant with a known performance profile is a true comparison of performance for that variant and not simply a difference in viral concentration in the samples, a normalisation of the viral concentrations was done. The viral concentrations seen in the VOC were adjusted to the viral concentrations seen in the variant with a known performance profile, following the steps below:

1. For each LFD type, each count/frequency of Omicron VOC cases in each viral concentration category is divided by the total cases over all viral concentration categories to create weights for Omicron LFD positive cases and respective total paired PCR positive results.

2. The weights are divided by an equivalent ratio for Delta for the same viral concentration category to create a scale factor.
3. The scale factor is multiplied by the observed Omicron VOC case frequencies to obtain normalised frequencies.
4. The normalised values are then used to calculate the normalised relative detection rates.

In eval 2, the analyses were done in relation to viral concentration >1 million. At >1 million RNA copies per mL, it is expected that LFD will detect close to 100% of positive B.1.617.2 Delta cases. In order to understand the LFD detection rate relative to the detection rate with viral concentration (VC) >1 million RNA copies per mL, the Biotime LFD at >1 million RNA copies per mL for the Delta variant was taken as the reference value. The normalised detection rate for each LFD kit for each variant at each viral concentration was then divided by this reference rate. The reference of the same LFD kit with the same variant at viral concentration >1 million RNA copies per mL was also used in the same way.

The analyses were not stratified by participant characteristics (for example, age, sex, presence of symptoms), or test setting (self-testing or assisted).

Results

Descriptive analysis

The query of the National Pathology Exchange (NPEX) database yielded 50,706,399 LFD results between 10 November 2021 and 10 January 2022 (analysis Omicron versus Delta); and 37,558,694 results registered between 19 January 2022 and 13 March 2022 (analysis BA.2 versus BA.1 sub-lineages of Omicron). Table 2 below shows the numbers of pairs of matched LFD and PCR results analysed in different comparisons. The viral variant Omicron was more frequent because it was the dominant variant during the period that the data were registered. Data related to the SureScreen LFDs had small numbers for the Omicron versus Delta analysis and was therefore not conducted.

Table 2. Number of pairs of matched LFD and PCR results in which the viral variants analysed were found, separately for LFD types of tests and analyses

Table 2a. Analysis: Delta versus Omicron

Analysis and viral variants	Biotime	Acon	Orient Gene	SureScreen
Delta (B.1.617.2) - reference	20,731	27,623	3,057	10
Omicron (B.1.1.529)	138,087	106,248	47,084	954

Table 2b. Further analysis: Delta versus Omicron

Analysis and viral variants	Biotime	Acon	Orient Gene	SureScreen
Delta (B.1.617.2) – reference	15,876	31,470	3,700	15
Omicron (B.1.1.529)	86,215	164,248	56,850	734

Table 2c. Analysis: Omicron BA1 versus BA.2

Analysis and viral variants	Biotime	Acon	Orient Gene	SureScreen
Omicron (B.1.1.529, BA.1) – reference	33,558	32,084	14,034	2,304
Omicron (B.1.1.529, BA.2)	10,799	7,671	3,373	582

Evaluation 1: Omicron (B.1.1.529) compared to Delta (B.1.617.2)

Detection rates

Figures 1, 2 and 3 show the detection rates for Omicron (B.1.1.529) and Delta (B.1.617.2) variants for the viral concentration category of the samples for each of the LFD kits (Biotime, Acon and Orient Gene). The findings suggest that the detection rates for both variants were similar up to the viral concentration of 10,000. Below 10,000, the rates with Biotime and Orient Gene diverge but with the detection rate for Omicron higher than for the rate for Delta.

Figure 1. Detection rate of Omicron and Delta for Biotime LFD kits at different viral concentrations

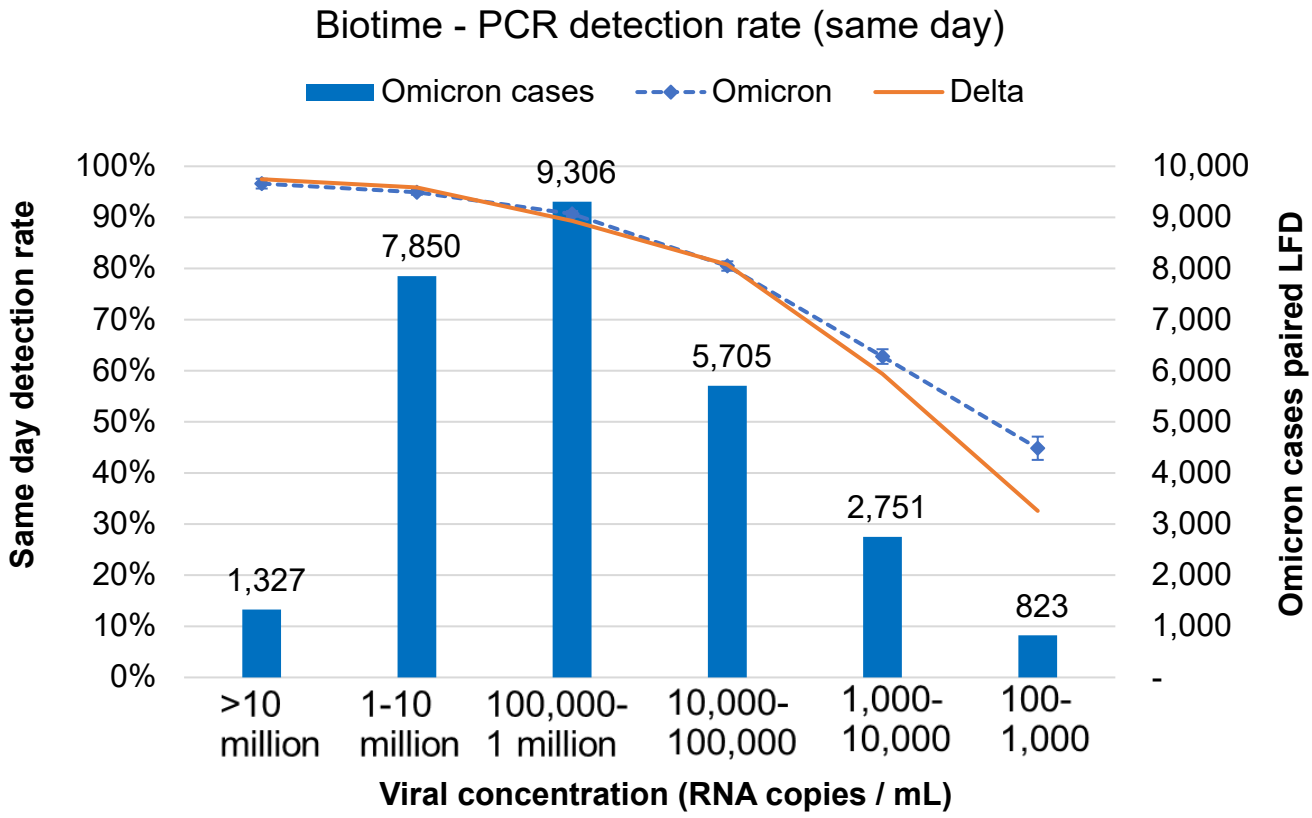


Figure 2. Detection rate of Omicron and Delta for Acon LFD kits at different viral concentrations

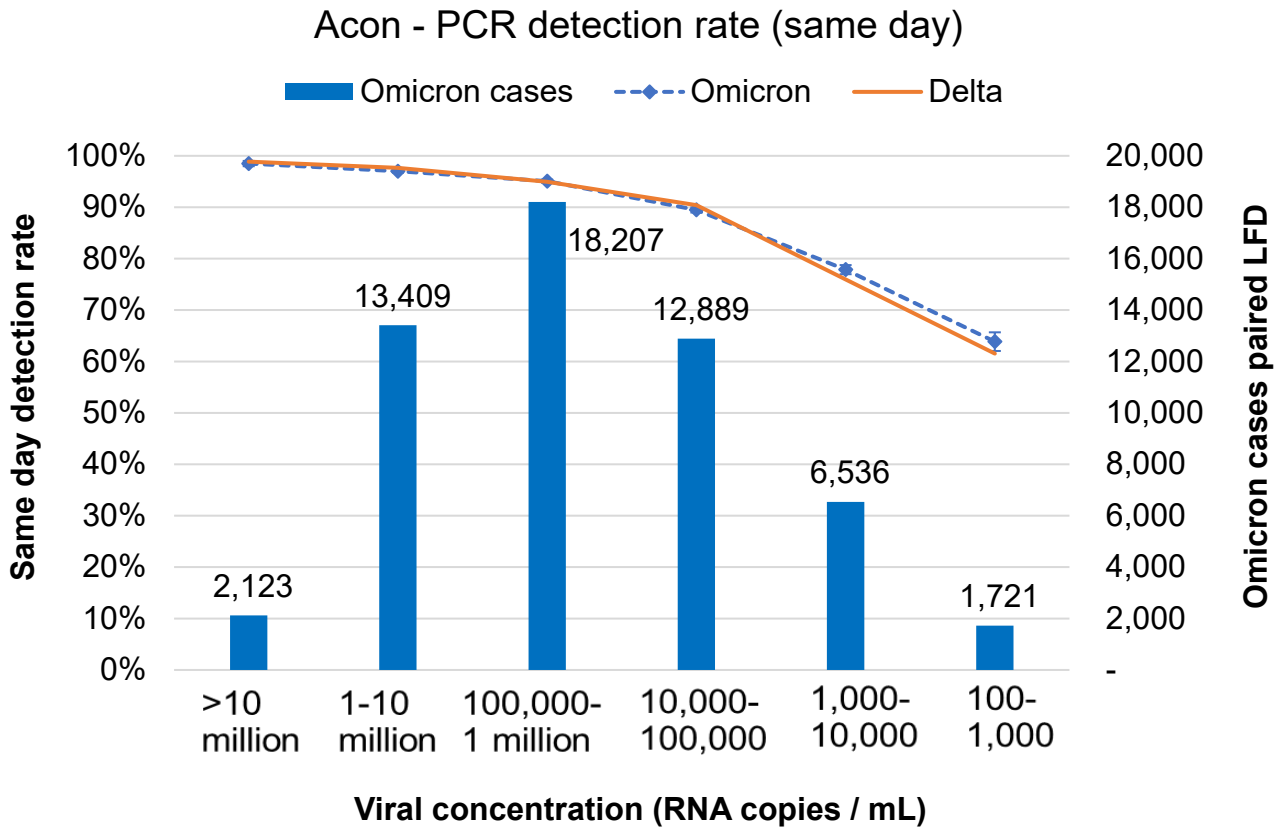
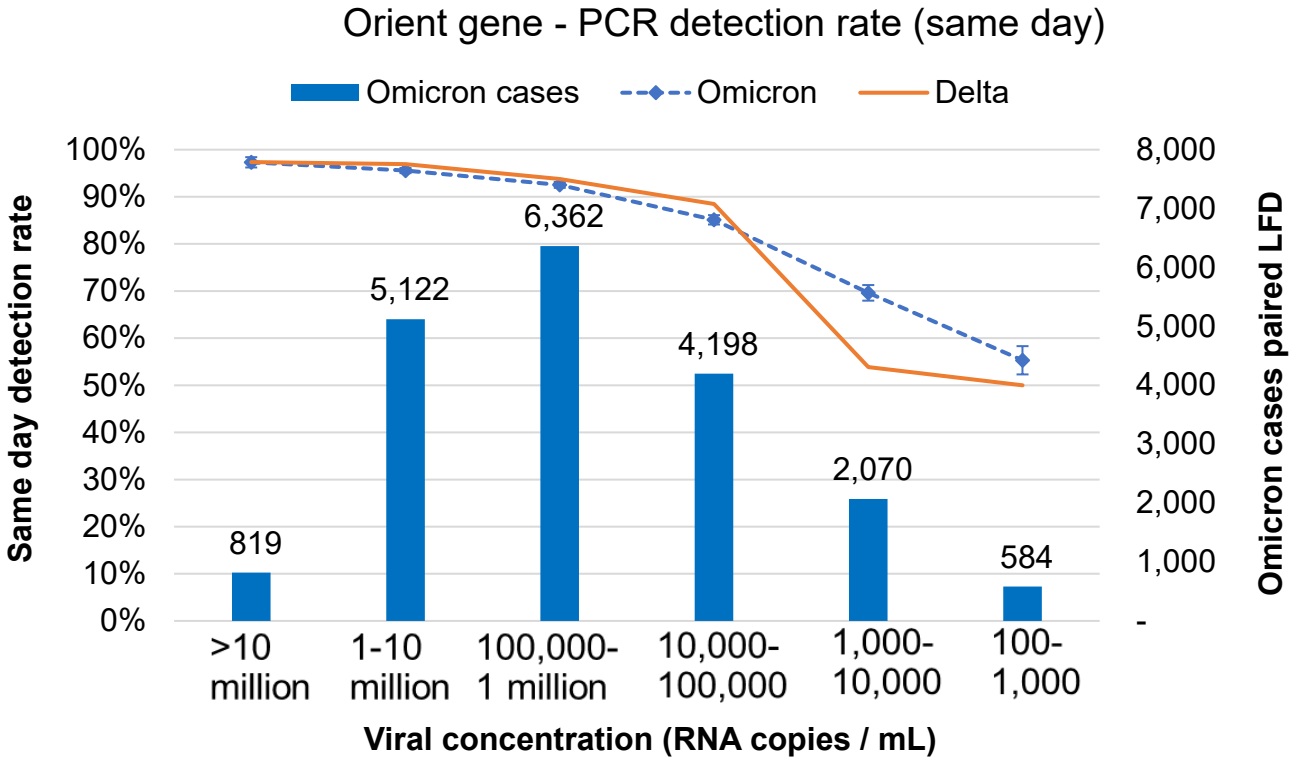


Figure 3. Detection rate of Omicron and Delta for Orient Gene LFD kits at different viral concentrations



Relative detection rates

Figure 4 shows the absolute difference between the detection rates for Omicron minus Delta in those samples with a viral concentration >10,000 RNA copies per mL and Figure 5 shows the normalised absolute difference. The absolute differences rates are presented as percentage points, separately for the type of LFD kit, with the 95% confidence intervals. A positive relative detection indicates that the detection rate for Omicron was higher than the detection rate for Delta, and a negative value that the detection rate for Omicron was lower than that for Delta.

The figures show that when the detection rates were normalised for viral concentration (Figure 5), the results for all LFD kits are similar between variants.

Figure 4. Relative detection rate for viral concentration >10,000 RNA copies per mL without normalised detection rates

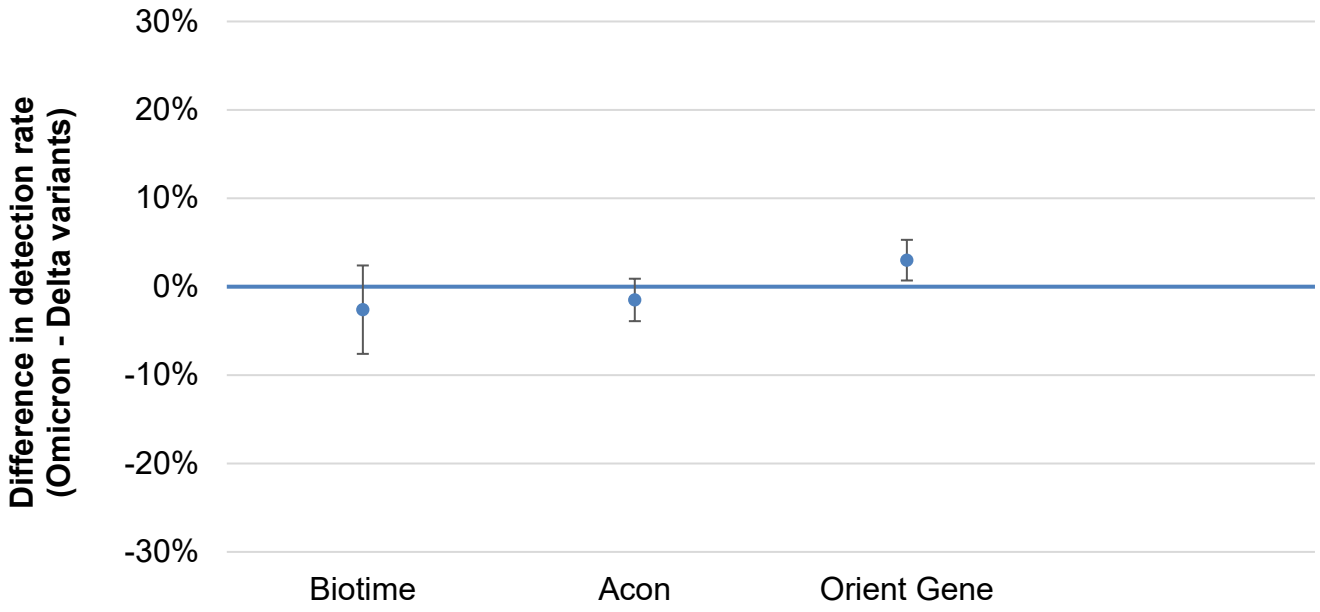
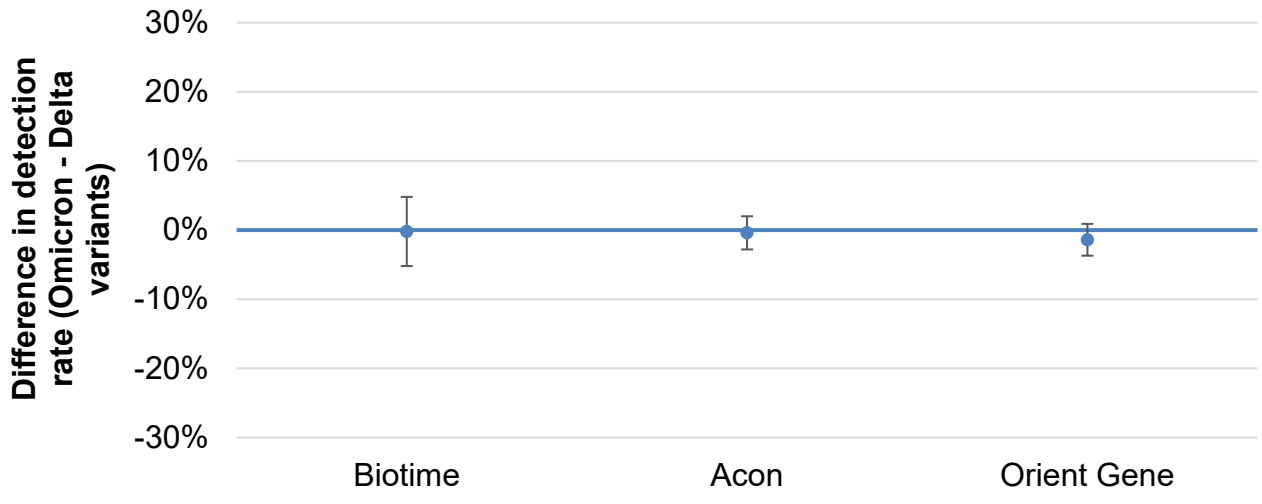


Figure 5. Relative detection rate for viral concentration >10,000 RNA copies per mL, with normalised detection rates



Evaluation 2: Analyses in relation to viral concentration >1 million

Figure 6 below presents the ratios between the normalised detection rate for each of the 3 LFD kits for each variant at each viral concentration category, over the detection rate for Biotime LFDs for Delta at >1 million RNA copies per mL (at which it would be expected that close to 100% of cases would be correctly identified). The horizontal line represents the ratio equal to 1.

Since LFD detection rate is known to vary by viral concentration (above), 4 viral concentration categories were chosen to assess the effect of the variant. The lower 2 categories were combined into <10,000 RNA copies per mL since individuals are unlikely to be infectious at this level. The top 2 categories were combined into >1 million RNA copies per mL since individuals are most infectious at this level and it would be expected that LFDs would detect close to 100% of positive cases at this viral concentration and if there was variation between variants it would be important to detect at this level. However, the category cut off points are arbitrary and simply designed to enable assessment of performance.

The findings suggest that, in each viral concentration category >10,000 viral copies per mL, there were no substantial differences in the detection rates for Omicron compared to Delta at each viral concentration.

Figure 6. Ratio of normalised detection rates divided by the rate for Delta variant with Biotime kit and viral concentration >1 million RNA copies per mL

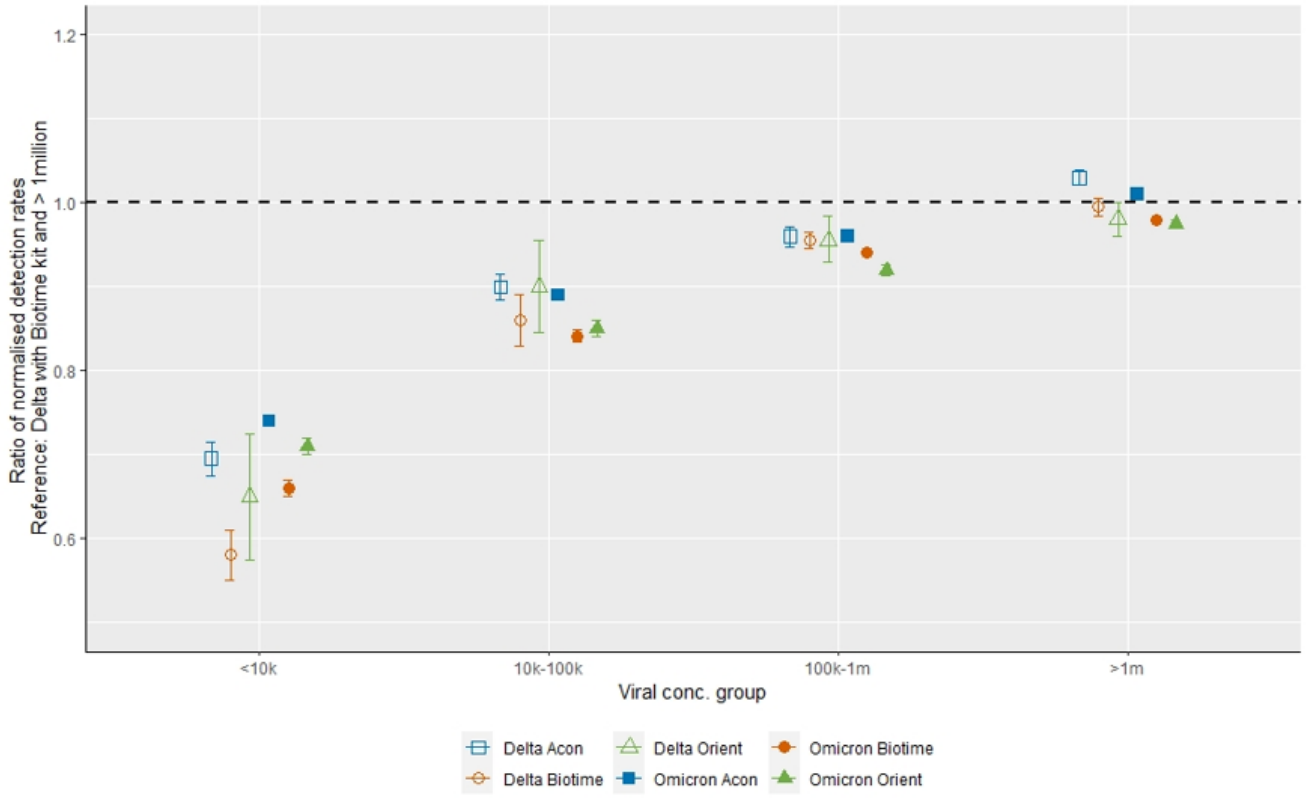
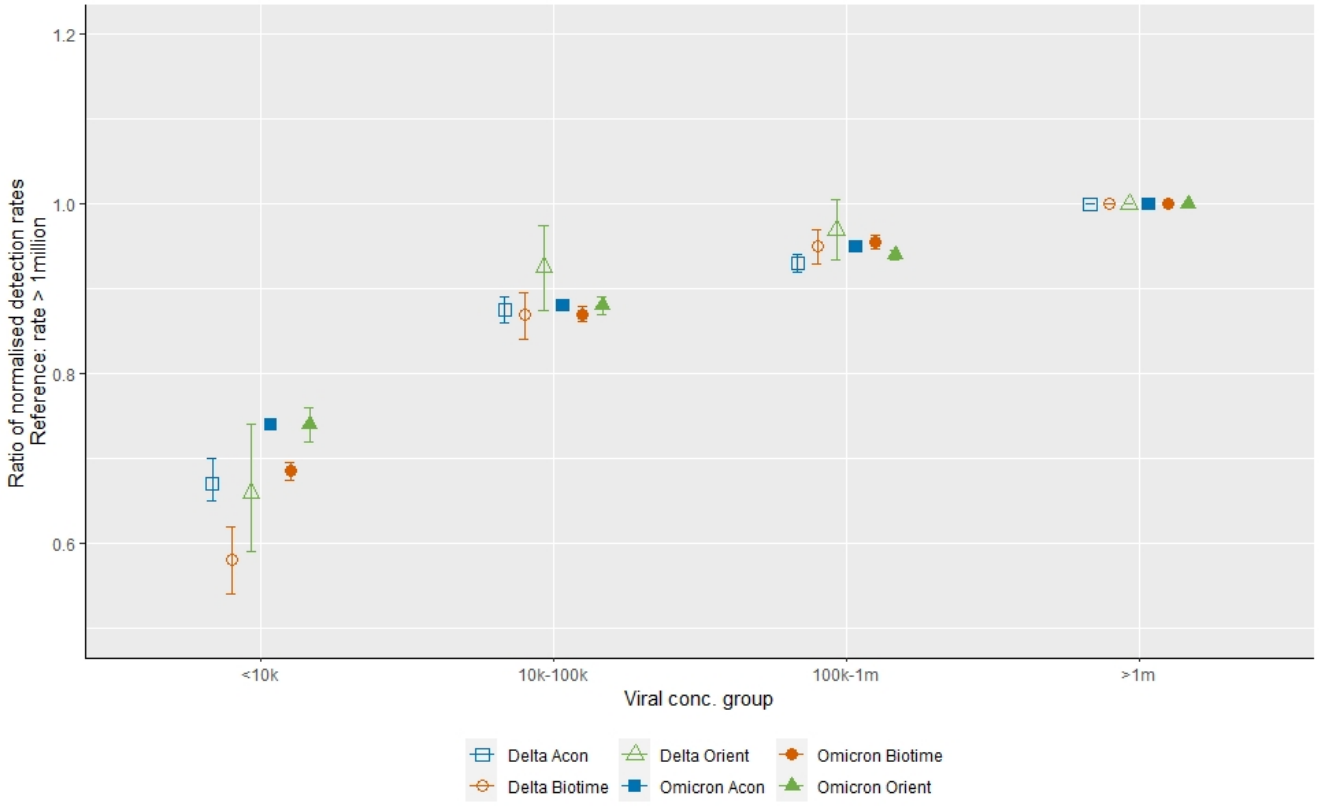


Figure 7 presents the ratios between the normalised detection rate for each of the 3 LFD kits for each variant at each viral concentration category, over the detection rate for the same viral variant and the same LFD kit at a viral concentration of >1 million RNA copies per mL (at which concentration the LFD would be expected to perform at the highest level).

In both figures, the findings suggest that all LFD kits perform similarly at the various viral concentration categories >10,000 RNA copies per mL regardless of the variant detected.

Figure 7. Ratio of normalised detection rates for each viral variant divided by the rate for the same LFD kit with the same variant at viral concentration >1 million RNA copies per mL, separately for viral concentration and LFD kit



Evaluation 3: sub-lineages of Omicron (B.1.1.529), BA.2 compared to BA.1

Detection rates

Figures 8, 9 and 10 below show the detection rates for Omicron sub-lineages BA.1 (orange line) and BA.2 (dash-dotted blue) for the viral concentration categories in the samples analysed and the type of LFD kit.

The findings suggest that the detection rates for both variants were similar above a viral concentration of 1,000. Below 1,000 the rates were different with the detection rate for Omicron lower than for the rate for BA.2 for Acon LFDs, and higher for Biotime and Orient Gene.

Figure 8. Detection rate of Omicron BA.2 and BA.1 strains for Biotime LFD kits

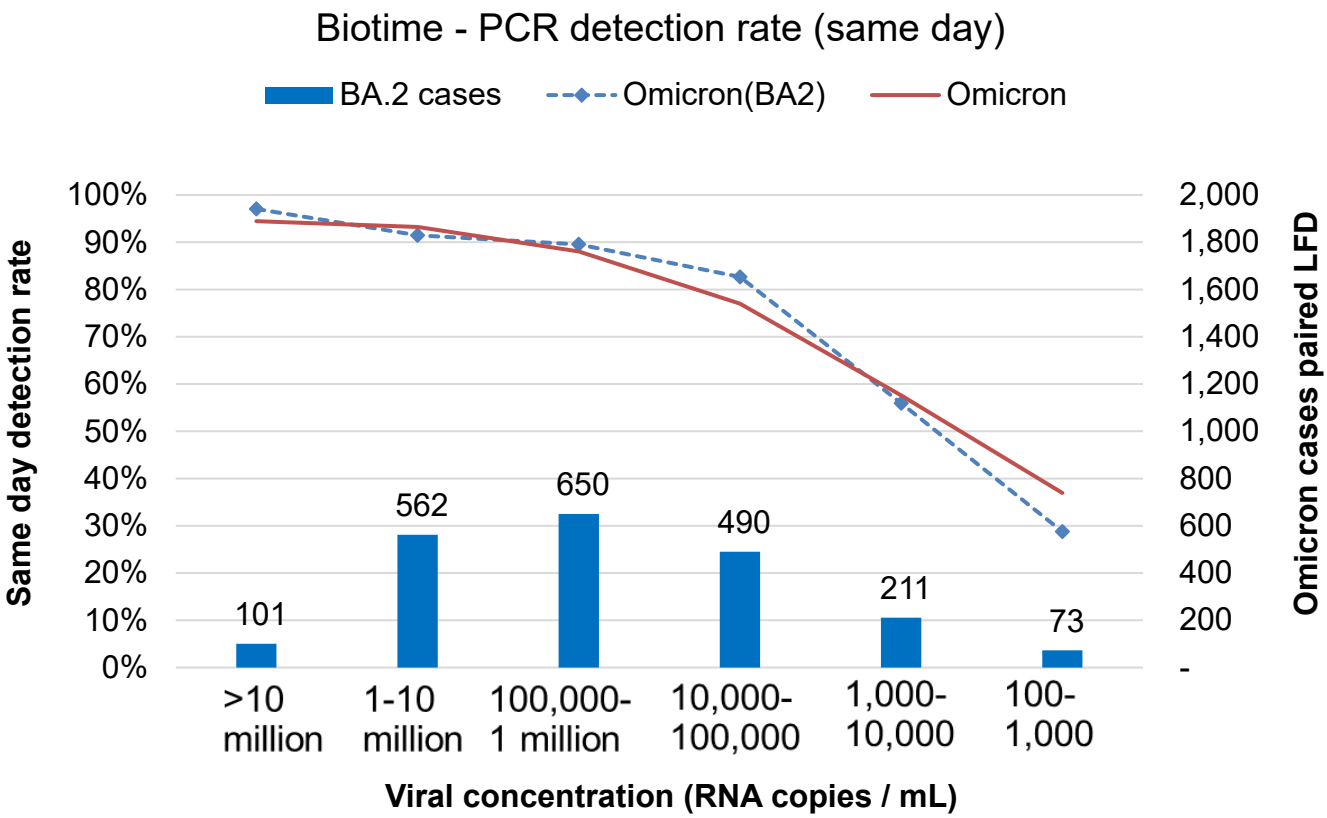


Figure 9. Detection rate of Omicron BA.2 and BA.1 strains for Acon LFD kits

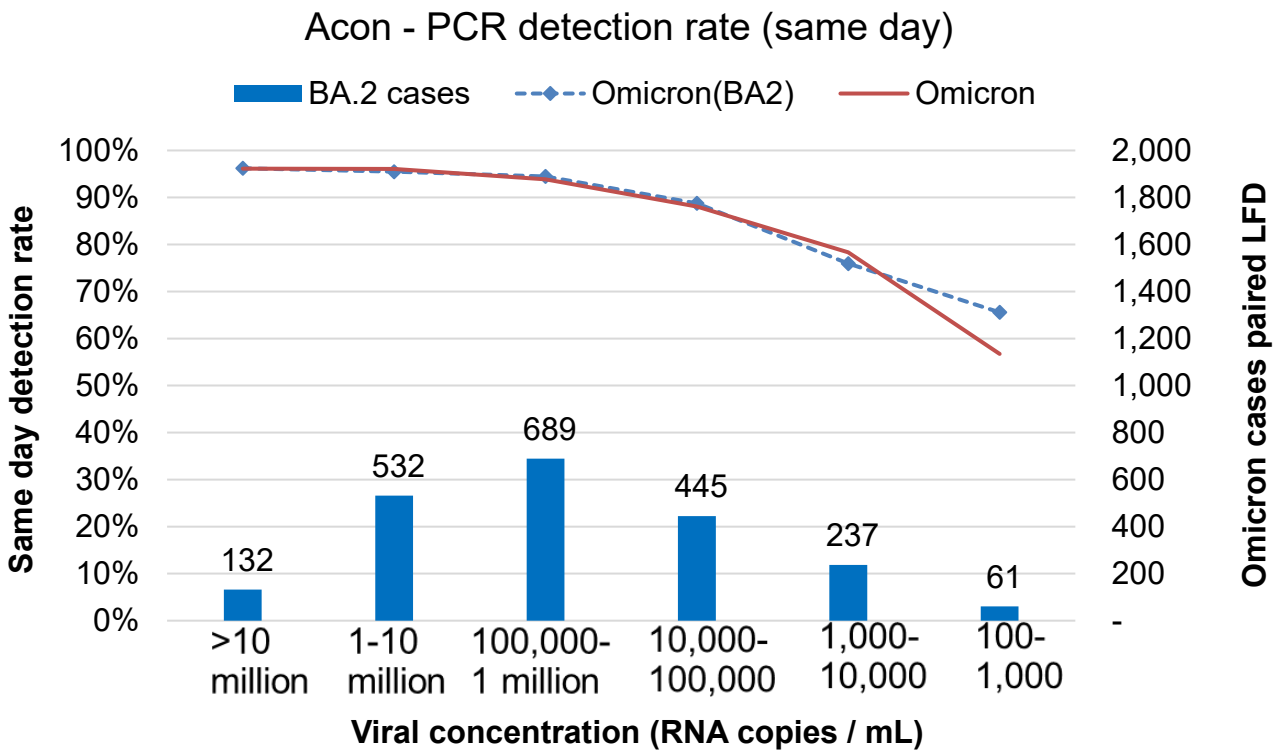
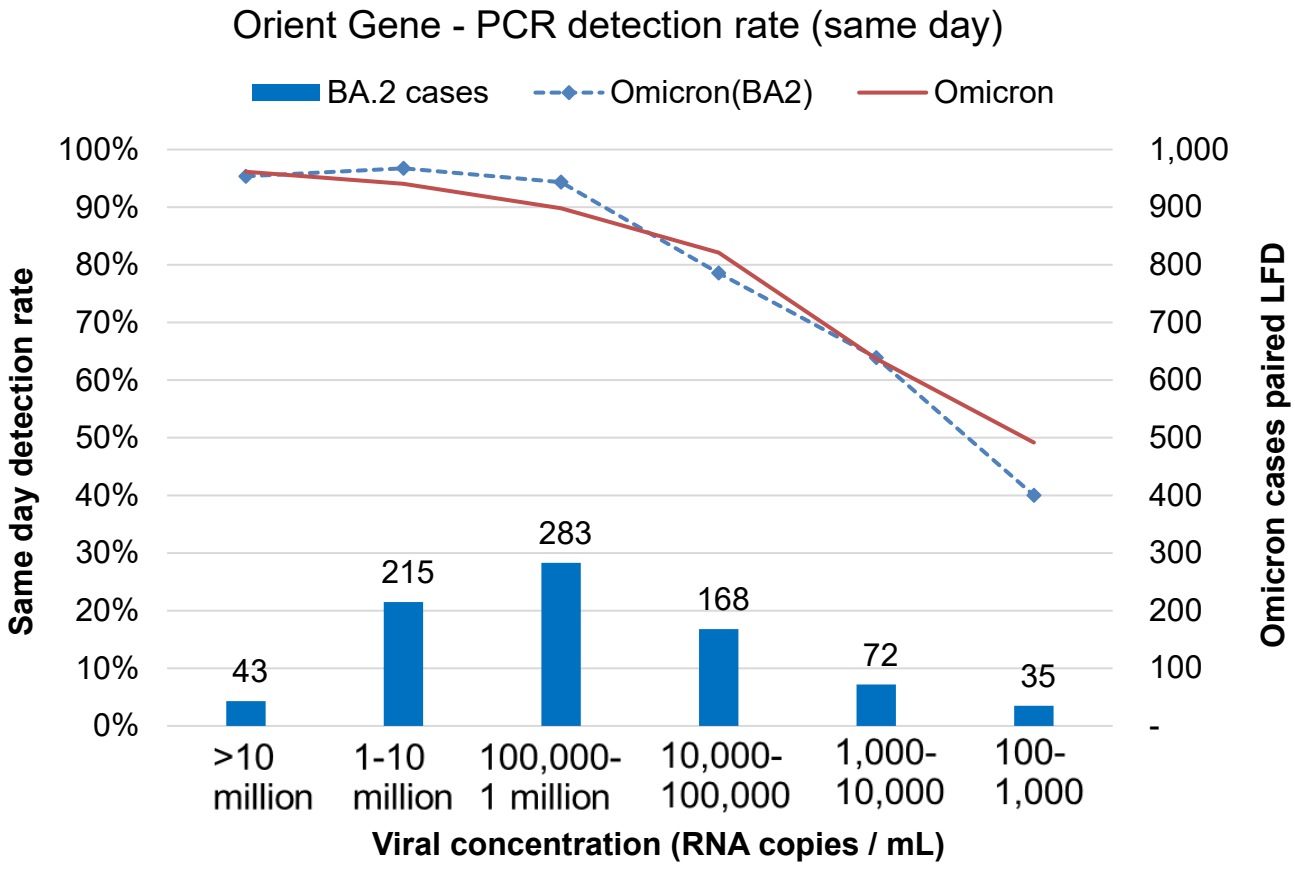


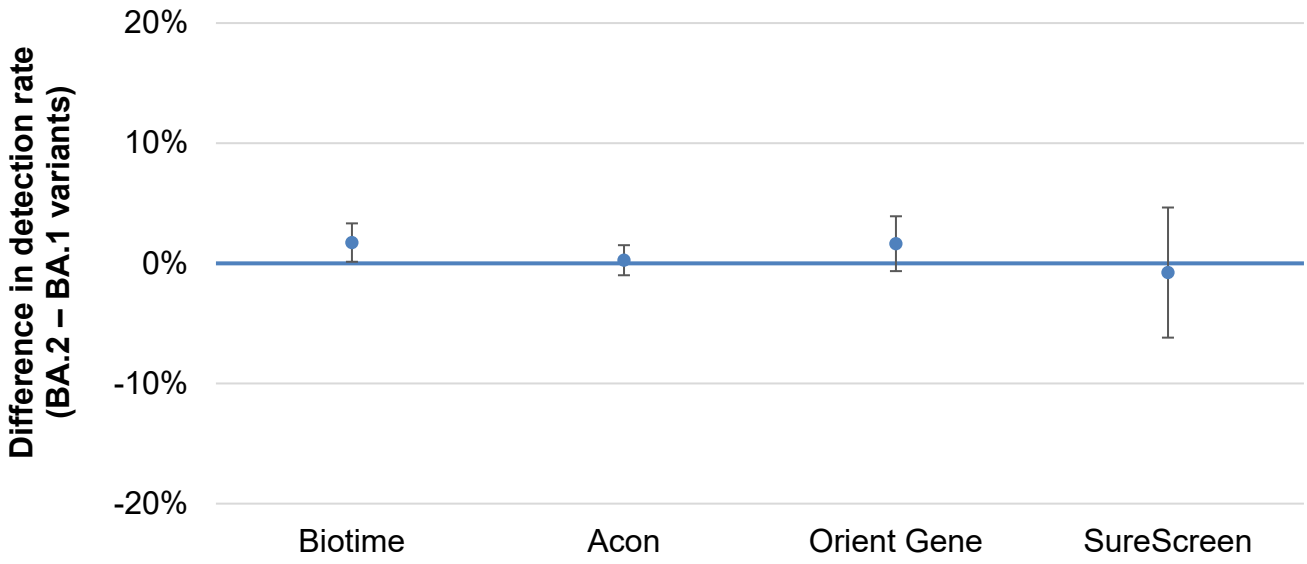
Figure 10. Detection rate of Omicron BA.2 and BA.1 strains for Orient Gene LFD kits



Relative detection rates

Figure 11 shows the absolute difference between normalised detection rates for Omicron variants (BA.2 – BA.1), when the viral concentration was >10,000 RNA copies per mL, for each LFD kit. There was only a small variation in the point estimates of the difference (range: -0.8 to 1.7%).

Figure 11. Relative detection rate for viral concentration >10,000 RNA copies per mL, with normalised rates: absolute difference between rates for Omicron sub lineages (BA.2 – BA.1)



Conclusions and some issues in the interpretation of the findings

The main findings of these analyses based on HER databases can be summarised in the following points:

1. There was no difference between the ability of the LFD kits to detect the variants Omicron (B.1.1.529) (detection rate) and Delta (B.1.617.2) when the viral concentration was ≥10,000 RNA copies per mL. Below 10,000, the rates with Biotime and Orient Gene were different but the detection rate for Omicron was higher than for Delta for all LFD kits analysed.
2. All LFD kits performed similarly regardless of variant at each VC category >10,000 RNA copies per mL.
3. There was no difference between the detection rates for Omicron sub-lineages BA.1 and BA.2 for all the LFD kits analysed.

Some issues and caveats to consider in the interpretation of the findings:

1. The LFDs were used in different settings and services.
2. Viral concentrations >10,000 were used in order to focus on infected individuals who were likely to account for 85% of transmissible cases and at which VC the LFD tests were likely to detect SARS-CoV-2 (6).
3. The number of cases tested with SureScreen LFD kits was below the required number for statistical power in the Omicron compared to Delta analysis and therefore the performance of this kit was not assessed.
4. Analysis was not broken down by the method of collection, that is, self or assisted testing.
5. At the time this analysis was conducted, due to the urgent need for the results the evaluations did not go through the full external review process; the results have subsequently been reviewed and approved.

Evaluation 4: sensitivity of Acon LFD for Omicron

Objectives

Primary objective

To estimate the sensitivity and other diagnostic performance measures of the ACON Flowflex LFD test (Acon) for detecting Omicron (B.1.1.529) (hereafter referred to as Omicron), taking the results of the polymerase chain reaction (PCR) test as the reference standard.

Secondary objectives

1. To estimate the sensitivity and other diagnostic performance measures, separately for presence of symptoms, vaccination status and viral concentration in the sample analysed with PCR.
2. To compare the sensitivity rate of the Acon LFD test for detecting Omicron with the previous results on the sensitivity rate of LFD tests for detecting the Delta variant.

Methodology

Study design

This evaluation was conducted as a diagnostic accuracy study with a cross-sectional design (10). The LFD test was the index test, and the real-time quantitative reverse transcription PCR, (hereafter referred to as PCR test) was the reference standard.

Tests

The Acon LFD test required collection with a nasal swab (both nostrils). The PCR sample was collected with the standard throat and nose swab in use within the NHSTT supply chain. qRT-PCR analyses of these samples were conducted using the ThermoFisher Applied Biosystems TaqPath COVID-19 CE-IVD RT-PCR kit deployed in the NHSTT Lighthouse Laboratories.

Participants

Participants were from a non-probabilistic sample, prospectively and consecutively recruited among those who visited 11 NHS Test and Trace Test sites in England (UK), listed below, between 11 January and 2 February in 2022. All those who visited the testing centres were considered eligible if they fulfilled the inclusion and exclusion criteria below, irrespective of vaccination status and presence of symptoms. The target population (the population in which the inference is intended to be made) was conceived as all those who visited these centres during the study period.

Inclusion criteria

1. Individuals attended an NHSTT Regional Test Site (RTS) for the purposes of receiving a diagnostic test irrespective of the reason.
2. Participants agreed to take part in one assisted throat and nose (1 x PCR test) and one assisted nasal (1 x LFD test) swab.
3. Participants were aged ≥16 years. Individuals such as the very elderly for whom a throat swab would not be possible were not recruited.
4. Participants understood that their LFD result was indicative and might differ from the result of their diagnostic test (PCR).
5. Participants consented to have the data from their PCR and LFD test used alongside data collected as part of their test booking, as part of this evaluation.
6. Participants agreed to self-isolate, irrespective of the LFD result, until they received their PCR result.

Exclusion criteria

1. Participants had cuts in their nose.
2. Participants had healing nose piercings.
3. Participants had eaten, drunk, smoked or vaped in the 30 minutes before the swabbing.

Study sites

The testing centres where the participants were recruited(all in England): Birmingham Airport, Croydon, Gatwick Airport, Humber Bridge Car Park, Leeds Temple Green, Leicester Birstall Park and Ride, Manchester Airport, Newcastle NGP Park, Preston, Stoke Bet365 Stadium, Worcester County Hall.

Sample size

Sample size was calculated originally assuming that the sensitivity rate would be 66%, with lower confidence intervals not inferior to 55%, and a study power of 90%. The estimated sample size was of approximately 3,618 participants, considering a positivity rate of 15% which would give 217 PCR+ results. However, as the positivity rate obtained was higher than what was expected, the number recruited was below the 3,618 originally estimated.

Data collection

The tester asked the participant if they had any COVID-19 symptoms, for which the responses were recorded as one, or a combination, of the following:

1. High temperature or fever
2. new continuous cough
3. Loss or change to sense of smell or taste
4. Runny nose

5. Headache
6. Fatigue
7. Sneezing
8. Sore throat
9. Feeling unwell
10. Other symptoms
11. No symptoms
12. Prefer not to say

The tester also asked the participant if they have received a COVID-19 vaccination, for which the responses were recorded as one of the following:

1. No – unvaccinated
2. Yes – partially vaccinated
3. Yes – fully vaccinated without booster
4. Yes – fully vaccinated with booster
5. Prefer not to say

Participants were attending an RTS to access symptomatic testing and were informed that their responses would be used purely for the purposes of analysis to help interpret the LFD results. They were further informed that any response would not affect their access to PCR testing at the RTS, even where their responses suggested they may not be eligible for the PCR testing.

Collection of samples

Samples for the LFD and PCR tests were collected on the same day consecutively. The samples were collected by trained staff, either a health professional or someone trained by the NHST&T to take the swabs, known as assisted testing, following the procedures:

First, a nasal swab (both nostrils) for the LFD using gentle rotation. The fabric tip of the swab was inserted less than 2.5cm from the edge of each nostril, according to the provided instructions for the LFD.

Secondly, a throat and nose swab for the PCR as per the RTS SOP (Regional Test Site Standard Operating Procedure). The technicians who processed the PCR tests did not know the LFD result at the time of processing the test.

Reading the LFD test results

The trained professional read and recorded the final LFD result 30 minutes after the test was taken, in line with the Information for Use (IFU) instructions. All results were confirmed by a colleague. The presence of any line at ‘T', even a faint one indicated a positive result. Results read after 30 minutes were not considered valid.

Statistical analysis

The sensitivity rate of Acon LFDs for detecting COVID-19 infection was expressed as percentage or proportion and estimated among those with valid data for both LFD and PCR, as the formula below:

$$\frac{LFD\ positive\ among\ those\ with\ a\ PCR\ positive\ for\ variant\ \mathrm{x}}{[(LFD\ negative + LFD\ positive)]\ among\ those\ with\ a\ PCR\ positive\ for\ variant\ \mathrm{x}}$$

Text equivalent of formula
LFD positive among those with a PCR positive for variant x divided by (LFD negative plus LFD positive) among those with a PCR positive for variant x.

Exact 95% confidence intervals were calculated for each sensitivity rate.

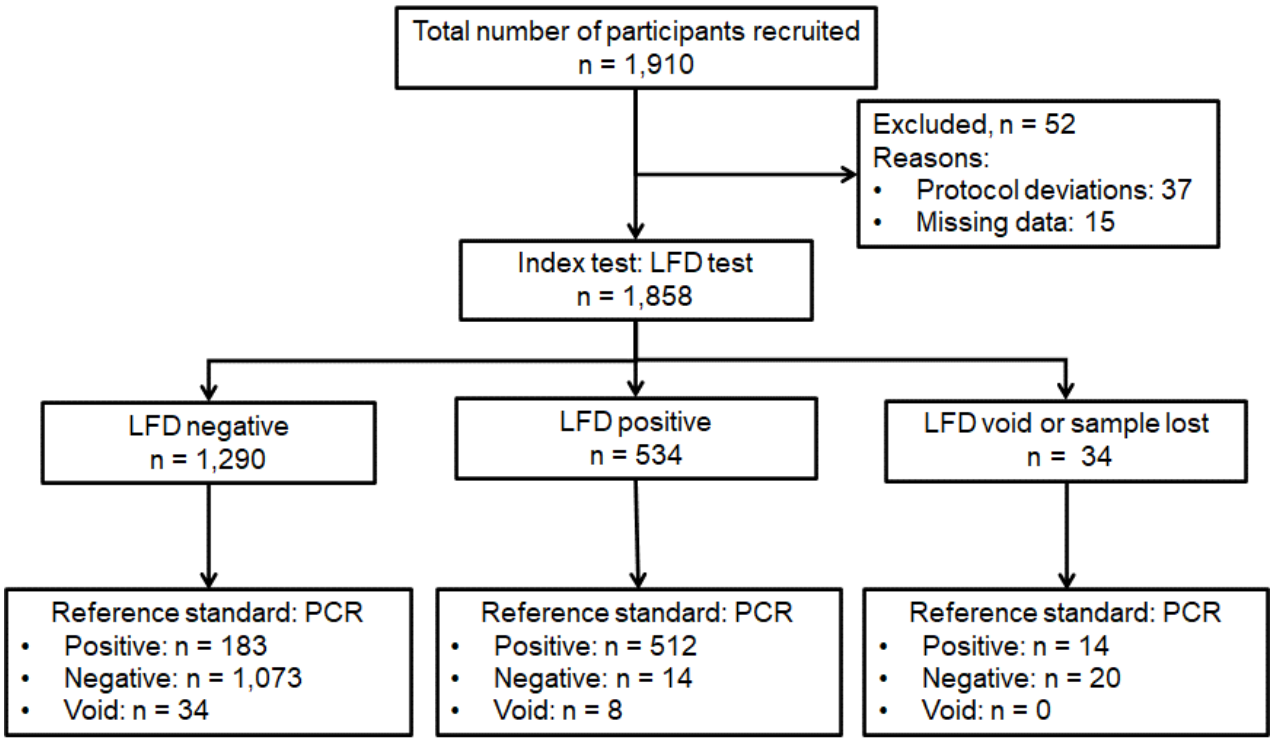
Results

Description of the study population

The flowchart (Figure 12) below shows the distribution of the number of participants from the recruitment and according to the LFD and PCR results. In total, 1,910 individuals were recruited, and 52 (2.7%) were excluded for different reasons. Of the remaining 1,858:

- 34 (1.8%) had lost or void (invalid or not interpretable result) LFD results
- 1,824 had valid LFD results, and 534 (29.3%) had a positive LFD result
- 1,816 had valid PCR results, and 695 (39.0%) had a positive PCR result

Figure 12. Flowchart of participant recruitment



Distribution of participants according to study characteristics

Among the 1,816 participants with valid PCR results, the number of participants with PCR positives and PCR negatives was similar in relation to age. Among those with a PCR positive (n = 709), the mean age was 44 (sd = 15.1), median of 43, range 16 to 97; among those with a PCR negative (n = 1,170), the mean age was 45 (sd = 15.5), median 44, range 16 to 86).

Table 3 below shows the distribution of other characteristics. The PCR+ and PCR- split were quite similar to each other in relation to sex and ethnic group. However, in comparison with those who were PCR negative, those who were PCR positive were more likely to report symptoms (77% versus 92%), and there were a lower proportion with 2 doses and booster vaccination (71% versus 80%).

Table 3. Characteristics of the 1,816 participants with valid PCR results for any viral variant

Table 3a. Ethnicity

Ethnicity	PCR positive n = 709 n (%)	PCR negative n = 1,107 n (%)	Total n = 1,816 n (%)
White British	550 (78)	902 (81)	1,452 (80)
White	29 (4)	41 (4)	70 (4)
Asian or Asian British	79 (11)	98 (9)	177 (10)
Black	15 (2)	13 (1)	28 (2)
Other ethnic groups	9 (1)	15 (1)	24 (1)
Mixed or multiple ethnic groups	3 (<1)	14 (1)	17 (1)
Not declared	24 (3)	24 (2)	48 (3)
Missing	0 (0)	0 (0)	0 (0)

Table 3b. Gender

Gender	PCR positive n = 709 n (%)	PCR negative n = 1,107 n (%)	Total n = 1,816 n (%)
Male	319 (45)	447 (40)	766 (42)
Female	387 (55)	658 (59)	1,045 (58)
Missing data	3 (<1)	2 (<1)	5 (<1)

Table 3c. Vaccination status

Vaccination status	PCR positive n = 709 n (%)	PCR negative n = 1,107 n (%)	Total n = 1,816 n (%)
Two doses and booster	505 (71)	883 (80)	1,388 (76)
Two does without booster	140 (20)	158 (14)	298 (16)
One dose	18 (3)	28 (3)	46 (3)
Unvaccinated	36 (5)	31 (3)	67 (4)
Missing data or not declared	10 (1)	7 (<1)	17 (1)

Table 3d. Presence of symptoms

Presence of symptoms	PCR positive n = 709 n (%)	PCR negative n = 1,107 n (%)	Total n = 1,816 n (%)
Any symptoms	652 (92)	853 (77)	1,505 (83)
Key symptoms	396 (56)	534 (48)	930 (51)
Other symptoms	256 (36)	319 (29)	575 (32)
Asymptomatic	57 (8)	254 (23)	311 (17)
Missing data	0 (0)	0 (0)	0 (0)

Positive PCR results according to viral variant

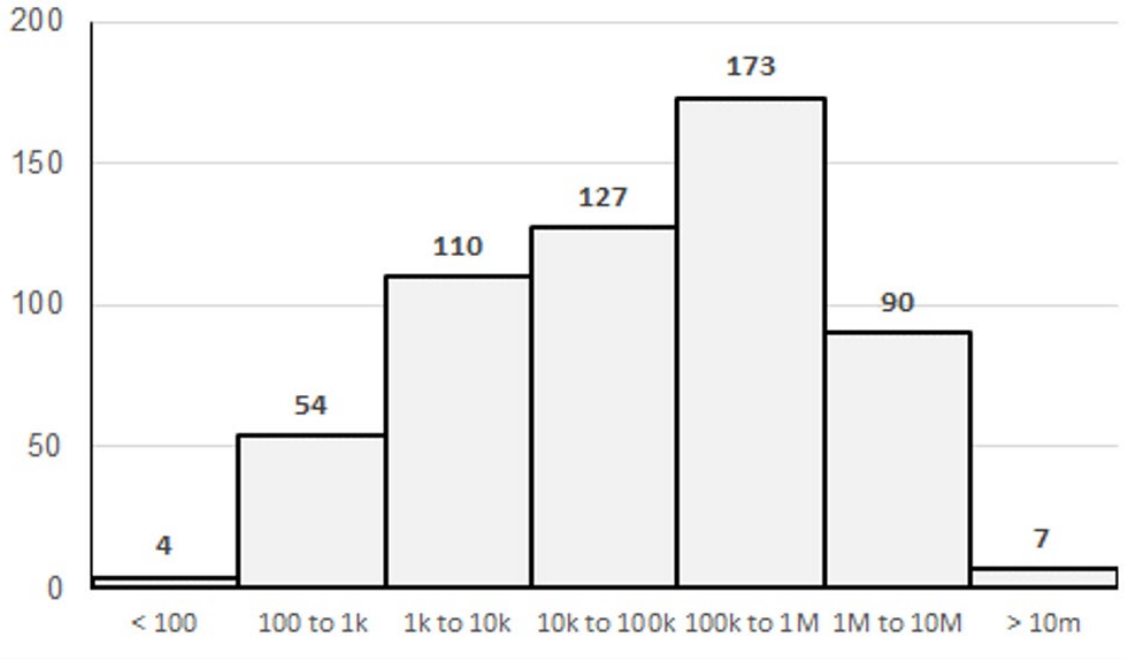
Among those with valid PCR results (1,816), the Omicron variant B.1.1.529 was detected in 567 (31%), corresponding to 80% of all PCR positives (n = 709).

Table 4. Number of positive PCR results with viral variant identified

Viral variant	Positive PCR results n = 709 n (%)
Omicron B.1.1.529	567 (80%)
Omicron BA.2	12 (<1)
Variant unknown	130 (18)
Total	709 (100)

Figure 13 below shows the distribution of viral concentration for cases with a PCR+ result for Omicron (B.1.1.529) (n = 565 out of 567, 2 participants with missing value for viral concentration).

Figure 13. Histogram with distribution of participants with positive PCR for Omicron B.1.1.529 (n = 565), separately for the estimate of viral concentration



Overall sensitivity rate

The table below (Table 5) shows the cross tabulation of positive and negative results from participants with valid results for both PCR and LFD tests (n = 1,782) with the estimates of the diagnostic performance measures.

Table 5. Comparison between LFD results (index test) and PCR results (reference standard) and diagnostic performance measures

LFD result	Positive by viral variant: For all variants	Positive by viral variant: Only for Omicron (B.1.1.529)	Positive by viral variant: For other variants[1]	Negative	Total
Positive	512	432	80	14	526
Negative	183	124	59	1,073	1,256
Total	695	556	139	1,087	1,782
Prevalence (%)	39.0	31.2	7.8		
FN (%)[2]	26.3	22.3	42.4		
FP (%)[3]				1.3	
Sensitivity (%)[4] (95% CI)	73.7 (70.2, 76.9)	77.7 (74.0, 81.1)	57.6 (48.9, 65.9)		
Specificity (%)[5] (95% CI)				98.7 (97.9, 98.5)	

Notes to Table 5

[1] This included those with and without identification of the viral variant.
[2] FN (%), percentage of false negatives.
[3] FP (%), percentage of false positives.
[4] Sensitivity = TP/(TP+FN), for all variants = 512 out of 695 (73.7%), only for Omicron (B.1.1.529) = 432 out of 556 (77.7%), for other variants = 80 out of 139 (57.6%)
[5] Specificity = TN/(TN+FP) = 1,073 out of 1,087 (98.7%)

Prevalence: the percentage within this sample with the COVID-19 infection for any variant was 39.0% (n = 695); the prevalence specifically for Omicron (B.1.1.529) was 31.2%, and for other variants it was 7.8%.

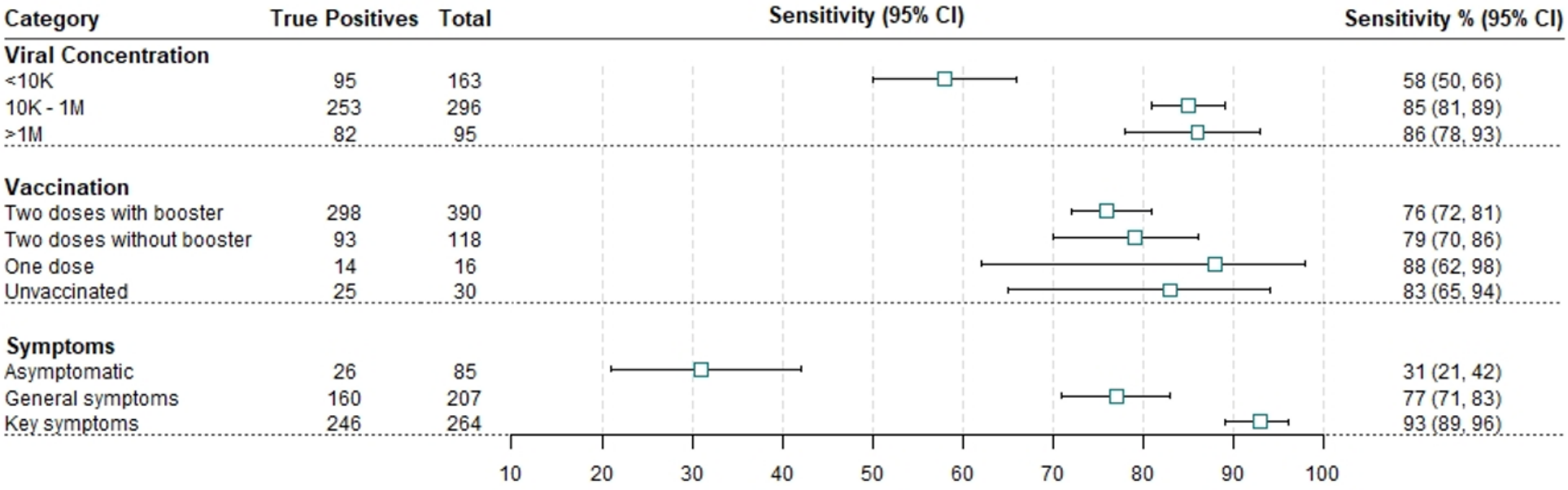
Sensitivity rate: among all those with COVID-19 due to any variant (695), the LFD test identified 512, giving a sensitivity of 73.7%. For Omicron (B.1.1.529), sensitivity was 77.7%, and for all other variants it was 57.2%. Specificity was 98.7%.

Among the 1,782 participants with valid results, the LFD tests had a discordant result in 183 + 14 = 197 participants (11.1%), or 1 in 10. Assuming that the PCR result is always correct, the percentage of tests with discordant results would be equal to the “error rate”, and therefore the interpretation is that in ten tests conducted, one would have an incorrect result. However, the error rate varies with the prevalence of the disease and so this interpretation is for a hypothetical population similar to these 1,782 participants analysed and it may not be generalisable for all populations (10).

Sensitivity separately for effect modifiers

The sensitivity rate was estimated separately for subgroups: viral concentration, vaccination status, and symptom status. The results are presented in the Figure 14 below.

Figure 14. Forest plot with the sensitivity rates of LFD test for Omicron (B.1.1.529), PCR as reference standard, separately for viral concentration of the sample analysed, vaccination status and symptoms of the participants

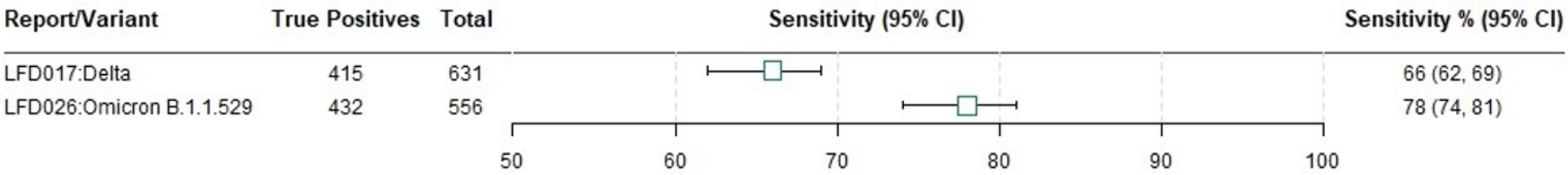


The sensitivity rate was higher when the viral concentration was above 10,000 RNA copies per mL, and with the presence of symptoms, especially with the key symptoms of COVID-19 infection. The overall sensitivity rate was 78%, which increased to around 85% for VC >10,000 RNA copies per mL, comparable with results previously described in the literature (6). The sensitivity rate for the asymptomatic was around 30% which increased to 93% for those with cardinal COVID-19 symptoms. This relationship between sensitivity and presence of symptoms has also been observed in other studies (9, 11, 12). There was no clear pattern in relation to vaccination status.

Comparison with sensitivity for Delta variant

The overall sensitivity rate for Omicron B.1.1.529 was compared to the sensitivity rate for the variant Delta estimated in a previous report (LFD017). As demonstrated in the figure below, the sensitivity rate was higher for the Omicron B.1.1.529 (78% versus 66%).

Figure 15. Forest plot with the sensitivity rates of LFD for Omicron B.1.1.529 as estimated in this report, and as estimated for Delta variant in previous report (LFD017)



Conclusion

The main findings of the analysis of the evaluation on the sensitivity of the Acon LFD tests for detecting Omicron (B.1.1.529) can be summarised in the following points:

1. The overall sensitivity was estimated as 77.7% (74.0, 81.1), and higher among those participants with key symptoms of COVID-19 (93%, 95% CI: 89, 96) and with viral concentration ≥10,000 RNA copies per mL (85%; 95% CI: 81, 89).
2. This overall sensitivity of Acon LFD for Omicron was higher than the sensitivity reported for the Delta variant in a previous analysis (66%; 95% CI: 62, 69).

Interpretation of these results should take into account that the participants were from a non-probabilistic sample.

Summary of findings and conclusions from all 4 evaluations

All findings and conclusion described below are based on the data from these 4 evaluations; there was no attempt to compare with findings in the literature.

The initial evaluations described in this report are based on the real-world use of LFDs within the National Testing Programme. They show that LFDs are most effective at viral concentrations above 10,000 RNA copies per mL, which has been shown as the level above which around 85% of transmissions are likely to occur (5). Further, they show that at these viral concentrations the detection of COVID-19 using LFD was similar regardless of viral variant (Delta and Omicron including sublineage BA.2) and LFD kit type. Analysis of Acon LFDs suggests that detection is highest for those who experience symptoms with highest sensitivity seen with main symptoms.

Questions and answers

Omicron (B.1.1.529) versus Delta (B.1.617.2): evaluations 1 and 2

Question 1: Was the ability of the LFD test to detect Omicron (B.1.1.529) infection different from the detection of Delta (B.1.617.2) infection?

Answer 1: With viral concentrations (VC) >10,000 RNA copies per mL, there was no evidence that the detection rates were different between the 2 viral variants.

Question 2: Was the detection rate for different LFD kits at different VC categories for Omicron (B.1.1.529) similar to the detection rate for VC >1 million for the same LFD kit and variant?

Answer 2: When VC was >10,000, the detection rate of LFD kits for each viral variant did not appear to differ.

Omicron (BA.2) versus Omicron (B.1.1.529): evaluation 3

Question 3: Was the ability of the LFD test to detect Omicron (BA.2) infection different from the detection of Omicron (B.1.1.529) infection?

Answer 3: With a VC >10,000 RNA copies per mL, there was no evidence that the detection rates were different between the 2 viral variants.

Sensitivity for Omicron (B.1.1.529) with Acon LFD test: evaluation 4

Question 4: What was the sensitivity of Acon LFDs for Omicron (B.1.1.529)?

Answer 4: The overall sensitivity for Omicron (B.1.1.529) was estimated at 77.7% (95% CI: 74 to 81). The point estimate was higher with VCs >10,000 RNA copies/mL (86%), and with cardinal symptoms (93%).

Question 5: How does the sensitivity of Acon LFDs for Omicron (B.1.1.529) variant compare with the previous report of the sensitivity of LFD tests for the Delta variant?

Answer 5: The sensitivity for Omicron (B.1.1.529) was higher than for Delta: 78% (74 to 81) versus 66% (62 to 69).

References

1. NHS Health Research Authority (2022). 'Is my study research?'
2. Department of Health and Social Care (DHSC) (2021). 'First UK Cases of Omicron Identified'
3. Office for National Statistics (2022). 'Coronavirus (COVID-19) latest insights'
4. UKHSA (2021). 'NHS Test and Trace: how we test your samples'
5. Lee LYW, Rozmanowski S, Pang M, Charlett A, Anderson C, Hughes GJ and others. 'Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectivity by viral Load, S gene variants and demographic factors, and the utility of lateral flow devices to prevent transmission.' Clinical Infectious Diseases 2022: volume 74, issue 3, pages 407 to 415
6. Peto T, UK COVID-19 Lateral Flow Oversight Team. 'COVID-19: rapid antigen detection for SARS-CoV-2 by lateral flow assay:aA national systematic evaluation of sensitivity and specificity for mass-testing'. EClinicalMedicine 2021: volume 36, page 100,924
7. DHSC (2021). 'Asymptomatic testing for SARS-CoV-2 using antigen-detecting lateral flow devices: evidence from performance data October 2020 to May 2021'
8. DHSC (2021). 'Technical report: in vitro and clinical post-market surveillance of Biotime SARS-CoV-2 lateral flow antigen device in detecting the SARS-CoV-2 Delta variant (B.1.617.2)'
9. UKHSA (2022). 'Evaluation of lateral flow device performance within the National Testing Programme'
10. Knottnerus JA, Buntinx F. 'The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research, Second Edition'. BMJ Books 2008
11. Takeuchi Y, Akashi Y, Kato D, Kuwahara M, Muramatsu S, Ueda A and others. 'Diagnostic performance and characteristics of anterior nasal collection for the SARS-CoV-2 antigen test: a prospective study'. Scientific Reports 2021: volume 11, issue 1, page 10,519
12. Schuit E, Veldhuijzen IK, Venekamp RP, van den Bijllaardt W, Pas SD, Lodder EB and others. 'Diagnostic accuracy of rapid antigen tests in asymptomatic and presymptomatic close contacts of individuals with confirmed SARS-CoV-2 infection: cross sectional study'. British Medical Journal 2021: volume 374, page number 1,676
13. UKHSA. 'COVID-19 variants identified in the UK: latest updates'

Glossary

Term	Explanation
Detection rate	Among those subjects with matched pairs of LFD/PCR, the number of subjects with positive LFD divided by the number of subjects with positive PCR. This metric can be presented as a proportion or as percentage.
HER	Routinely collected health electronic records (databases), such as the databases used for cross-sectional analysis described in this report
Real-World Performance Monitoring (RWPM)	A programme of post-market surveillance operated by the NHS Test and Trace (NHSTT) aimed to monitor the performance of the diagnostic tests used by the NHSTT via routine data (real world data).
Real-world data (RWD)	RWD are defined as data relating to patient health status or delivery of health care collected outside of a clinical study. Sources of RWD include healthcare electronic records (HER) defined as structured, digital collections of patient level medical data, primary and secondary care records, disease registries, and administrative data on births and deaths.
Relative detection rate	Absolute difference between 2 detection rates, taken the detection rate for a known performance as the reference: detection rate for viral variant ‘x’ minus the detection rate for a viral variant with known performance. This metric is presented as percentage points.
Variants of concern (VOC)	A variant of concern is a viral variant with changes in transmissibility, severity, or immune evasion compared to the current dominant variant, and/or a growth rate potentially compatible with the eventual replacement of the current dominant variant (13)
Viral concentration	The number of SARS-CoV-2 RNA viral copies present per mL of viral transport medium calculated by converting the Ct value from qRT-PCR into a viral concentration using the laboratory’s specific conversion formula. This is a proxy for the amount of virus present in a person’s nasal or oral cavity rather than a direct measure. It depends both on the quality of the swabbing technique and the efficiency of the release of the virus from the swab into the transport medium. Other reports and papers may refer to this as viral load.

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