Clinical and laboratory evaluation of SARS-CoV-2 lateral flow assays for use in a national COVID-19 seroprevalence survey

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ABSTRACT

Background Accurate antibody tests are essential to monitor the SARS-CoV-2 pandemic. Lateral flow immunoassays (LFIA) can deliver testing at scale. However, reported performance varies, and sensitivity analyses have generally been conducted on serum from hospitalised patients. For use in community testing, evaluation of finger-prick self-tests, in non-hospitalised individuals, is required.

Methods Sensitivity analysis was conducted on 276 non-hospitalised participants. All had tested positive for SARS-CoV-2 by reverse transcription PCR and were ≥21 days from symptom onset. In phase I, we evaluated five LFIA in clinic (with finger prick) and laboratory (with blood and sera) in comparison to (1) PCR-confirmed infection and (2) presence of SARS-CoV-2 antibodies on two ‘in-house’ ELISAs. Specificity analysis was performed on 500 prepandemic sera. In phase II, six additional LFIA were assessed with serum.

Findings 95% (95% CI 92.2% to 97.3%) of the infected cohort had detectable antibodies on at least one ELISA. LFIA sensitivity was variable, but significantly inferior to ELISA in 8 out of 11 assessed. Of LFIA assessed in both clinic and laboratory, finger-prick self-test sensitivity varied from 21% to 92% versus PCR-confirmed cases and from 22% to 96% versus composite ELISA positives. Concordance between finger-prick and serum testing was at best moderate (kappa 0.56) and, at worst, slight (kappa 0.13). All LFIA had high specificity (97.2%–99.8%).

Interpretation LFIA sensitivity and sample concordance is variable, highlighting the importance of evaluations in setting of intended use. This rigorous approach to LFIA evaluation identified a test with high specificity (98.6% (95%CI 97.1% to 99.4%)), moderate sensitivity (84.4% with finger prick (95% CI 70.5% to 93.5%)) and moderate concordance, suitable for seroprevalence surveys.

INTRODUCTION

There are currently more commercially available antibody tests for SARS-CoV-2 than any other infectious disease. By May 2020, over 200 tests were available or in development. Accurate antibody tests are essential to monitor the COVID-19 pandemic at population level, to understand immune response and to assess individuals’ exposure and possible immunity from reinfection with SARS-CoV-2. Serology for national surveillance remains the fourth key pillar of the UK’s national testing response.

Access to high-throughput laboratory testing to support clinical diagnosis in hospitals is improving. However, the use of serology for large-scale seroprevalence studies is limited by the need to take venous blood and transport it to centralised laboratories, as well as assay costs. Lateral flow...
immunoassays (LFIs) offer the potential for relatively cheap tests that are easily distributed and can be either self-administered or performed by trained healthcare workers. However, despite manufacturers’ claims of high sensitivity and specificity, reported performance of these assays has been variable2–9 and their use is limited to date.

In the UK, the Medicines and Healthcare Products Regulatory Agency (MHRA) requires that clinical sensitivity and specificity must be determined for each claimed specimen type, and that sample equivalence must be shown.10 For antibody tests intended to determine whether an individual has had the virus, the MHRA recommend a sensitivity >98% (95% CI 96% to 100%) (on a minimum of 200 known positive specimens, collected 20 days or more after symptom onset) and specificity >98% on a minimum 200 known negatives.10 To date, no LFIs have been approved for use by these criteria. However, LFIs with lower sensitivity can still play an important role in population seroprevalence surveys,11 in which individual results are not used to guide behaviour, provided specificity (and positive predictive value) is high. Such tests will need to have established performance characteristics for testing in primary care or community settings, including self-testing.

As part of the REACT (Real Time Assessment of Community Transmission) programme,12 we assessed LFIs for their suitability for use in large seroprevalence studies. This study addresses the key questions of how well LFIs perform in people who do not require hospitalisation, and how finger-prick self-testing compares with laboratory testing of serum on LFIs and ELISA.

**METHODS**

A STARD checklist (of essential items for reporting diagnostic accuracy studies) is provided in the online supplementary section.

**Patient recruitment and selection of sera**

Between 1 and 29 May 2020, adult NHS workers (clinical or non-clinical), who had previously tested positive for SARS-CoV-2 by PCR, but not hospitalised, were invited to enrol into a prospective rapid antibody testing study, across four hospitals in two London NHS trusts. Participants were enrolled once they were at least 21 days from the onset of symptoms, or positive swab test (whichever was earlier). Sera for specificity testing were collected prior to August 2019 as part of the Airwaves study13 from police personnel.

**Test selection**

LFIs were selected based on manufacturer’s performance data, published data, where available, and the potential for supply to large-scale seroprevalence surveys. Initially, five LFIs were assessed, with a view to using the highest performing test in a national seroprevalence survey commencing in June 2020 (phase I). After selection of an initial candidate, further evaluation was undertaken of LFIs to be considered for future seroprevalence surveys (phase II, ongoing). For all LFIs, sensitivity analysis was conducted on a minimum 100 sera from the assembled cohort. LFIs with >80% sensitivity underwent further specificity testing, and those with specificity >98% are being evaluated in clinical tests in detecting IgG antibodies to SARS-CoV-2, at least 21 days from symptom onset. For consistency, in the three kits which had separate IgM and IgG bands, only IgG was counted as a positive result (ie, ‘MG’ or ‘G’ but not ‘M’, distinct from manufacturer guidance).

**Study procedure**

Each participant performed one of five LFIA self-tests with finger-prick capillary blood, provided a venous blood sample for laboratory analysis, and completed a questionnaire regarding their NHS role and COVID-19 symptoms, onset and duration (see online supplementary table i: flow of participants). Participants were asked to rate their illness as asymptomatic, mild, moderate or severe, based on its effect on daily life, and record symptoms based on multiple choice tick box response. Baseline characteristics are shown in table 1 and in the supplement.

The LFIA self-tests were performed using instructions specific to each device (see online supplementary table i) observed by a member of the study team. Results were recorded at the times specified in the product insert. Participants were asked to grade their illness as asymptomatic, mild, moderate or severe, based on its effect on daily life, and record symptoms based on multiple choice tick box response. Baseline characteristics are shown in table 1 and in the supplement.

### Table 1  Baseline characteristics

| Participant characteristics | All individuals (n=315) |
|----------------------------|------------------------|
| Age                        | 37 (29–47)             |
| Female, n (%)              | 221 (71)               |
| Role, n (%)                | 13 (4)                 |
| Doctor                     | 111 (36)               |
| Nurse or midwife           | 114 (37)               |
| Other clinical             | 51 (17)                |
| Non-clinical               | 31 (10)                |
| Comorbidities, n (%)       |                        |
| Organ transplant recipient | 1 (0)                  |
| Diabetes (type I or II)    | 7 (2)                  |
| Heart disease or heart problems | 6 (2)              |
| Hypertension               | 20 (6)                 |
| Overweight                 | 50 (16)                |
| Anaemia                    | 7 (2)                  |
| Asthma                     | 33 (11)                |
| Other lung condition       | 1 (0)                  |
| Weakened immune            | 3 (1)                  |
| Depression                 | 14 (4)                 |
| Anxiety                    | 23 (7)                 |
| Psychiatric disorder       | 1 (0)                  |
| No comorbidity             | 198 (63)               |
| COVID-19 characteristics    |                        |
| Self-assessed disease severity, n (%) |                  |
| Asymptomatic               | 7 (2)                  |
| Mild                       | 56 (18)                |
| Moderate                   | 163 (52)               |
| Severe, not hospitalised   | 87 (28)                |
| Duration of symptoms, days | 13 (9–23)              |
| Time since symptom onset, days | 44 (35–53)         |

Results are median (IQR), unless otherwise stated. Percentages are calculated from non-missing values. Symptom feedback incomplete for two participants.
intensity of the result band(s) from 0 (negative) to 6 according to a standardised scoring system on a visual guide (see online supplementary figure ii). Invalid tests were repeated. A photograph of the completed test was emailed to the study team.

The first 77 participants enrolled to the study all used the same device. Subsequent participants used different LFIAIs according to the study site attended (i.e. consecutive allocation). As new LFIAIs became available, participants were invited for a second visit to perform an alternative LFIA. A simultaneous venous sample for laboratory analysis was taken at all visits.

To assess concordance, each finger-prick self-test in the clinic was performed with the same participant’s serum in the laboratory. Test evaluations were conducted according to manufacturer’s instructions, by a technician blinded from the clinic result or patient details. Any invalid tests in the laboratory were repeated. Initially, scoring was performed independently by two individuals, but this practice ceased after inter-rater scoring was found to be almost perfect by 7-point categorical score (0–6) (kappa=0.81)14 and perfect on binary outcome (positive/negative) (online supplementary table 4).

Given uncertainties over the proportion of individuals who develop antibodies with non-hospitalised disease, additional serological testing was performed with two laboratory ELISAs: spike protein ELISA (S-ELISA) and a hybrid spike protein receptor binding domain double antigen bridging assay (hybrid DABA). Both ELISAs were shown to be highly specific. Details of these methodologies and their prior specificity testing are available in the supplementary section. Sensitivity of each LFIA in clinic and laboratory was assessed versus PCR-confirmed cases, versus S-ELISA and versus hybrid DABA.

Sample size
Sample size for individual tests was calculated using exact methods for 90% power and a significance level α=0.05 (one sided). To detect an expected sensitivity of 90% with a minimal acceptable lower limit of 80%, a sample size of 124 was targeted. For specificity, a sample size of 361 is required based on an expected specificity of 98% and a lower limit of 95%.

Performance analysis
The primary outcome was the sensitivity and specificity of each rapid test. For sensitivity, tests were compared against two standards: (1) PCR-confirmed clinical disease (via swab testing) and (2) positivity in patients with either a positive S-ELISA and/or hybrid DABA in the laboratory.

LFIA performance was assessed with (1) finger-prick self-testing (participant interpretation); (2) finger-prick self-testing (trained observer interpretation); and (3) serum in the laboratory. Specificity of LFIAIs was evaluated against the known negative samples, with all positives counting as false positives. The analysis included all available data for the relevant outcome and are presented with the corresponding binomial exact 95% CI.

Positive predictive value (PPV) and negative predictive value (NPV) are calculated for a range of population seroprevalence (from 0.1% to 20%). For the purposes of this calculation, we use LFIA sensitivity scores with serum in laboratory (rather than fingerprick) to ensure sample consistency with the pre-pandemic sera used for specificity analysis.

For comparison of individual test performance between clinic and laboratory, we compare cases where paired results from an individual were available from both settings. We calculate sensitivities and 95% CI and test differences using the McNemar test for dependent groups. Agreement between the testing methods was assessed using the Kappa statistic. Interpretation of kappa values is as follows: <0, poor agreement; 0.00–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and >0.8, almost perfect agreement.14

All data were analysed using Stata (V.14.2, StataCorp, Texas, USA), and a p value<0.05 was considered significant.

Patient and public involvement
As part of the REACT programme, there has been extensive input into the study from a patients’ panel, identified through the Patient Experience Research Centre (PERC) of Imperial College and IPSOS/MORI. This has included feedback around study materials, methods, questionnaires and extensive usability testing of LFIAIs through patient panels. User-expressed difficulties interpreting results motivated us to investigate agreement between self-reported and clinician-reported results. Usability data from this public outreach will be published in an additional study. Results of the study, once published, will be disseminated to Imperial College Healthcare NHS staff.

RESULTS
We assessed LFIA sensitivity on sera from 276 NHS workers with confirmed SARS-CoV-2 infection at a median 44 days from symptom onset (range 21–100 days). Seventy-two per cent reported no, mild or moderate symptoms, 28% reported severe symptoms and none were hospitalised (table 1). The most common symptoms described were lethargy (78%), loss of smell (66%), fever (61%), myalgia (61%) and headache (61%) (online supplementary table iii). Less than half reported persistent cough (46%) or dyspnoea (41%). Median symptom duration was 13 days.

Evidence of antibody response was found in 94.5% (95% CI 91.4% to 96.8%) sera assayed using the S-ELISA, 94.8% (95% CI 91.6% to 97.1%) on hybrid DABA, and 95.2% (95% CI 92.2% to 97.3%) using a composite of the two (table 2). Agreement between the two laboratory ELISAs was very high (online supplementary figure i). Seven of 11 LFIAIs assessed with serum detected less than 85% of samples positive on either ELISA (<85% sensitivity vs laboratory standard). Four LFIAIs detected >85% positive sera. The most sensitive test identified antibodies in 93% (95% CI 86.3% to 96.5%) of positive samples from composite ELISA testing.

Of the five LFIAIs tested in laboratory and clinic, sensitivity of two of the tests was reduced in a clinical setting using finger-prick self-testing, giving positive results for 21.9% (95% CI 13.1% to 33.1%) (80% in laboratory) and 61.2% (95% CI 46.2% to 74.8%) (71% in laboratory) of individuals whose sera tested positive with the ELISAs (figure 1). To explore whether this discrepancy was due to sample type (serum vs blood), or influenced by test operator (participant vs laboratory technician), we also tested four of the LFIAIs with whole blood in laboratory (online supplementary table iv). The least sensitive test was significantly inferior with whole blood (57.1% (95% CI 45.4% to 68.4%)) versus composite of laboratory ELISAs than with serum (79.8% (95% CI 70.2% to 87.4%)), but the other three LFIAIs were broadly similar with both whole blood and serum.

The two LFIAIs that showed higher sensitivity with serum detected 95.6% (95% CI 84.9% to 99.3%) and 84.4% (95% CI 70.5% to 93.5%) composite laboratory ELISA positives from finger-prick self-testing in clinic.
### Table 2  Results for all LFIAs analysed

| Lateral flow assay | Sensitivity | Specificity |
|--------------------|-------------|-------------|
|                     | Sera (vs positives on S-ELISA and/or hybrid DABA) | Finger-prick self-test (vs positives on S-ELISA and/or hybrid DABA) |
|                     | Sensitivity | 95% CI      | n/N | Sensitivity | 95% CI      | n/N | Police force sera Nov 2019 (all positives considered false) |
| Phases phase I      |             |             |     |             |             |     |                                                               |
| WONDFO (IgM/IgG combined) | 80% | 70.2 to 87.4 | 75/94 | 22% | 13.1 to 33.1 | 16/73 | 99.4% | 98.3 to 99.9 | 497/500 | 0% (0) |
| MENARINI (separate IgM and IgG) | 93% | (86.3 to 96.5) | 112/121 | 96% | 84.9 to 99.5 | 43/45 | 97.8% | 96.1 to 98.9 | 489/500 | 0.6% (3) |
| FORTRESS (separate IgM and IgG) | 88% | 83.3 to 91.2 | 255/291 | 84% | 70.5 to 93.5 | 38/45 | 98.6% | 97.1 to 99.4 | 493/500 | 0.6% (3) |
| BIOPANDA I (separate IgM and IgG) | 65% | 56.7 to 72.2 | 101/156 | 67% | 55.5 to 76.6 | 56/84 | 99.8% | 98.9 to 100.0 | 499/500 | 0% (0) |
| BIOSURE/MOLOGIC I (IgG only) | 71% | 62.2 to 77.9 | 98/139 | 61% | 46.2 to 74.8 | 30/49 | 97.2% | 95.3 to 98.5 | 486/500 | 1.6% (8) |
| Phases phase II     |             |             |     |             |             |     |                                                               |
| SURE-BIOTECH (separate IgM and IgG) | 68% | 57.3 to 77.1 | 63/93 | 61% | 46.2 to 74.8 | 30/49 | 97.2% | 95.3 to 98.5 | 486/500 | 1.6% (8) |
| BIOSURE/MOLOGIC II (IgG only)* | 48% | 40.8 to 55.9 | 87/180 | 98.4% | 96.5 to 99.2 | 442/450 | 0% (0) |
| BIOPANDA II (separate IgM and IgG) | 82% | 75.7 to 86.4 | 151/184 | 97.8% | 96.1 to 98.9 | 489/500 | 0% (0) |
| BIOMERICA (separate IgM and IgG) | 81% | 74.7 to 86.4 | 149/184 | 99.8% | 98.9 to 100 | 499/500 | 0% (0) |
| SURESCREEN (separate IgM and IgG) | 88% | 81.8 to 91.9 | 161/184 | 99.8% | 98.9 to 100 | 499/500 | 0% (0) |
| ABBOTT (separate IgM and IgG) | 91% | 85.6 to 94.5 | 167/184 | 99.8% | 98.9 to 100 | 499/500 | 0% (0) |

| Reference assays | Laboratory test | vs PCR-confirmed cases |
|------------------|-----------------|------------------------|
| S-ELISA          | 94.5%           | 91.4 to 96.8           | 293/310 |
| RBD hybrid DABA  | 94.8%           | 91.6 to 97.1           | 274/289 |
| Composite ELISA/hybrid DABA positivity | 95.2% | 92.2 to 97.3 | 296/311 |

Biosure/Mologic II was tested with 5 µL serum in phase II (in accordance with instructions provided at time). Manufacturer advises test should be performed with 10 µL serum.

DABA, Double antigen bridging assay; IgG, immunoglobulin G; IgM, immunoglobulin M; RBD, Receptor binding domain; S-ELISA, spike ELISA.
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Findings from the matched clinic and laboratory results are presented in Table 3. Concordance between LFIA performance in clinic, with finger prick, and in laboratory, with serum, on the same participants, was variable, with three tests showing ‘moderate’ agreement (kappa 0.41, 0.54, 0.56), according to Landis and Koch interpretation,14 one showing ‘fair’ agreement (kappa 0.34) and the other only ‘slight’ (kappa 0.13) (Table 3). Of the tests performed in the clinic, results reported by participants were consistent with those reported by a trained observer in four out of the five LFAs. In one LFIA, observer-read positive results were frequently reported as negative by study participants.

Specificity was high for all LFAs assessed (Table 2), ranging from 97.2% to 99.8% in phase I and from 97.8% to 99.8% in phase II. For the purposes of this evaluation, in the LFAs that had separate IgM and IgG bands, IgM alone was counted as a negative result. Counting IgM alone (without IgG) as a positive result made no difference in performance for most LFAs, with the exception of the Fortress and Biomerica. In both these tests, specificity was reduced to 96% when IgM counted as positive.

PPV (probability that a positive test result is a true positive) was highest for the LFAs with highest specificity and fell below 85% at 10% seroprevalence for two of the LFAs tested in phase I.

| Table 3 Matched samples from clinic versus laboratory |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | WONDFO          | MENARINI        | FORTRESS        | BIOPANDA I      | BIOSURE/MOLOGIC I |
| Matched LFIA results between clinic and laboratory, n | 76              | 47              | 48              | 68              | 44              |
| Days since symptom onset, median (IQR)              | 37 (32–47)      | 41 (33–47)      | 59 (40–69)      | 44 (35–54)      | 40 (32–49)      |
| Sensitivity (%) against reference assays (95% CI)  |                 |                 |                 |                 |
| Sensitivity vs PCR confirmed                         |                 |                 |                 |                 |
| Clinic (finger prick)                                | 21.1 (12.5 to 31.9) | 91.5 (79.6 to 97.6) | 79.2 (65.0 to 89.5) | 64.7 (52.2 to 75.9) | 56.8 (41.0 to 71.7) |
| Laboratory (serum)                                  | 73.7 (62.3 to 83.1) | 93.6 (82.5 to 98.7) | 87.5 (74.8 to 95.3) | 75.0 (63.0 to 84.7) | 79.5 (64.7 to 90.2) |
| p<0.001                                              | p<0.001         | p=0.219         | p=0.167         | p=0.006         |
| Kappa                                                | 0.13 (0.03 to 0.24) | 0.54 (0.08 to 1.00) | 0.56 (0.25 to 0.86) | 0.34 (0.11 to 0.58) | 0.41 (0.16 to 0.65) |
| Sensitivity (%) vs S-ELISA and/or hybrid DABA       |                 |                 |                 |                 |
| Clinic (finger prick)                                | 21.9 (13.1 to 33.1) | 95.6 (84.9 to 99.5) | 84.4 (70.5 to 93.5) | 67.7 (54.9 to 78.8) | 60.0 (43.3 to 75.1) |
| Laboratory (serum)                                  | 76.7 (65.4 to 85.8) | 95.6 (84.9 to 99.5) | 93.3 (81.7 to 98.6) | 76.9 (64.8 to 86.5) | 85.0 (70.2 to 94.3) |
| p<0.001                                              | p<0.001         | p=0.219         | p=0.238         | p=0.002         |

Sample is individuals with only matched clinic and laboratory results for the specific LFAs. 95% CI, 95% binomial exact CI. P value compares clinic and laboratory sensitivity using McNemar’s $\chi^2$ test. Kappa is the inter-rater agreement between the self-test result and the serum test result.

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Figure 1  Sensitivity of lateral flow immunoassays with (A) finger-prick (self-read), (B) finger-prick (observer read) and (C) serum in laboratory compared with (1) PCR-confirmed cases or (2) individuals testing positive with at least one of two laboratory assays (spike protein ELISA and hybrid spike protein receptor binding domain double antigen bridging assay).
Any invalid tests were repeated. For one LFIA, 8 out of 508 (1.6%) results were invalid, two tests had 3 out of 503 (0.6%) invalid results, and the remaining six tests had no invalid results on specificity testing (table 3).

**DISCUSSION**

LFIAs offer an important tool for widespread community screening of immune responses to SARS-CoV-2. They have already been used for large regional and national seroprevalence surveys in the USA and Europe.13–17 However, to allow robust estimates of seroprevalence, a better understanding is needed of (1) the performance of LFIAs in the general population, where most infected patients have not been hospitalised (and may have lower antibody responses associated with asymptomatic or paucisymptomatic infection);18–22 (2) the performance of LFIAs in finger-prick self-testing; and (3) the reliability of LFIA user interpretation.

Specificity of the rapid tests was high. For six (of nine) LFIs assessed, specificity exceeded 98% (the minimum standard recommended by MHRA for clinical use). All had sufficient specificity to be considered for seroprevalence studies. However, all 11 LFIAs assessed (in phase I and phase II) had lower sensitivity to be considered for seroprevalence studies. However, it is not possible to generalise these findings to all LFIAs, particularly as manufacturers continue to develop better assays and housings. However, these results emphasise the need to evaluate new tests in the population of intended use and demonstrate that laboratory performance cannot be assumed to be a surrogate for finger-prick testing.

In summary, this study describes a systematic approach to clinical testing of commercial LFIA kits. Based on a combination of kit usability, high specificity (98.6% (95% CI 97.1% to 99.4%)), moderate sensitivity (84% with fingerprick (95% CI 70.5% to 93.5%), 88% with serum (95%CI 83.3% to 91.2%)), high PPV (87% (95% CI 76.9% to 93.5%)), moderate sample concordance (kappa 0.56 (95% CI 0.25% to 0.86%)) and availability for testing at scale, the Fortress test was selected for a further validation study in over 5000 police force personnel (REACT Study 4) and in a large, nationally representative seroprevalence study. The REACT seroprevalence study commenced in England in June 2020. Further analysis of additional LFIAs from phase II will be used to inform subsequent rounds of seroprevalence studies, as test performance continues to improve.

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**Competing interests** All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no financial relationships with any organisations that might have an interest in the submitted work in the instructions are provided. However, it should be noted that although many participants were healthcare workers (from a range of areas including both clinical and non-clinical staff), they may not be representative of the general population. Further work is underway to assess the tests with a study group better representing the general population.
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previous three years; no other relationships or activities that could appear to have influenced the submitted work.

**Patient consent for publication** Not required.

**Ethics approval** The study’s conduct and reporting is fully compliant with the World Medical Association’s Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. This work was undertaken as part of the REACT 2 study, with ethical approval from South Central–Berkshire B Research Ethics Committee (REC ref: 20/SC/0206; IRAS 283805). Samples for negative controls were taken from the Airwave study approved by North West–Haydock Research Ethics Committee (REC ref: 19/NW/0054).

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**Data availability statement** Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. Anonymised data with results of positive/negative individual tests can be provided on request through contact with study team. Email b.flower@imperial.ac.uk; ORCID ID: 0000-0002-2659-544X.

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