Public Health England

Protecting and improving the nation's health

Laboratory Protocol for the evaluation of commercial Lateral Flow Devices to detect SARS-CoV-2 antibodies

PHE National Infection Service

Background and objective of the study

1. Commercial providers have developed rapid SARS-CoV-2 antibody tests in response to the COVID-19 pandemic, some of which may be suitable for home use testing. This evaluation will examine Lateral Flow Devices that have been selected by the DHSC for further assessment. The primary purpose of this project is to provide an objective assessment of the diagnostic capability of LFDs to identify individuals with or without antibodies to SARS-CoV-2, with reference to currently available laboratory-based SARS-CoV-2 assays. User testing of the devices will follow completion this evaluation.

2. The EDSAB-HOME study (Evaluating Detection of SARS-CoV-2 AntiBodies at HOME) aims to develop a characterised volunteer group, and a plasma bank derived from them, for the assessment of home-based SARS-CoV-2 serodiagnostic technologies. A total of 2693 participants (1147 Police and Fire & Rescue staff and 1546 healthcare workers) have been recruited, which form the plasma bank for testing of home antibody kits.

   An additional 154 participants, with known previously positive PCR result, have been recruited to form the "known positives". A total of 1,995 pre-pandemic blood samples have also been obtained from the COMPARE study, a blood donor study conducted in 2018, forming the "known negatives".

Acquisition of product, duration of assessment and training of evaluator

3. The evaluator will require sufficient kits to test a panel of specimens, and quality control samples to be run at the start of each testing session (Table 1). The manufacturer should provide sufficient kits to run the specimen panel and controls, and any retests that may be required (see section below on 'Determination of status and discordant results'). The evaluator will also require any ancillary reagents and consumables that are recommended by the manufacturer. The kits will be used in conjunction with any other equipment that is either provided by the manufacturer or available at PHE and has been agreed to meet the requirements of the manufacturer's representative.

4. Prior to commencement of the assessment, the manufacturer will sign to confirm they are satisfied with the study protocol and agreement and will be invited to train the evaluators in the use of the kits. The 'company trained PHE-evaluators' will cascade training to other staff when subsequent changes in operators or readers cannot be avoided. The company will be responsible for providing on-going support should any problems arise in the use of the kits or associated reagents, consumables or equipment provided by the company.

5. It is anticipated that, assuming satisfactory performance, that the laboratory work, data analysis and production of a draft report will be completed within 1 week for Stage 1 and a further two weeks for Stage 2 (see paragraph 7). The evaluations will be staggered with the priority determined by the DHSC. Each kit evaluation session will be arranged so that there are two operators and three readers, with scaling up of teams as required. The manufacturers will be kept informed of progress.

Specimen panel

6. A panel of specimens has been assembled for evaluation as shown in Table 1 below. The evaluation is organised in two stages with the first stage comprising results on 504 specimens, to be followed by results on the remaining specimens. One production lot is being assessed for this evaluation.

7. A panel of 4,842 specimens has been assembled available for evaluation (Table 1), comprising 504 (154 known positives and 350 known negatives) for Stage 1, and "two-gate" and 2693 additional samples for Stage 2 "one-gate". We also have a larger panel of 1,645 negative plasma samples which may be analysed depending on the results of Stage 1.

Table 1: Specimen panel for the evaluation of SARS-CoV-2 Lateral Flow Devices

Stage	Clinical samples	Number
1	"Known positives" (recruited through EDSAB-HOME via a separate stream only for individuals who have had a previously positive PCR result)	154
1	"Known negatives" (COMPARE study – pre-pandemic plasma)	350 randomly selected samples (from 1,995)
2	EDSAB-HOME samples (frontline workers)	2,693
2+	"Known negatives" (COMPARE study – pre-pandemic plasma)	1,645
	TOTAL	**4,842**

Note: Two quality control samples will be used in each testing session: QC1 sample (strong/medium reactive) and QC2 sample (weakly reactive)

The quality control samples are prepared separately for each device as required, to be used as run controls for each testing session. The NIBSC QC1 control (code 20/B764) will be used as one of the controls, as appropriate.

Conduct of the evaluation

8. The product will be used exactly as laid down in the manufacturer's instructions. Any modifications to the instructions provided with the kit, during the training period or any subsequent changes and other limitations of which we should be aware, must be confirmed in writing.

9. All reactions will be read visually by three readers working independently. The current WHO scoring system[1] for subjectively read assays will be implemented to allow a standardised system to be employed across different devices.

Table 2 – Scoring system for subjectively read assays

Scoring index	Intensity reading scale
0	Negative
1	Very Weak, but Definitely Reactive
2	Medium to Strong Reactivity
7	Invalid (including no control line/band/dot/spot visible, or obviously defective test device, no flow, debris present)

10. Where the device offers both IgG and IgM bands, we will record the results as follows:

G: IgG+, IgM -
M: IgG-, IgM+
GM: IgG+, IgM+
NR: IgG-, IgM-

Where the device offers only and IgG band, we will record the results as follows:

R: IgG+ve band

NR: no IgG band seen

11. An image of the stable end point of the devices will be obtained using a camera and stored as an unmodified file. All original hard-copy score-sheets will be retained together with a digital image of the score-sheet which will be stored on a backed-up network drive.

12. Scans of the devices using commercially available lateral flow device readers may also be obtained and stored.

13. In the event of any unexpected findings or problems encountered during the assessment, laboratory work will cease, and the kit supplier will be informed. Laboratory work will only re-commence once the problem has been resolved satisfactorily by both parties.

[1] https://www.who.int/diagnostics_laboratory/evaluations/alter/protocols/en/ (e.g refer to HIV and HBsAg protocols)

Storage of samples

14. The serum/plasma specimens are stored in aliquots in plastic tubes with screw-cap lids with O-ring seals. The aliquots are stored below -20°C until required and at 2-8°C during the assessment. Five freeze-thaw cycles will be permitted. Thawing will be carried out at room temperature, following which each specimen will be well mixed.

Determination of status and discordant results

15. The object of this assessment is to assess the ability of the devices to detect serological evidence of SARS-CoV-2 infection in human serum or plasma specimens. A discrepancy will arise when a result by the kit under assessment disagrees with laboratory-based assay results held on the specimen(s) by the PHE. If this occurs, tests will be repeated in duplicate on the same aliquot of the specimen. Retesting will take place after data analysis has been undertaken at the end of each stage of testing. If any specimens give invalid results (i.e. the control band does not form) these will be tested again singly at the time of evaluation.

16. Where the device reports only an IgG band, we will regard the result as positive where IgG band is reactive.

17. Where the device offers both IgM and IgG bands, we will report as a primary analysis the algorithm recommended in the Instruction for Use for interpretation.

    Where such algorithms are not stated explicitly, we will report two different strategies for determining status.

#	Strategy	Positive if result is
1	Ignore IgM band; only read IgG band. Report positive if IgG band reactive.	G or GM
2	Report positive if either IgG or IgM band positive	G,M,GM

18. We may use images from the evaluation to train and validate electronic algorithms to read the devices, e.g. in partnership with NHSX.

19. If a validated electronic reader exists, deemed suitable for deployment by the manufacturer, and it is available to the investigation, we may report results from this as a primary outcome.

Other aspects of the evaluation

20. The manufacturer must inform PHE in writing if the kits have any limitations of which we should be aware before the evaluation starts, and which are not detailed in the Instructions for Use. For example:

    - Incubation temperature limits;
    - Maximum number of devices that should be set up simultaneously by a single operator;
    - Stability of the end-point, i.e. for how long after the test is complete, may it be read?
    - Can stored samples be used?
    - Is centrifugation of samples recommended/necessary before testing?
    - Are both serum and plasma samples acceptable?
    - Number of freeze/thaw cycles.

21. The following features of the kits may be remarked on in the report:

    - The packaging and labelling of the materials
    - The clarity of the operating instructions
    - The ease of use and reliability of the products, including equipment supplied for the assessment
    - Ease of result interpretation
    - Stability of result end point
    - Health and safety considerations
    - Storage requirements
    - Shelf-life

Analysis of results and report

22. Raw data will be transferred from the scoring sheets onto a database or spreadsheet specifically prepared for this assessment. The data entry will be checked by a second person.

23. PHE, together with DHSC, will review the results of the Phase I evaluation (n=504), results of which will be shared with the manufacturer. A decision as to whether to proceed to Phase 2 will be made by PHE/DHSC in the light of factors including comparisons with results obtained with other products on the same data set, and the MHRA's target product profiles for such devices. Whether additional negative control samples (n=1,650) will be included in the Phase 2 will be decided based on Phase I data.

24. Analysis will proceed as described in the main study protocol.

25. The results may subsequently be published without seeking further permission if the kit has a CE Mark and is on the market in the same unmodified form as tested during assessment.

Version history

Version	Date	Modified by	What changed
1.0	20200730	Keith Perry	Initial draft
1.2	20200805	Keith Perry	Reviewed internally, shared with RTC
1.3	20200914	David Wyllie, Keith Perry	Instructions for dual band reading specified