Department of Health & Social Care

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)

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Summary

This document summarises the findings of routine *in vitro* and clinical post-market surveillance focusing on the performance of the Biotime LFD test[1] to detect the Delta (B.1.617.2) and Alpha variants (B.1.1.7).

In vitro assessment of performance of the Biotime LFD in detecting the Delta variant using live virus isolated and cultured from clinical specimens identified possible reduced sensitivity (namely reduced band strength and hence readability) of the test for lower viral concentrations.

Routine clinical post-market surveillance between 1st April and 2nd June 2021 did not detect significant differences in the ability of the Biotime LFD to detect the Delta and Alpha variants.

- Performance monitoring of real-world asymptomatic testing using the Biotime LFD, where a positive LFD result is confirmed with a qRT-PCR assay[2] on the same day, identified 2,042 cases of the Delta variant between 1st April and 26th May 2021.

- There was no correlation between the proportion of positive PCR tests identified as the Delta variant and the LFD/ PCR positivity ratio when analysed by local authority area, comparing areas with high and low Delta prevalence; this is consistent with there being no clinically significant change in LFD sensitivity for this variant.

- Logistic regression modelling of paired testing data (i.e. qRT-PCR and Biotime LFD tests performed by the same person on the same day) from select 'surge' testing sites[3] provides further confidence that no statistical or clinical difference exists between the Biotime LFD's ability to detect the Delta variant compared to the Alpha variant across the range of viral concentrations.

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[1] Xiamen Biotime is manufacturer of the SARS-CoV-2 Lateral Flow Antigen Test as used in the Innova SARS-CoV-2 Lateral Flow Antigen Test Kit for professional use and DHSC COVID-19 Self-Test Kit for 'home' self-test use.

[2] Further subjected to genomic sequencing.

[3] 'Surge' testing is a public health intervention initiated in response to elevated prevalence rates in a local area. In surge testing asymptomatic PCR testing is conducted to identify the presence of any variants of concern. Surge testing sites deploy PCR testing alongside an LFD take away model known as 'LFD collect' where individuals are provided concurrent LFD testing. This supports performance analysis for different variants.

- People who self-tested and were assisted-tested with Biotime LFDs in April to June 2021 detected Alpha and Delta variants with the same degree of competence as expert users in the FALCON C-19 study detected the wild type in October 2020[4].

The Biotime LFD remains suitable for deployment as part of the asymptomatic testing programme to identify infectious individuals in the population and to reduce onward transmission risk. There is no difference in performance in its ability to detect the Delta variant in comparison to the Alpha variant.

As further new variants emerge, and with increased diversity of LFD product manufacturers planned for deployment in the future, the combination of routine *in vitro* and clinical post-market surveillance will remain a critical tool for rapid surveillance of device performance in a changing landscape.

[4] Peto et al. COVID-19: Rapid antigen detection for SARS-CoV-2 by lateral flow assay: A national systematic evaluation of sensitivity and specificity for mass-testing. E Clinical Medicine 2021 May 30;100924

1. Introduction

NHS Test & Trace operates a programme of post-market surveillance comprising clinical evaluation via routine monitoring of performance using 'real-world' data (i.e. data collected on a routine basis as part of the testing programme). This is essential to detect early signals of changes in performance with emerging variants. It is carried out alongside the wider PHE Porton Down validation programme where *in vitro* assessment using live virus cultured from clinical samples gives the ability to monitor LFD test's ability to detect emerging variants.

The Delta variant (B.1.617.2) was first detected in the UK in March 2021[5]. As with previous Variants of Concern (VOC), *in vitro* evaluation has been performed on LFD antigen tests from multiple manufacturers in parallel with routine monitoring of clinical performance data for the Biotime LFD[6] as the predominant LFD antigen test in use in the UK from April through to June 2021.

[5] Public Health England. Variants: distribution of cases data, 20 May 2021. Variants: distribution of cases data, 20 May 2021 - GOV.UK (www.gov.uk)

[6] Biotime LFD test: Xiamen Biotime SARS-CoV-2 Lateral Flow Antigen Test as used in the Innova SARS-CoV-2 Lateral Flow Antigen Test and DHSC COVID-19 Self-Test Kit

2. In vitro laboratory evaluation

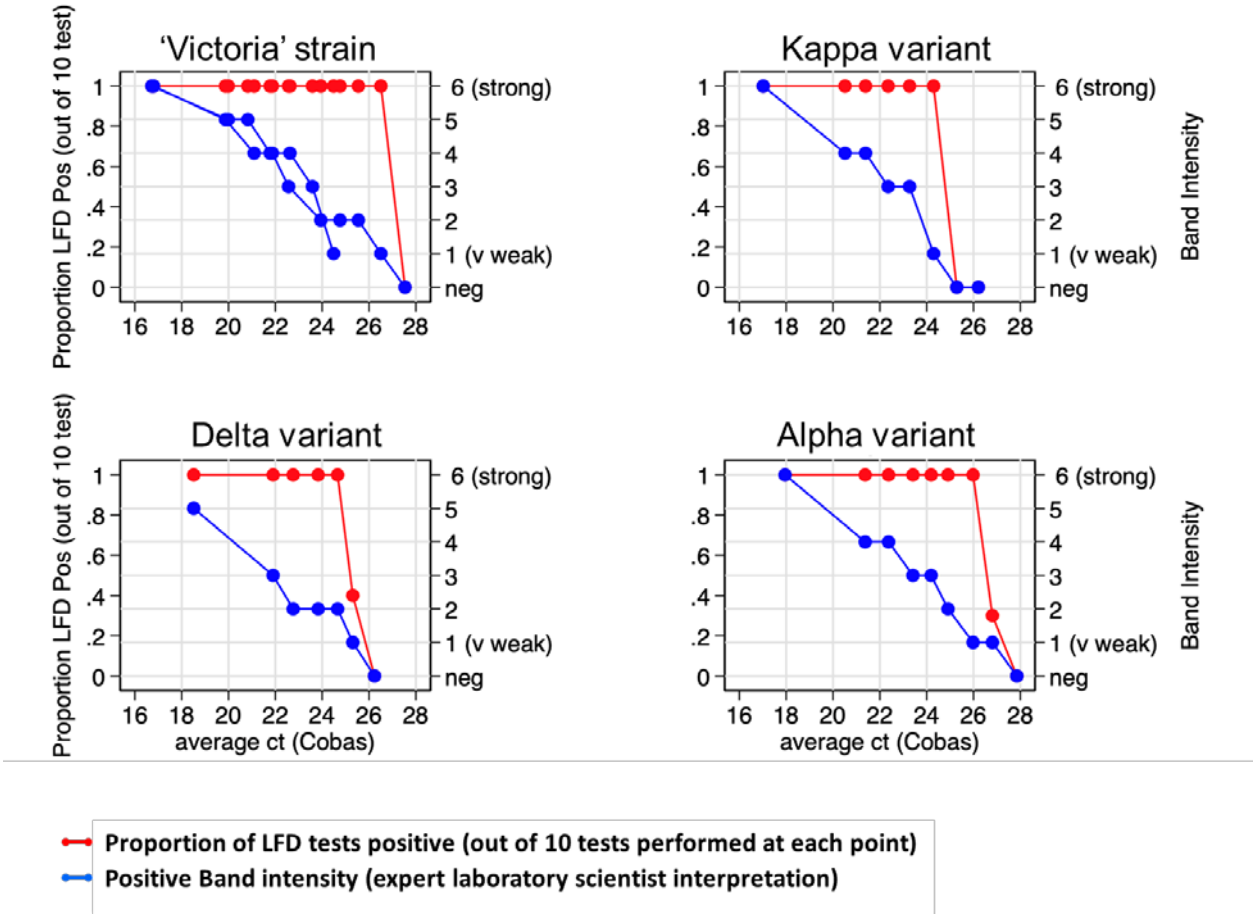
DHSC has an established validation process, commissioned from PHE and Oxford University and overseen by an LFD Oversight Group, for all LFD antigen tests proposed for use within the national testing programme[7]. The protocol is delivered in 3 phases. Phase 1 is a desk-top assessment of product viability carried out by DHSC. Phase 2 is a futility test at PHE Porton Down to prioritise products for further assessment by identifying kit failure rate, whether known negative samples give a negative result (indicator of specificity), whether known positive samples give a positive result (indicator of sensitivity), and an initial view on usability of each test. Spiked samples are serially diluted and assessed for each test. Phase 3 involves evaluation of each LFD antigen test against a larger clinical reference panel (1,000 true negatives and 200 true positives). For those tests that pass Phase 2, an assessment using 3 seasonal coronaviruses is carried out to identify any cross-reactivity (human seasonal coronaviruses 229E, OC43 and NL63).

All LFD antigen tests that pass Phase 3 of the validation process are subjected to repeated assessment with live SARS-CoV-2 virus identified as VOC – with a focus on tests that are in active national deployment (i.e. the Biotime SARS-CoV-2 Lateral Flow Antigen Device and the Orient Gene Coronavirus Ag Rapid Test Cassette). Live virus cultured from clinical samples / grown from isolates and serially diluted is used to assess device performance[8]. Previous evaluations have demonstrated that the Biotime LFD successfully detected Alpha (B1.1.7), Beta (B.1.351) and Gamma (P.1) variants. The *in vitro* results for the Delta VOC experiment are presented in Figure 1.

[7] Guidance: Protocol for evaluation of rapid diagnostic assays for specific SARS-CoV-2 antigens (lateral flow devices). Updated 3 June 2021. Protocol for evaluation of rapid diagnostic assays for specific SARS-CoV-2 antigens (lateral flow devices) - GOV.UK (www.gov.uk)

[8] PHE Porton Down use a 0-6 point scale on band intensity for readability of the indicator line; 0 is negative and 6 is the strongest response. The band intensity is assessed by expert laboratory scientists.

Figure 1 - Extended dilution series assessment of the Biotime LFD detection rate and band intensity for 'Victoria' strain[9], Kappa, Delta and Alpha variants. Red lines are the categorical LFD results (positive / negative) and the blue lines show the band strengths at each dilution

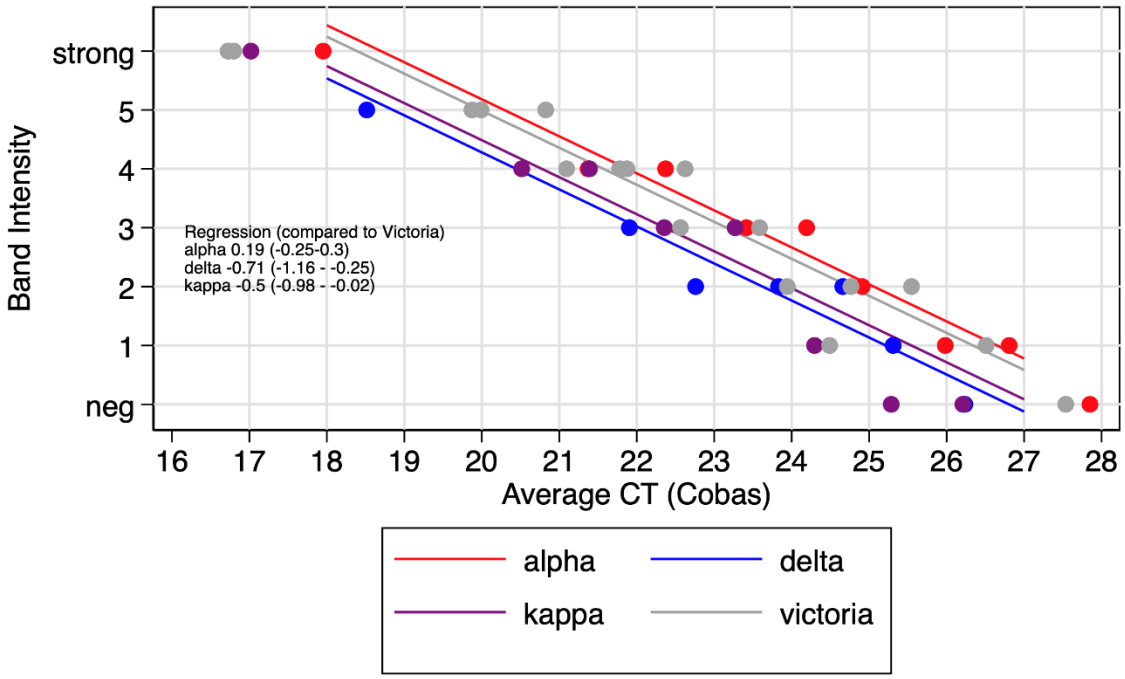


Regression modelling of band strength outcomes from the extended dilution series is shown in Figure 2. This shows that the identification of Alpha and the 'Victoria' strain are similar, and Delta and Kappa are similar. At each viral load Delta is approximately 0.7 band strengths weaker than the 'Victoria' strain. An alternative summary of this difference is that the Delta strain is 1 Ct[10] (1.7-0.3) units less sensitive when compared *in vitro* (equivalent to 0.33 (0.10-0.56) log viral load units).

[9] 2019-nCoV/Victoria/1/2020: Live virus isolate considered synonymous with the wild type. Nomenclature: SARS-CoV-2 Victoria/01/2020. Provided by The Doherty Institute, Melbourne, Australia at P1 and passaged twice in Vero/hSLAM cells 405 [ECACC 04091501]. Caly, L. et al. Isolation and rapid sharing of the 2019 novel coronavirus (SARS702 CoV-2) from the first patient diagnosed with COVID-19 in Australia. n/a, 703 doi:10.5694/mja2.50569. PHE Porton Down received the virus from early February 2020.

[10] I.e. 1 cycle of amplification in a qRT-PCR assay

Figure 2 - Sensitivity of Biotime LFD by SARS-CoV-2 variants (band intensity). Regression estimated from band intensities 1-5



3. LFD performance monitoring of asymptomatic testing services

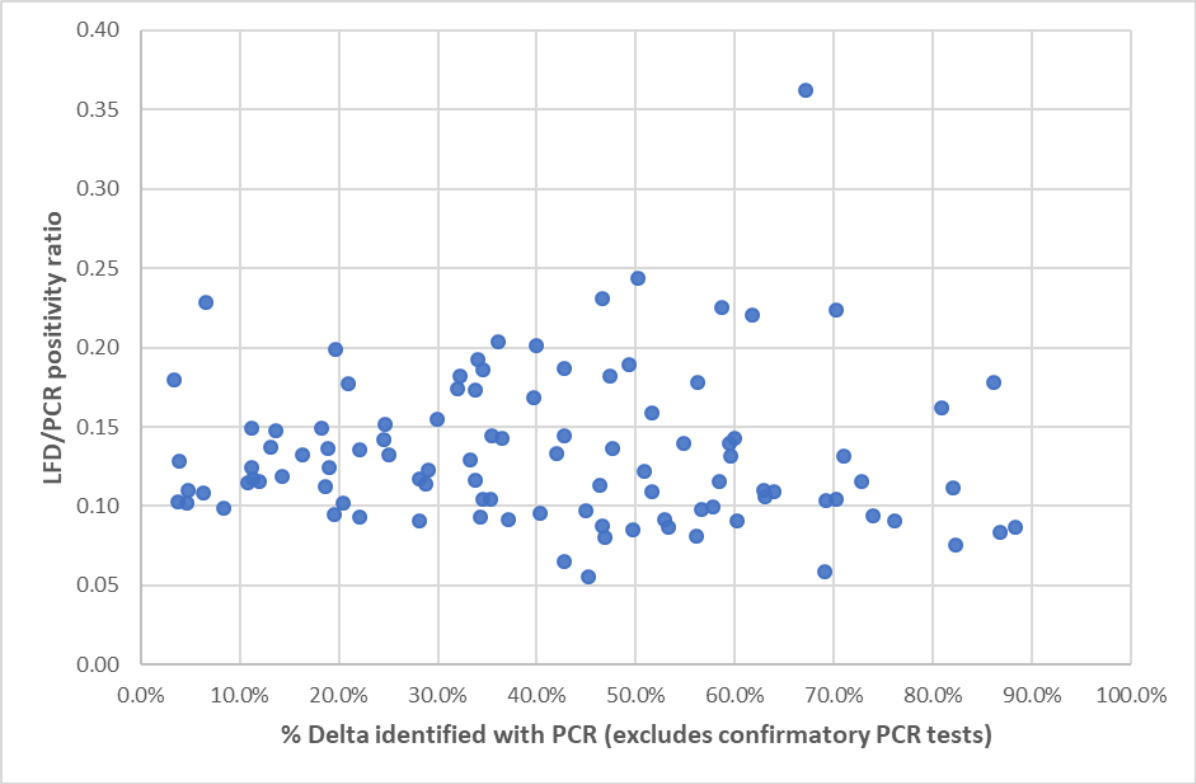
LFD test performance is routinely monitored through real-world data generated from asymptomatic testing services[11]. Real-world data refers to data captured routinely as part of asymptomatic testing services. This data is segmented by device, service team and site, and incorporates positivity, void, and confirmatory PCR rates for positive LFD tests, alongside a summary of any variants detected.

From 1st April to 26th May 2021, asymptomatic testing with the Biotime LFD combined with confirmatory PCR testing for positive LFD results had detected **2,042** cases of Delta variant with a further **28** cases of Kappa variant (B.1.617.1) and **2** cases of B.1.617.3 variant[12].

This real-world data from both assisted testing and self-test services was used to investigate whether LFD test positivity rates are significantly lower than PCR positivity rates in locations where prevalence of the Delta variant is high. This would be a high-level signal of the ability of the Biotime LFD to detect the Delta variant. If the ability of the Biotime LFD to detect the Delta variant is reduced, a lower LFD/PCR positivity ratio would be expected within areas of high prevalence for the Delta variant. This is because the LFD test would not be detecting the proportionally higher number of Delta variant cases in these areas compared to the baseline PCR positivity rate, which is unaffected by variant status. For the analysis, LFD and PCR tests performed from 1st April to 6th June 2021 were included. The analysis focussed only on Biotime LFD tests[13] and excluded PCR tests likely to have been performed as confirmatory PCR in order to ascertain two distinct populations[14]. Figure 3 shows there was no correlation between the proportion of sequenced positive tests identified as the Delta variant in comparison to the LFD /PCR positivity ratio.

[11] Real world performance monitoring refers to data that is captured only through analysis of testing within the wider testing programme and not performed under a specific evaluation protocol or as part of paired testing regime conditions (see section 5).
[12] All variants have been confirmed by genomic sequencing.
[13] Barcode prefixes are used to identify LFD test kits in the dataset.
[14] There is no direct identifier for confirmatory PCR tests as this data is not collected during test registration. Within this analysis, confirmatory PCR tests are identified by matching positive LFD tests with the first non-void PCR result within 3 days. There may be more confirmatory PCR tests that were not excluded if they were not taken within a 3-day period of the LFD, if the algorithm prioritised one PCR over another due to being closer to the LFD test or if the algorithm failed to detect the confirmatory PCR due to the inability of matching an individuals' data.

Figure 3 - LFD/PCR positivity ratios in comparison to proportion of Delta variant cases identified by positive PCR results; stratified by local authority area. Inclusive of both assisted testing and self-test services



4. Evaluation settings with routine paired testing (LFD & PCR)

In addition to the real-world performance data, analysis has been conducted on data from surge testing sites where the testing regime is one of paired testing. Each person receives both a PCR and a self-test LFD test, which can be compared to give an evaluation of LFD performance. LFD results are reported to NHS Test & Trace irrespective of a negative or positive PCR result. Surge testing sites are sites set up to increase testing capacity for asymptomatic PCR testing within local areas with raised prevalence, where a VOC is suspected. This provides a representative population on which to calculate detection rate as a proxy for sensitivity. The analysis focused on results where both tests have been performed and recorded on the same day[15]. Comparison of the Biotime LFD detection rate for the Delta variant has been compared to the detection rate for the Alpha variant predominant within the population at the time[16].

Over the period 1st April – 2nd June 2021 the surge testing sites identified **1,030** cases of the Delta variant and **637** cases of the Alpha variant with a paired same-day PCR and LFD test result. Table 1 provides the full analysis of SARS-CoV-2 variants identified within the sample population. It is known that as viral load increases, the infectiousness of a person increases. The degree to which infectiousness increases with viral load is dependent on the 'closeness' of the contact, with household contacts being most at risk[17]. Of the Delta variant cases, 77.3% were categorised as having a viral load of the over 1 million dC/ml viral, whilst 18.3% and 4.4% were categorised in the 10,000 – 1 million dC/ml and under 10,000 dC/ml viral concentration categories respectively. The sample size was large enough to provide meaningful conclusions on performance.

Table 1 - Analysis of SARS-CoV-2 variants detected within the paired same-day PCR and LFD testing (surge testing sites) sample population

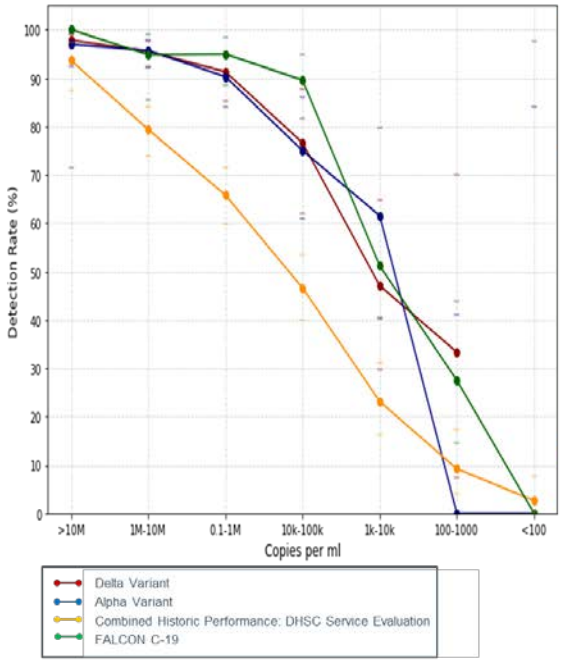
SARS-CoV-2 variant / strain	Number of tests
Alpha variant	637
Beta variant	7

[15] And where the qRT-PCR test was analysed in a laboratory allowing for the conversion of sample qRT-PCR Ct value to estimated viral load (dC/ml) and viral copy categorisation. Relevant laboratories are Glasgow (GLS), Milton Keynes (MK), Alderley Park (AP), Plymouth (SW), and Health Services Laboratories (HSL).
[16] Variants and strains predominant at the time available through the Wellcome Sanger Institute: Lineages (raw) | COVID-19 Genomic Surveillance – Wellcome Sanger Institute
[17] Lee et al. An observational study of SARS-CoV-2 infectivity by viral load and demographic factors and the utility lateral flow devices to prevent transmission. Pre-print published 31 March 2021.

SARS-CoV-2 variant / strain	Number of tests
Delta variant	1030
Other variant / strain	5
No known strain identified	34
Not sequenced	218

Review of the recent surge testing same-day PCR and LFD population dataset suggests no statistical difference in detection rates between Delta variant and Alpha variant across the range of viral concentrations, as shown in Figure 4[18].

Figure 4 - DHSC post-market surveillance analysis of SARS-CoV-2 detection rate for paired same-day LFD and PCR testing (surge testing sites)



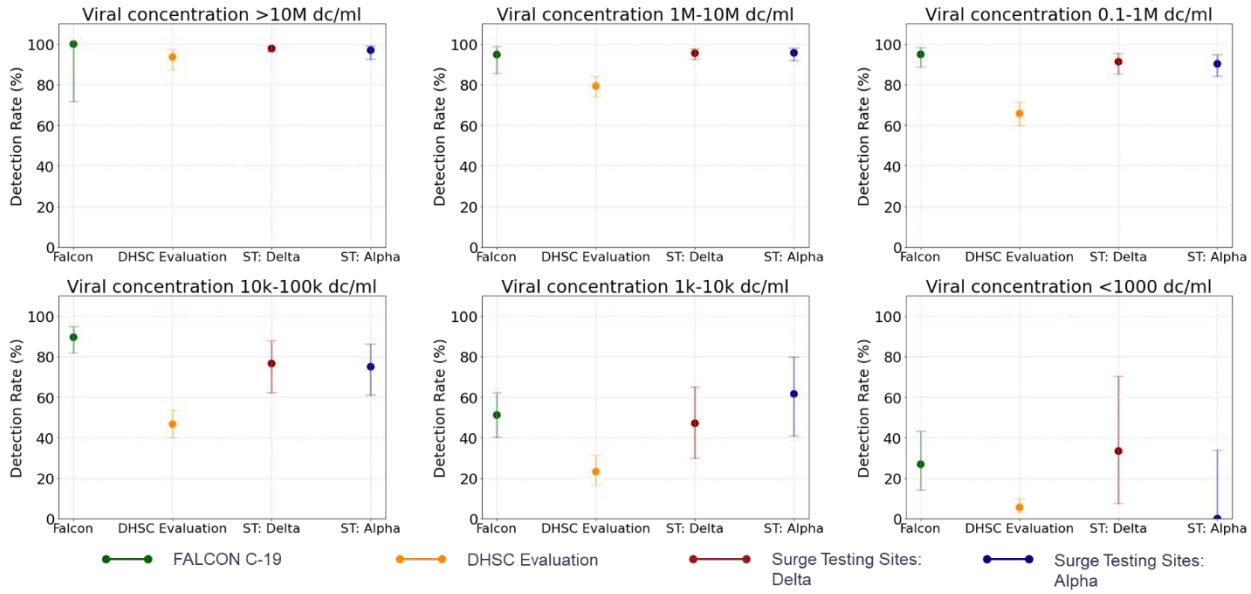
Estimated viral concentration (dC/ml)	Delta Variant			Alpha Variant		
	Detection Rate (%)	False Negative (LFD -, PCR +)	True Positive (LFD +, PCR +)	Detection Rate (%)	False Negative (LFD -, PCR +)	True Positive (LFD +, PCR +)
> 10M	97.9 (95% CI 96.2 – 98.9)	11	514	97.0 (95% CI 92.5 – 99.2)	4	129
1M – 10M	95.5 (95% CI 92.3 – 97.7)	12	256	95.7 (95% CI 92.0 – 98.0)	9	200
0.1M – 1M	91.3 (95% CI 85.3 – 95.4)	12	126	90.3 (95% CI 84.0 – 94.7)	13	121
10k – 100k	76.6 (95% CI 62.0 – 87.7)	11	36	75.0 (95% CI 61.1 – 86.0)	13	39
1k – 10k	47.1 (95% CI 29.8 – 64.9)	18	16	61.5 (95% CI 40.6 – 79.8)	10	16
100 – 1000	33.3 (95% CI 7.49 – 70.1)	6	3	0.00 (95% CI 0.00 – 41.0)	7	0
< 100	-	0	0	0.00 (95% CI 0.00 – 84.2)	2	0

Estimated viral concentration (dC/ml)	Historic performance evaluation: combined estimate of Biotime LFD antigen test performance evaluation across service evaluations. Sensitivity estimate and 95% CI, stratified by PCR sample estimated viral copies/ml. Predominant strains at the time of evaluations: Wuhan and Alpha.	FALCON C-19
	Estimated Sensitivity (%)	Estimated Sensitivity (%)
>10M	93.6 (103/110; 95% CI 87.3 – 97.4)	100 (11/11; 95% CI 71.5 – 100)
1M-10M	79.4 (200/252; 95% CI 73.8 – 84.2)	94.8 (55/58; 95% CI 85.6 – 98.9)
0.1M-1M	65.8 (179/272; 95% CI 59.8 – 71.4)	95.0 (94/99; 95% CI 88.6 – 98.3)
10K-100K	46.6 (103/221; 95% CI 39.9 – 53.4)	89.6 (86/96; 95% CI 81.7 – 94.9)
1K-10K	23.1 (31/134; 95% CI 16.3 – 31.2)	51.2 (44/86; 95% CI 40.1 – 62.1)
100-1000	9.2 (8/87; 95% CI 4.1 – 17.3)	27.5 (11/40; 95% CI 14.6 – 43.9)
<100	2.7 (3/113; 95% CI 0.6 – 7.6)	0.0 (0/1; 95% CI 0.0 – 97.5)

[18] Fisher's exact test; no significant difference between the Delta and Alpha variant Biotime LFD detection rates (p-value>0.05)

The current performance of the test can be compared with previous Biotime LFD evaluations carried out from September 2020 to January 2021. The evaluation of performance is measured relative to a reference standard of qRT-PCR[19], with the primary objective to understand the diagnostic performance of the device or method when deployed within the intended NHS Test and Trace setting. The original FALCON C-19 evaluation[20] used expert operators (clinical research nurses and laboratory scientists) to undertake tests, while other evaluations at symptomatic and asymptomatic test sites used a combination of trained operatives, and self-testing. As can be seen in Figure 5, while 2020 studies showed a better performance when Biotime LFDs were used by expert operators, the ability to ascertain and interpret positives results for Alpha and Delta variants in the recent analysis has improved to approach the results of the original FALCON C-19 evaluation. This suggests a growing competence among users since the introduction of LFD tests in asymptomatic testing services in late 2020.

Figure 5 - DHSC post-market surveillance analysis of SARS-CoV-2 detection rate for paired same-day LFD and PCR testing (surge testing sites), FALCON C-19, combined historic DHSC service evaluation performance



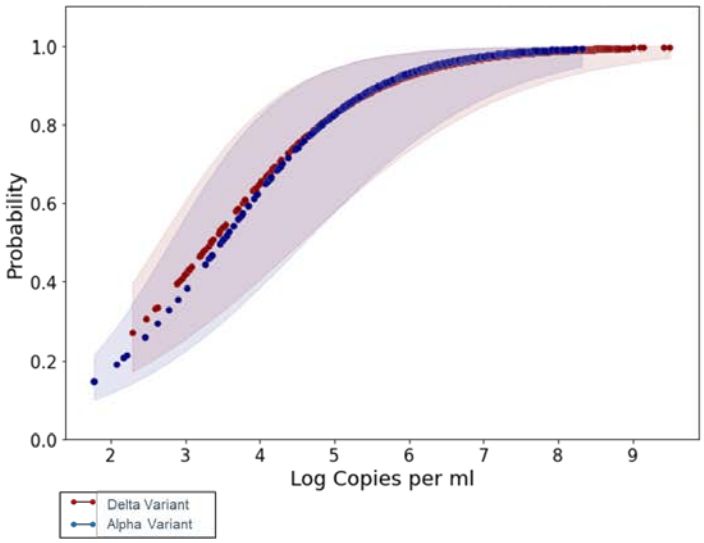
Logistic regression modelling has been performed on this data, as shown in Figure 6. This provides a deeper way to understand of the Biotime LFD device's performance along the

[19] The service evaluation programme performed evaluation of the Biotime LFD at symptomatic and asymptomatic test sites and incorporated evaluation when performed by trained operatives, and when performed as a self-test.

[20] FALCON-C19 observational evaluation (Facilitating Accelerated Clinical validation Of Novel diagnostics for COVID-19, 20/WA/0169, IRAS 284229)4. The evaluation incorporated use of the Biotime LFD by healthcare professionals on individuals recruited and re-tested within 5 days of a PCR confirmed diagnosis of SARS-CoV-2 infection during the period September – October 2020.

full range of viral loads than can be achieved by estimating performance in discrete categories. There was no statistical difference in detection rate between the two variants' logistic regression curves (logistic regression comparison between the Alpha and Delta variant: p-value = 0.622).

Figure 6 - Logistic regression modelling of the select surge testing site data for Delta and Alpha variants



Log viral concentration (dC/ml)	Viral concentration (dC/ml)	PPP*: Delta	PPP*: Alpha
2	100	21.9% (95% CI: 14.5-31.7)	17.9% (95% CI: 11.7-26.4)
3	1000	42.0% (95% CI: 25.4-60.5)	37.9% (95% CI: 22.4-56.4)
4	10000	65.1% (95% CI: 40.7-83.6)	63.1% (95% CI: 38.6-82.3)
5	100000	82.8% (95% CI: 57.9-94.4)	82.8% (95% CI: 57.8-94.4)
6	1000000	92.6% (95% CI: 73.4-98.2)	93.1% (95% CI: 74.9-98.4)
7	10000000	97.0% (95% CI: 84.7-99.5)	97.4% (95% CI: 86.7-99.5)

*PPP stands for predicted proportion positive, based on the logistic regression model.

1. LFD test result ~ log copies per ml * delta variant
2. Positive tests with a variant/strain other than Alpha or Delta were removed (12/1,645 PCR positive sequenced tests)
3. 95% confidence intervals have been calculated using the standard error associated with log copies per ml from the logistic regression (shaded area).

5. Conclusion

The routine laboratory assessment suggesting a lower sensitivity of the Biotime LFD did not translate into a difference in real-world performance within asymptomatic LFD testing or select surge testing sites operating a paired LFD-PCR testing regime.

The Biotime LFD remains suitable for deployment as part of the asymptomatic testing programme to identify infectious individuals in the population and to reduce onward transmission risk at a local and national population level. There is no difference in performance in its ability to detect the Delta variant in comparison to the Alpha variant.

With further variants expected to appear in the future and LFDs from other manufacturers planned for introduction into the testing programme, routine *in vitro* and post-market surveillance remains critical as part of a multi-level strategy to continually assess LFD antigen devices.

Appendix A: Glossary

Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR): qRT-PCR is used to identify the presence of genetic material through a process of genome amplification. qRT-PCR is able to detect very small amounts of RNA through this process. The process of qRT-PCR initially involves the extraction of the viral RNA from the sample. This is then purified, stabilised, and concentrated. The extract is then added to specific enzymes and primers. The primers are short stretches of nucleic acid that match to specific genome targets. Additionally, the 'building blocks' are added (nucleotide bases). Fluorescently labelled probes (short stretches of nucleic acid) that attach to the reaction product are also added and act as a reaction indicator for analysis. Through a cycle of repeated heating and cooling, thermal cycles, copies are made of the viral RNA. The primers attach allowing the enzyme to add the nucleotides and form a complementary DNA strand. With the thermal cycles the copies are doubled with each cycle leading to an exponential rise. The fluorescent probes then enable detection of these copies. The cycle at which fluorescent signal exceeds that of the background (and therefore passes the threshold for positivity) is known as the Cycle Threshold level (Ct), and varies depending on the starting viral load of the sample. Through the use of fixed sample input, and verified standard dilutions run alongside, quantification of the viral load (i.e. concentration of viral particles) in the sample can also be performed.

Cycle Threshold (Ct): qRT-PCR detects the presence of small amounts of an organism's DNA or RNA by amplifying through a number of cyclical steps until it reaches a measurable level. The number of cycles taken to reach this level is termed the Cycle threshold (Ct) and bears an inverse relationship to the amount of RNA present in the original sample (i.e. low Ct corresponds to high amounts of RNA).

Digital copies per ml (dC/ml): The estimated number of genetic material fragments (RNA) in a millilitre of sample analysed by qRT-PCR. The qRT-PCR Ct value can be mathematically converted to the concentration of virus particles present in the sample - a proxy for how 'present' they are in the individual, although it can also be affected by how well the sample has been collected. This concentration is called the estimated 'viral load'.

Sensitivity: The sensitivity of a test is a measure of how good the test is at detecting true positive cases. A high sensitivity test is unlikely to return a negative result for someone who is truly positive (a 'false negative').

Specificity: The specificity is a measure of how good the test is at discerning true negative cases. A high specificity test is unlikely to return a positive result for someone who is truly negative (a 'false positive').

Prevalence: The disease prevalence, which is a measure of how many positive cases there are in a population at any one given time. This interacts with a test's sensitivity and

specificity to determine the absolute number of false negative and false positive cases returned by a test. In general, for any given test, the number of false negative results is higher when prevalence is higher. By contrast, when prevalence is lower the number of false positives is higher, while the total number of true positive tests results will be lower, making false positives a greater share of all positive test results.

Real World Performance Monitoring (RWPM): Real world performance monitoring refers to data that is captured only through analysis of testing within the wider Test programme and not performed under a specific evaluation protocol.

In vitro: Assessment performed in the laboratory environment.

Victoria strain: Victoria strain (2019-nCoV/Victoria/1/2020) is a live virus isolate considered synonymous with the wild type. Nomenclature: SARS-CoV-2 Victoria/01/2020. Provided by The Doherty Institute, Melbourne, Australia at P1 and passaged twice in Vero/hSLAM cells 405 [ECACC 04091501]. Caly, L. et al. Isolation and rapid sharing of the 2019 novel coronavirus (SARS702 CoV-2) from the first patient diagnosed with COVID-19 in Australia. n/a, 703 doi:10.5694/mja2.50569. PHE Porton Down received the virus from early February 2020.

Alpha variant: Alpha (Pango lineage B.1.1.7) first documented in the United Kingdom in September 2020 and designated 18th December 2020.

Delta variant: Delta (Pango lineage B.1.617.2) first documented in India in October 2020 and designated 4th April 2021.

Kappa variant: Kappa (Pango lineage B.1.617.1) first documented in India in October 2020 and designated 4th April 2021.

Gamma variant: Gamma (Pango lineage P.1) first documented in Brazil in November 2020 and designated 11th January 2021.

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