THE LANCET
Infectious Diseases

Supplementary appendix

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Supplement to: Eyre DW, Futschik M, Tunkel S, et al. Performance of antigen lateral flow devices in the UK during the alpha, delta, and omicron waves of the SARS-CoV-2 pandemic: a diagnostic and observational study. *Lancet Infect Dis* 2023; published online March 28. https://doi.org/10.1016/S1473-3099(23)00129-9.

Performance of antigen lateral flow devices in the UK during the alpha, delta, and omicron waves of the SARS-CoV-2 pandemic: a diagnostic and observational study

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Supplementary Methods

Swab details and assays

LFDs evaluated were the (i) Innova SARS-CoV-2 Lateral Flow Antigen Test (Innova, in original packaging or repacked with individual buffer containers, also known as Biotime); (ii) Orient Gene COVID-19 Ag Rapid Test Cassette LFD antigen tests (Orient Gene); and (iii) Acon Flowflex SARS-CoV-2 antigen rapid test (Self-Testing) kit (Acon). These tests remained the same during the study period. Innova LFDs were performed using a combined anterior nose and throat swab. Acon and Orient Gene LFDs were performed on anterior nose swabs. Tests were performed according to the manufacturer's instructions, including pre-specified interpretation of positive, negative, and void results.

Evaluated LFDs were selected from those in use the UK's national testing programme. SureScreen LFDs were included in the initial dataset, but the number performed (n<30) was too low to provide reliable estimates and results were only available from November 2021, and so these results are not presented. The process for assessing if LFD kits meet the standards set for inclusion in the national COVID-19 testing programme is outlined here: Assessment and procurement of coronavirus (COVID-19) tests - GOV.UK (www.gov.uk). Passing this standard is a requirement for inclusion in the procurement exercises undertaken, which are based on several criteria including cost.

PCR testing was undertaken by routine laboratories within the NHS Test and Trace laboratory network. It was performed predominantly using the Thermo Fisher SARS-CoV-2 TaqPath assay, and also using the Randox COVID-19 qPCR kit, the Applied Biosystems TaqMan Fast Virus 1-step RT-PCR assay, the PerkinElmer New Coronavirus Nucleic Acid Detection Kit, the PerkinElmer SARS-CoV-2 RT qPCR Reagent Kit, the Nonacus VirPath SARS-CoV-2 Multiplex qRT-PCR kit and the Clarigene SARS-CoV-2 assay. Thresholds used to determine a positive PCR test were identical to those used for routine clinical reporting by each accredited laboratory. PCR testing was performed on combined anterior nose and throat swabs, however nose only swabs were considered acceptable if swabbing both the throat and nose was not possible, e.g., in a distressed child.

Sample pairs with a void PCR and/or void LFD result were excluded from the analysis. Similarly, sample pairs without valid PCR and LFD results were excluded. Sample pairs with missing covariate

data were excluded from the regression analyses described below, where covariate data was required.

Where results of evaluation samples indicated a previously undetected infection, participants were asked to act on that result and follow relevant national guidelines. No reported adverse events occurred during the evaluations.

Statistical analysis

LFD performance

Data were linked within UKHSA systems and subsequently deidentified prior to being extracted for analysis. For LFD performance evaluations the infecting variant in PCR-positive infections was assigned based on sequencing or PCR-based genotyping where available and if not, based on the dominant variant in sequenced samples from the same week in the participant's local region (Lower Tier Local Authority) if >50% of samples were of a single variant and the address of the participant was known. Where the address was unknown if >50% of sequenced samples nationally were of a single variant then this variant was assigned (before 19 May 2021, Alpha/Pre-Alpha; 19 May – 12 December 2021, Delta; after 12 December 2021, Omicron), otherwise the variant was set to unknown. Based on the sequenced or genotyped samples in the dataset (n=517), we estimated the precision of this approach (i.e., the percentage of correctly predicted variants) to be 96%. A higher setting of the threshold for the percentage of cases due to a single variant could increase the precision to over 99% but only at the expense of a substantial loss of coverage to 60%. Similarly, to assess the specificity of LFDs according to time epochs defined by the dominant variant, local and national incidence data were used to assign a variant epoch to each sample.

Real time PCR cycle threshold (Ct) values were used to estimate SARS-CoV-2 viral loads in copies/mL using conversion formulae derived for each laboratory using calibrant samples (Qnostics SCV2AQP01 quantitative SARS-CoV-2 standards panel, calibrated in digital droplet PCR copies per mL; Table S2). Sample pairs only tested by endpoint PCR were excluded.

PCR-positive samples were used for analyses of LFD sensitivity. Univariable and multivariable logistic regression was used to model the relationship between LFD positivity and $\log_{10}$ viral load and other covariates. Covariates included LFD device, study setting, assisted vs. self-testing, self-reported symptom status (symptomatic, i.e., any of fever, cough or anosmia/ageusia, otherwise asymptomatic), vaccination status by number of doses (0, 1, 2 or more) and viral variant (Alpha [B.1.1.7] / pre-Alpha [B.1.177], Delta [B.1.617.2], Omicron [BA.1 and BA.2]; or Other / Unknown). PCR-negative samples were used to analyse LFD specificity using univariable and multivariable logistic regression and the same covariates. Non-linear effects of continuous variables were allowed for using natural cubic splines (with up to 5 default-placed knots). These were included in the final model if they improved model fit based on a reduction of >2 in the Bayesian information criterion. We also refitted models with an interaction between $\log_{10}$ viral load and LFD device to allow any differences between devices in the relationship between sensitivity and viral load to be visualised.

Samples sizes for specific UKHSA sub-studies (e.g., assisted testing in asymptomatic participants in community settings using the Innova LFD at a specific time point) were determined to provide enough samples to detect an absolute change in sensitivity of >10% compared to pre-deployment performance with 80% power (two-sided alpha=0.05). This was determined to be 154 PCR-positive cases in each sub-study. However, as all available data were pooled in this current study, no specific sample size calculation was used for the whole dataset.

Contact tracing data analysis

We also estimated the proportion of infectious individuals potentially detectable by LFDs. We used contact testing data and logistic regression to estimate the relationship between index case symptom status and PCR Ct values/viral loads, and positive results in PCR/LFD-tested contacts. We used the same Ct value to viral load conversions used above (Table S2).

We followed a similar approach to previous analyses[1,2] adjusting for index case age, sex, vaccination status (partial, two doses, boosted), as well as contact event type, contact age, sex, vaccination status, and calendar time (as a proxy for changes with time, incidence, and circulating variants). Natural cubic splines were used to account for non-linearity in continuous variables (5 knots; except calendar time, 9 knots). Pre-specified interactions based on previous analyses [1] were included between contact event type and index case age, contact event type and contact age, index case sex and contact sex, index case age and contact age. Interactions were generated by multiplying each spline term for non-linear continuous variables.

We used index case-contact pairs plausibly related by transmission to estimate the proportion of infectious index cases potentially detected by LFDs. Each index case was included in the analysis only once, however the total number of linked PCR/LFD-positive contacts per index case was also recorded to allow evaluation of whether performance differed in more infectious index cases. Each index case was included only once as the focus of the analysis was how many potential infectious index cases might be detectable by LFD, rather than how many transmission events might be averted. We performed a separate analysis by month from 01 January 2021 to 11 January 2022, using data from all index cases with at least one PCR/LFD-positive contact. The analysis was ended on 11 January 2022 as after this date the requirement for positive LFDs to be confirmed by PCR was dropped, meaning both index cases and infected contacts were less well ascertained. To estimate the probability of each index case being LFD-positive, we applied the estimated performance in community testing of the most widely used LFD (Innova), accounting for viral load, the symptom status of the index case. Results are plotted according to the nationally dominant circulating variant at each index case's diagnosis (01 January 2021 to 18 May 2021, Alpha; 19 May to 12 December 2021, Delta; 13 December 2021 to 11 January 2022, Omicron).

We used non-parametric bootstrap sampling with replacement of index cases (1000 iterations) to estimate 95% confidence intervals. To ensure our estimates reflected the uncertainty in LFD performance estimates we also applied bootstrap sampling to the LFD performance data, re-estimating LFD performance for each iteration.

Ethics

Within the context of the pandemic public health response and roll out of testing interventions, after review using the Health Research Authority (HRA) tool and further discussions with HRA it was determined that this evaluation would not require HRA research ethics approval. After an initial period, it was determined to gain Public Health England's Research Ethics and Governance Group (PHE REGG) approval (then separate to NHS Test and Trace) as service evaluations for subsequent studies to ensure further external scrutiny and assurance on this approach. Approval was obtained for an umbrella framework and associated participant-facing materials for the prospective data collection elements of Service Evaluation and Ongoing Evaluation. This was reviewed and approved under REGG R and D 438.

Supplementary figures

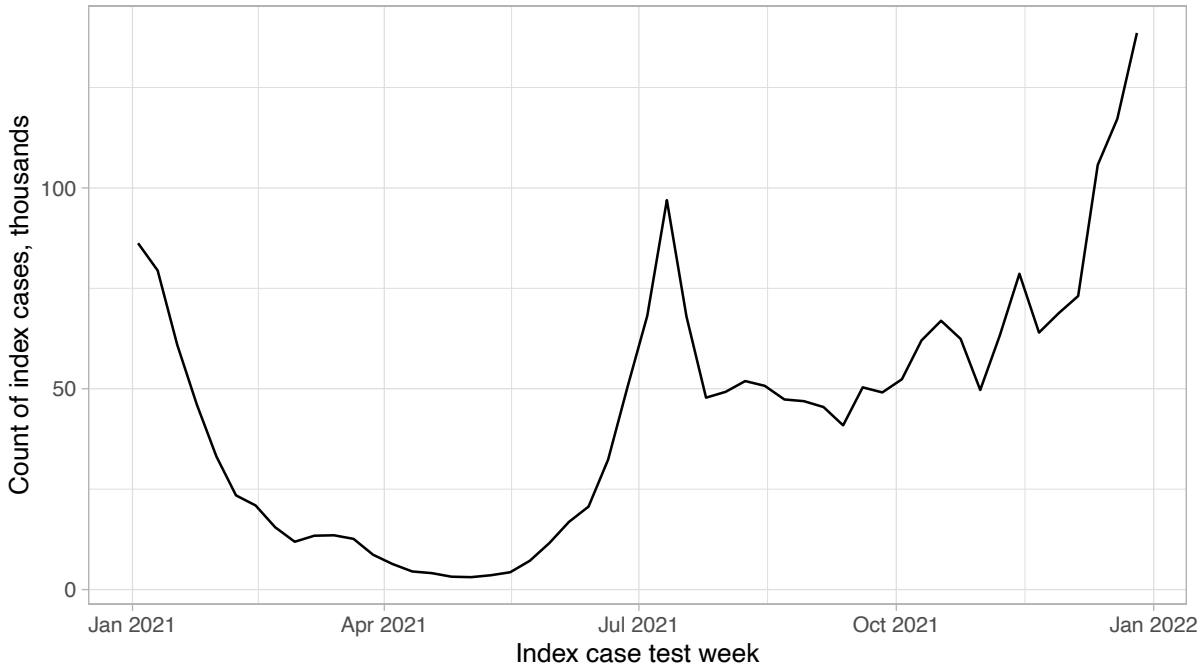

Figure S1. SARS-CoV-2 contact tracing index cases per week detected at three national testing "Lighthouse" laboratories in Milton Keynes, Alderley Park and Glasgow.

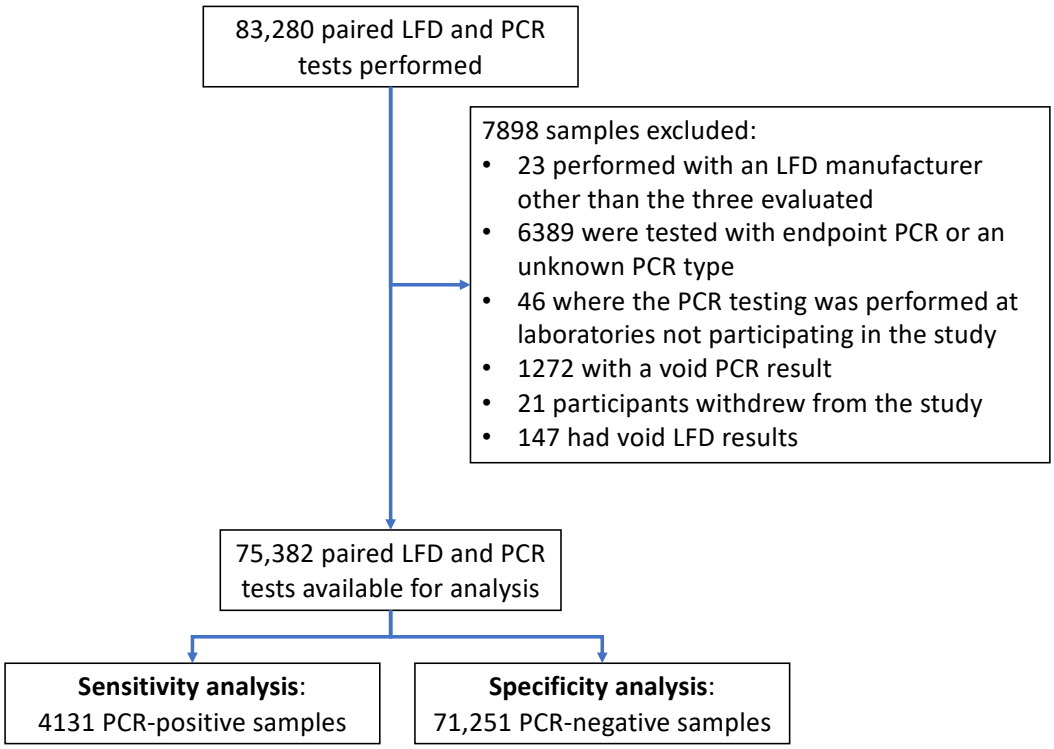

Figure S2. SARS-CoV-2 lateral flow device (LFD) and PCR paired samples.

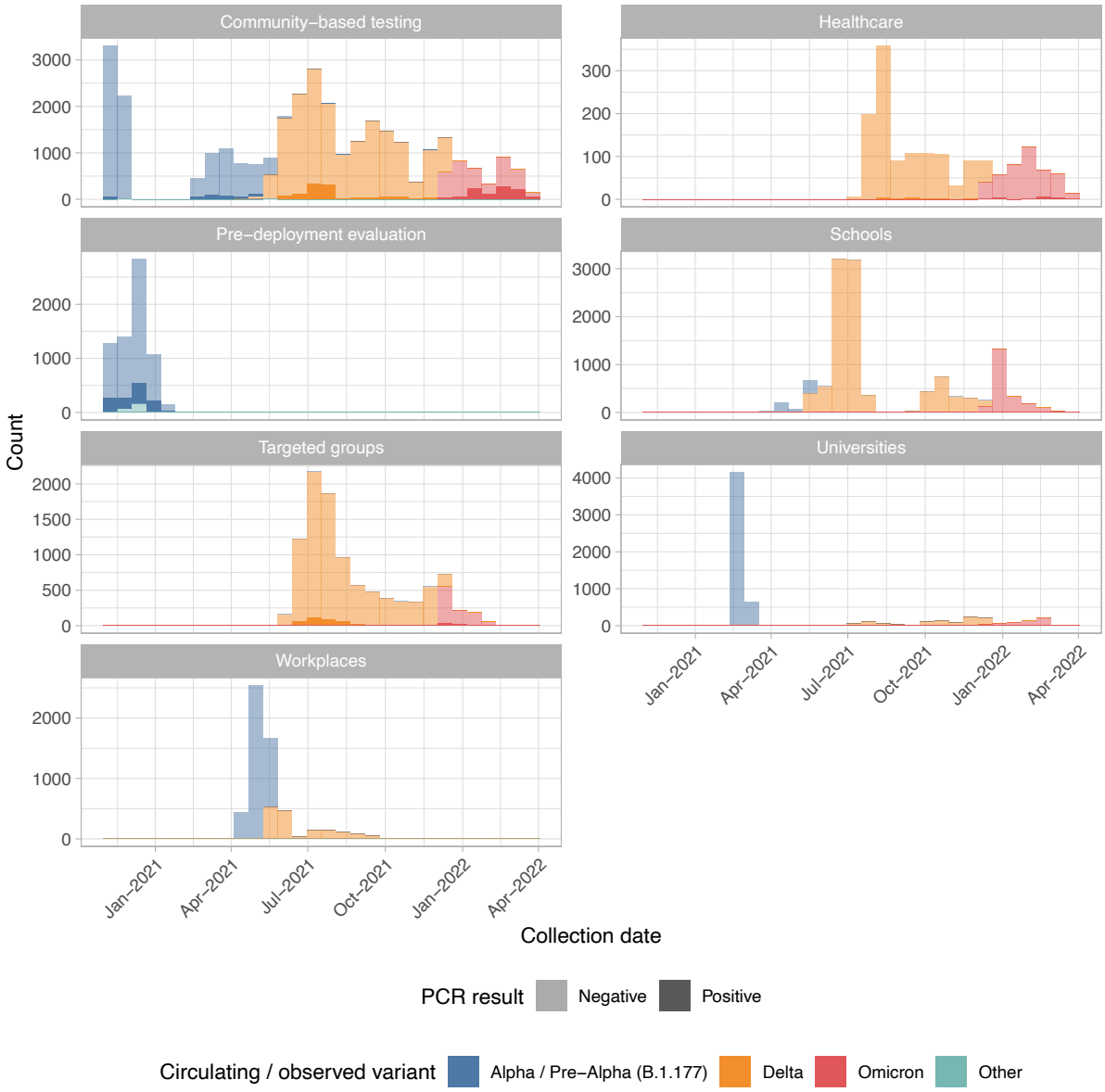

Figure S3. Lateral flow device evaluation samples by study setting, PCR result and circulating / observed variant.

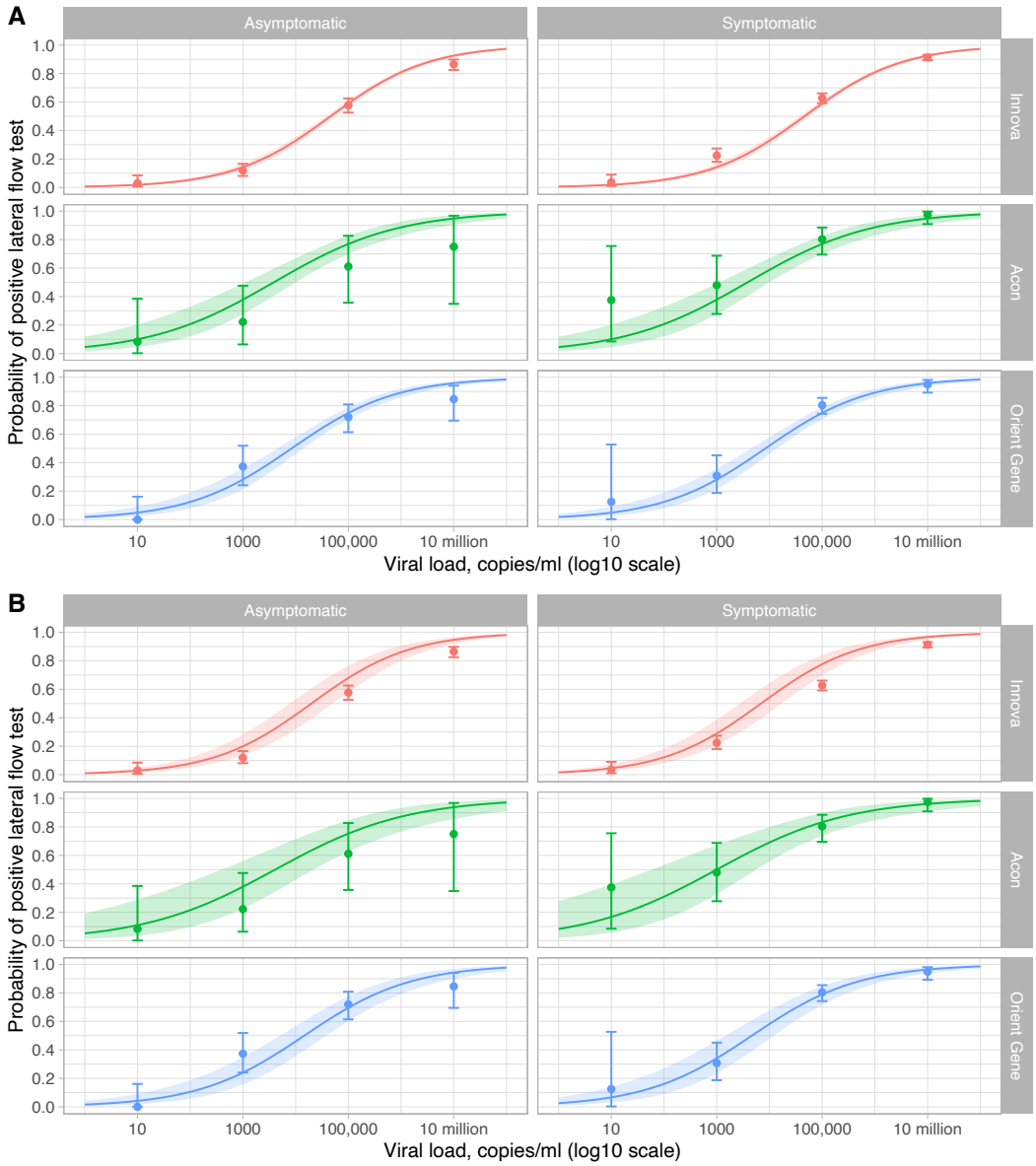

Figure S4. Sensitivity of SARS-CoV-2 lateral flow devices, by viral load and patient symptoms. Points (with error bars indicating exact binomial confidence intervals) are the observed data for <100 copies/ml, 100 to <10,000 copies/ml, 10,000 to <1 million copies/ml and 1 million to <100 million copies/ml. Model estimates are shown by the continuous lines; the model results were determined by fitting viral load on a continuous scale (the observed data are shown as categorical for visualisation purposes only). Panel A shows unadjusted estimates from a model containing only $\log_{10}$ viral load, lateral flow device and symptom status. Panel B shows estimates also adjusted for test setting (predictions are shown for community-based testing), assistance performing the test (self-performed), vaccination status (unvaccinated), and variant (Alpha/Pre-Alpha (B1.1.177)). In both models an interaction term between viral load and lateral flow device is included to allow the shape of the curves plotted to vary by device. The difference in model fit compared to the observed data between panels A and B reflects the impact of confounders adjusted for in panel B. There was no evidence that adjusted models allowing for a non-linear relationship between the log odds and $\log_{10}$ viral load (using splines with up to 5 knots) improved model fit based on the Bayesian information criterion.

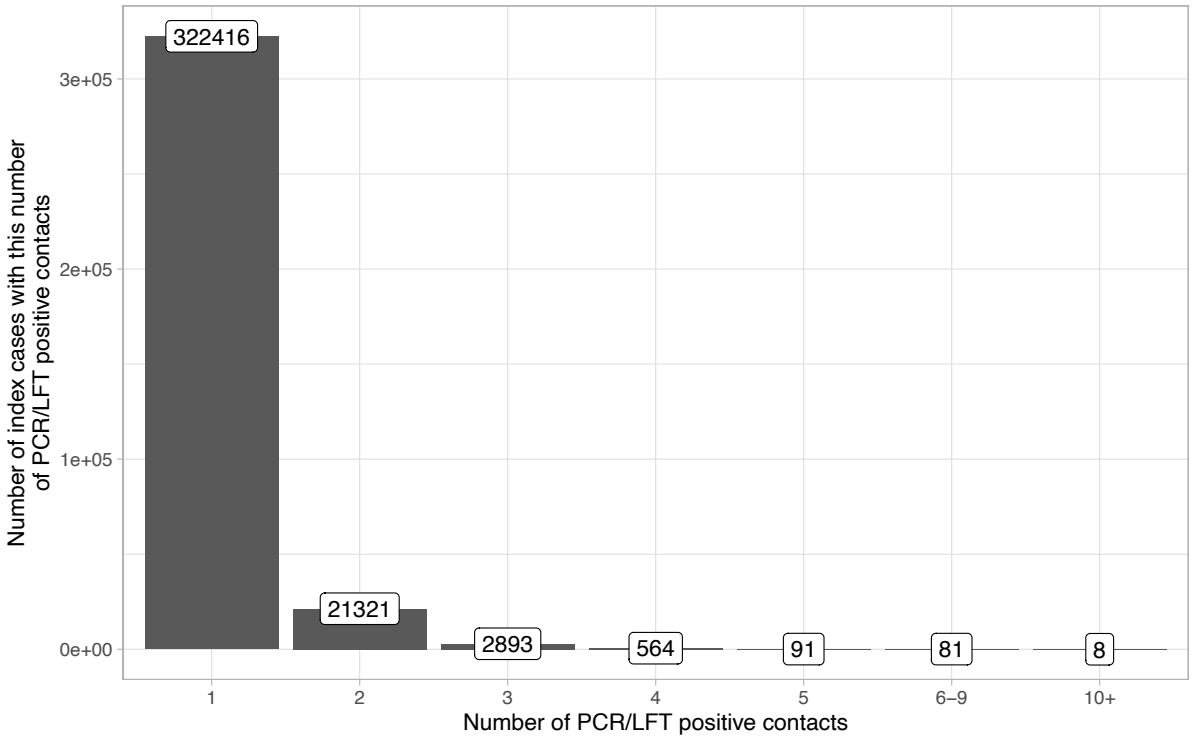

Figure S5. Number of PCR/LFD-positive contacts per index case in national contact testing data.

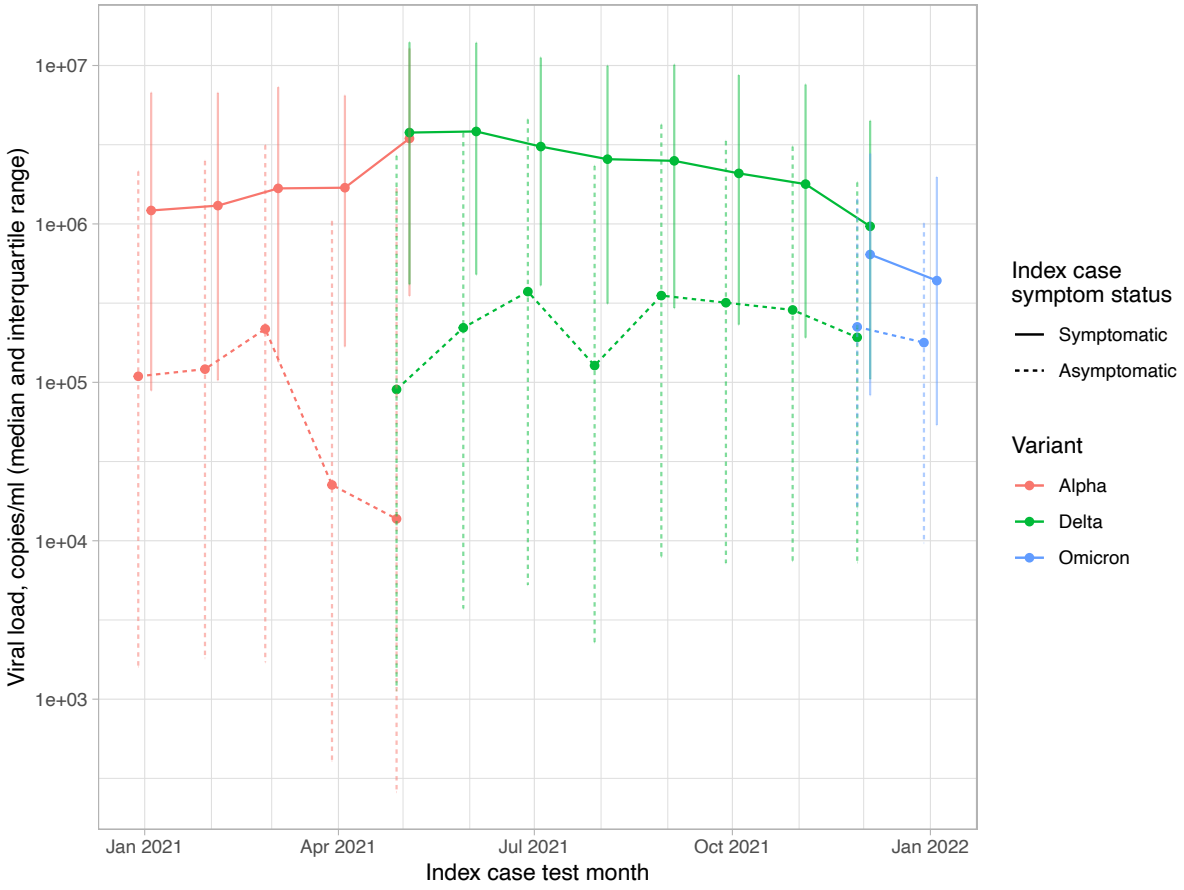

Figure S6. Index case viral load in probable case-contact transmission pairs. The median viral load is plotted with vertical bars indicating the interquartile range. Data are plotted aggregated by month with lines coloured by the nationally dominant circulating variant at each index case's diagnosis (01 January 2012 to 19 May 2021, Alpha; 19 May to 12 December 2021, Delta; 12 December 2021 to 11 January 2022, Omicron). The line type indicates index case symptom status.

Supplementary Tables

Study ID	LFDs evaluated	Test sites	Collection dates	Sampling	Inclusion criteria	Publications citing this study
LFD001	Innova Assisted testing	Haydock RTS, Leeds RTS, Manchester Airport RTS, Newcastle RTS	4 Nov 2020 to 18 Dec 2020	All subjects arriving at the symptomatic RTS (regional test site) for a PCR diagnostic test were invited to take part in the evaluation.	• Aged ≥18 years old • Subjects consented to take part and to have their data used for evaluation of LFDs	b
LFD002	Innova Self-testing	Manchester Etihad RTS, York RTS	23 Nov 2020 to 9 Jan 2021	All subjects arriving at the symptomatic RTS for a PCR diagnostic test were invited to take part in the evaluation.	• Subjects were willing to self-collect throat and nose swab samples (or, for children aged under 12 the parent or guardian was willing to administer the swab on their behalf) • Subjects (or their parent or guardian) consented to take part and to have their data used for evaluation of LFDs	b
LFD011 a.k.a. Accel	Acon, Innova, Orient Gene Self-testing	LTS/RTS across England	18 Feb 2021 to 31 Jul 2021	All subjects arriving at the symptomatic RTS/LTS (local test site) for a PCR diagnostic test were invited to take part in the evaluation.	• Aged ≥16 • Subjects consented to take part and to have their data used for evaluation of LFDs	c**
Liverpool MAST a.k.a. LFD Smart	Innova Assisted testing	Local asymptomatic test sites (ATS) across Liverpool	8 Nov 2020 to 29 Nov 2020	All subjects who arrived at the ATS were invited to take part in the evaluation.	• Aged ≥18 years old • The subjects were asymptomatic • Subject consented to take part and have their data used for evaluation of LFDs	a, b*
LFD101	Innova Assisted testing	RTS/LTS across England	12 Feb 2021 to 4 Mar 2022	Service teams were responsible for determining which sites to select for participation in the ongoing evaluation process, and what volumes to request from each site. Volumes could be completed in one day, or over several days depending on what works best for each site. Generally, it was assumed that all eligible subjects at a participating site on a day the ongoing evaluation process was running would be asked to participate so that the selection of participants provided a representative sample of subjects attending the site for testing.	• Aged ≥16 • Subject consented to take part and have their data used for evaluation of LFDs	

LFD102	Acon, Innova, Orient Gene Self-testing	Self-testing with kits collected from the workplace (public and private), schools, universities, independent health providers, and targeted community testing sites.	29 Mar 2021 to 21 Mar 2022	Service teams were responsible for determining which sites to select for participation in the ongoing evaluation process, and what volumes to request from each site. Volumes could be completed in one day, or over several days depending on what worked best for each site. Generally, it was assumed that all eligible subjects at a participating site on a day the ongoing evaluation process was running would be asked to participate so that the selection of participants provided a representative sample of subjects attending the site for testing.	• Aged ≥16 except in schools • Subject consented to take part and have their data used for evaluation of LFDs	
LFD103	Acon, Innova, Orient Gene Self-testing	Self-testing via kits ordered online. Tests were either posted to the individual's home or the individual collected the kit from the pharmacy	20 May 2021 to 21 Mar 2022	Home testing sampling: 1 in 10 orders were also sent an invitation to take part in ongoing evaluation which included a swab for taking a PCR plus instructions for use. Pharmacy sampling: ongoing evaluation kits were distributed to participating pharmacies on a rota system each week. The pharmacies were asked to randomly give out the kits.	Home testing: •Anyone who was eligible to request LFDs online was considered eligible for participation in ongoing evaluation Pharmacies: • Aged ≥18 • Asymptomatic (at the time of collecting the LFDs) For both pharmacies and home testing the participant consented to participate and to have their data used for evaluation of LFDs	
LFD104	Acon, Innova, Orient Gene Self-testing	LTS/RTS across Scotland	8 Jan 2022 to 21 Mar 2022	All subjects who attended a symptomatic LTS/RTS for a PCR test were offered a box of LFD tests to use at home and asked to take and report one of these LFD tests as part of ongoing evaluation.	• Aged ≥18 • Subject consented to take part and have their data used for evaluation of LFDs	

Table S1. Evaluations included and details of previous publications. Exclusion criteria for all studies were age outside of the inclusion criteria and absence of capacity and willingness to consent. There were no other exclusion criteria, however the operational delivery of testing always took precedence so, if for example, sites became particularly busy the evaluation would be suspended so testing continued to be delivered efficiently. Please see Table S2 for details of the number of samples in each study.

Publications

a) García-Fiñana et al. 2021. Performance of the Innova SARS-CoV-2 antigen rapid lateral flow test in the Liverpool asymptomatic testing pilot: population based cohort study. *BMJ* **374**:n1637.

b) Department of Health and Social Care. 2021. Asymptomatic testing for SARS-CoV-2 using antigen-detecting lateral flow devices: evidence from performance data October 2020 to May 2021. Available at https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/999866/asymptomatic-testing-for-SARS-CoV-2-using-antigen-detecting-lateral-flow-devices-evidence-from-performance-data-Oct-2020-to-May-2021.pdf

c) Department of Health and Social Care. 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). Available at https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/999867/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.pdf

* Data presented for the Liverpool MAST study are based on results found in reference b). These differ to those originally reported in reference a). The results in reference b) were based on reappraised LFD results after the LFD interpretations were independently reassessed by member of the NHS Test and Trace central data management team based on available photographic images of the LFDs. Thus, the results differ from the those published in reference a) which were based on on-site read-out of the LFDs.

**Only a small subset (56 paired tests) of the included data has been previously used for published reports. In particular, only data from 1 April 2021 to 2 June 2021 from surge testing (LFD011) of asymptomatic participants with verified variant of concern by sequencing were included in the technical report.

Study ID	Total paired samples	PCR positive	PCR positive, LFD positive	PCR positive, LFD negative	Sensitivity	Sensitivity 95% CI	PCR negative	PCR negative, LFD negative	PCR negative, LFD positive	Specificity	Specificity 95%CI
LFD001	4286	699	381	318	54.5	50.7 - 58.2	3587	3572	15	99.6	99.3 - 99.8
LFD002	2473	424	212	212	50	45.1 - 54.9	2049	2030	19	99.1	98.6 - 99.4
LFD011 a.k.a. Accel	7961	1098	791	307	72	69.3 - 74.7	6863	6813	50	99.3	99.0 - 99.5
Liverpool MAST a.k.a. LFD Smart	5534	74	39	35	52.7	40.7 - 64.4	5460	5456	4	99.9	99.8 - >99.9
LFD101	21583	494	245	249	49.6	45.1 - 54.1	21089	21067	22	99.9	99.8 - 99.9
LFD102	14134	111	51	60	45.9	36.4 - 55.7	14023	14006	17	99.9	99.8 - 99.9
LFD103	17761	489	350	139	71.6	67.4 - 75.5	17272	17197	75	99.6	99.5 - 99.7
LFD104	1650	742	540	202	72.8	69.4 - 76.0	908	900	8	99.1	98.3 - 99.6

Table S2. Evaluations included and LFD performance metrics. The sample numbers provided are for the analysed samples only, see Figure S2 for exclusions. See Table S1 for descriptive details for each study. LFD, lateral flow device; CI, confidence interval.

Lab (Assay)	Ct (denoted x) to log_{10}(viral load, copies per ml, denoted y)			
	ORF1ab	N-Gene	S-Gene	E-Gene
Randox Assay AQP1-A (Randox COVID-19 qPCR kit)	y = -0.3065x + 12.477	-	-	y = -0.3103x + 12.850
Randox Assay PE (Randox COVID-19 qPCR kit)	y = -0.2909x + 11.921	y = -0.3355x + 13.334	-	-
Alderley Park (Thermo Fisher TaqPath)	y = -0.3035x + 11.599	y = -0.3120x + 11.881	-	-
Glasgow (Thermo Fisher TaqPath)	y = -0.3050x + 11.372	y = -0.3096x + 11.449	y = -0.2894x + 11.221	-
Milton Keynes (Thermo Fisher TaqPath)	y = -0.3181x + 11.859	y = -0.3241x + 12.119	y = -0.3641x +13.372	-
HSL UCL (Applied Biosystems TaqMan Fast Virus 1-step RT-PCR)	-	y = -0.2915x + 14.049	-	-
Newcastle (PerkinElmer New Coronavirus Nucleic Acid Detection Kit)	y= -0.2831x + 11.354	y = -0.3161x + 11.882	y = -0.3857x + 14.003	-
Plymouth (PerkinElmer SARS-CoV-2 RT qPCR reagent kit)	y = -0.2971x + 12.649	y = -0.3441x + 14.637	-	-

Table S3. Testing laboratories, assays and conversion formulae from SARS-CoV-2 PCR results to viral load. Results were determined using the Qnostics SCV2AQP01 quantitative SARS-CoV-2 standards panel, and linear regression models. For LFD performance assessments the mean Ct value used as input was calculated across all detected targets. For transmission analyses, index case viral loads were calculated using the mean Ct value for the ORF1ab and N genes. Conversion formulae are only shown for laboratories with at least one PCR-positive result. Other PCR assays used in evaluations included the Nonacus VirPath SARS-CoV-2 Multiplex qRT-PCR kit and Clarigene SARS-CoV-2 assay.

Scenario	Total population	Population prevalence	Prevalence of symptoms due to other reasons	Number symptomatic for any reason	Proportion of infections symptomatic	Proportion of symptomatic cases who transmit	Proportion of asymptomatic cases who transmit	Number of potential symptomatic transmitters	Number of potential asymptomatic transmitters	LFD sensitivity in symptomatic transmitters	LFD sensitivity in asymptomatic transmitters	Number needed to test to detect one symptomatic transmitter	Number needed to test to detect one asymptomatic transmitter
Baseline	100000	1%	0.5%	1000	50%	6%	4%	30	20	79%	57%	42	8684
Increased prevalence	100000	**2%**	0.5%	1500	50%	6%	4%	60	40	79%	57%	32	4320
	100000	**5%**	0.5%	3000	50%	6%	4%	150	100	79%	57%	25	1702
Increased symptoms for other reasons	100000	1%	**1.0%**	1500	50%	6%	4%	30	20	79%	57%	63	8640
	100000	1%	**5.0%**	5500	50%	6%	4%	30	20	79%	57%	232	8289
Increased transmission	100000	1%	0.5%	1000	50%	**20%**	**10%**	100	50	79%	57%	13	3474
Comparable LFD performance regardless of symptoms	100000	1%	0.5%	1000	50%	6%	4%	30	20	79%	79%	42	6266
Perfect LFD performance	100000	1%	0.5%	1000	50%	6%	4%	30	20	**100%**	**100%**	33	4950
	100000	**5%**	0.5%	3000	50%	6%	4%	150	100	**100%**	**100%**	20	970

Table S4. Number of LFD tests needed to detect symptomatic and asymptomatic cases that would otherwise go on to transmit. Different scenarios are shown for an example population of 100,000 people. The factors changed in each scenario are shown in bold. For illustrative purposes, a total of 50% of infections are assumed to be asymptomatic. We assume that 6% of index cases went on to transmit and that asymptomatic cases were around 0.7-times as infectious as symptomatic cases, based on estimates from this study, accepting these rates depend on tests being sought by contacts between 1 and 10 days following the index cases' diagnosis and are therefore likely to be somewhat underestimated (a scenario with higher transmission rates is also shown). Estimates of LFD sensitivity in symptomatic and asymptomatic sources of onward transmission are taken from this study.

References

1. Eyre DW, Taylor D, Purver M, Chapman D, Fowler T, Pouwels KB, et al. Effect of Covid-19 Vaccination on Transmission of Alpha and Delta Variants. New Engl J Med. 2022;386: 744–756. doi:10.1056/nejmoa2116597

2. Lee LYW, Rozmanowski S, Pang M, Charlett A, Anderson C, Hughes GJ, 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; ciab421-. doi:10.1093/cid/ciab421