Oxford COVID-19
Evidence Service

Molecular and antibody point-of-care tests to support the screening, diagnosis and monitoring of COVID-19

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VERDICT

Moving diagnostic testing for COVID-19 from laboratory settings to the point of care is potentially transformative in the rate and quantity of testing that could be performed. Eleven diagnostic tests that are potentially suitable for testing for COVID-19 at the point-of-care are described: six molecular tests, and five antibody-based tests. Some devices show high diagnostic accuracy during controlled testing, but performance data from clinical settings, and a clear understanding of the optimal population and role for these tests in the care pathway, are currently lacking.

BACKGROUND

The need for increasing levels of testing for COVID-19 has been identified by both the World Health Organisation and by the UK government.

Currently, most COVID-19 testing is performed in the laboratory environment. Guidance for virus testing in NHS laboratories is available here, and the WHO also provides technical

guidance for laboratory testing. A comparison of oropharyngeal and nasopharyngeal swabs for laboratory diagnosis has been previously reported.

Accurate and scalable point-of-care (POC) tests for the diagnosis of COVID-19 would increase the scope for diagnosis to be made in the community and outside the laboratory setting (Wang et al (1), Nguyen et al (2)). They would have the potential to reduce the time to obtaining an actionable result, could support early identification of those with COVID-19 and could also support appropriate use of isolation resources, infection control measures, and recruitment into clinical trials of treatments.

In this report, we summarise the characteristics of current molecular and antibody diagnostic tests available to support the diagnosis and management of patients with suspected COVID-19. We consider assays that could run on analysers near to the patient, rather than those that would typically be placed within a laboratory. Many of these POC tests are molecular-based PCR-type tests, but others are serological assays, which detect the presence of antibodies in a blood sample.

Reference test

The current reference test for diagnosis of active infection by SARS-CoV-2 is a real time reverse transcriptase polymerase chain reaction (rRT-PCR) assay (Corman et al (3)). The rRT-PCR assay utilises viral RNA extracted from patient samples (e.g. material collected by NP/OP swab), synthesises complementary DNA (cDNA) through the action of the reverse transcriptase enzyme, and amplifies target sequences of the viral genome from the cDNA template. rRT-PCR can be interpreted in a semi-quantitative manner, with the speed of target amplification dependent on the concentration and quality of viral RNA in the initial sample, and thus amplification rate can be used as a proxy for sample viral load.

Failure to amplify can be interpreted as a negative result, but could also be attributable to poor quality of the clinical sample or to early disease status. These assays can be run on standard rRT-PCR thermocyclers or large automated or semi-automated diagnostic platforms. Testing in patients suspected of having COVID-19 involves sending a respiratory sample (e.g. oro/nasopharyngeal swab, sputum or bronchoalveolar lavage in seriously unwell patients) to a reference laboratory for rRT-PCR testing. The time between sample collection and generation of results can range from 24 to 72 hours, but could be much faster with a streamlined approach from sample to answer for urgent clinical scenarios.

Molecular point-of-care tests utilise the same basic methodology as the laboratory assay, but essentially automate a varying number of the steps required. As they could be operated in near-patient settings rather than on the laboratory bench, they might be expected to provide a shorter time to result.

Serological and antigen tests

Serological tests, using enzyme-linked immunosorbent assays, detect the presence of antibodies to coronavirus in a whole blood, plasma or serum sample (Xiao et al (4)). These tests

detect immunoglobulins M and G (IgM and IgG). IgM is the largest immunoglobulin, and is the first to appear after initial exposure to an antigen. IgG is the most common antibody found in the body, which will appear later but will be generated in abundance. These tests can determine whether a patient has previously been infected with coronavirus, as they will stay positive after active infection has gone.

Currently, serological testing is not routinely offered as part of the screening or diagnosis of COVID-19, as no validated assays are available. These tests will not be positive until the body has started to make antibodies to fight the virus, typically 5-10 days post-infection. The widespread use of such a test could reveal what percentage of the population has had the virus, but these tests are less likely to detect cases in the early stages of disease. In cases where the molecular test is negative but there is a strong clinical suspicion of COVID-19 disease, serological testing could support a diagnosis once validated assays become available.

Antigen tests (Khan et al (5)) may also offer additional information before or at the time of taking a sample for molecular screening, but there are no commercially available antigen tests for COVID-19 available at the time of writing.

For a more detailed overview of relevant laboratory methods, see Loeffelholz & Tang (6).

CURRENT EVIDENCE

We accessed the websites listed in the Search Strategy (below) on 26/03/2020 and extracted the list of POC tests available.

We recorded the following information as recorded on manufacturers' websites and assay package inserts. We also attempted to obtain information about diagnostic performance by contacting the manufacturers directly, but as little extra was obtained we report here publicly-available information only.
• Device type
• Target (e.g. SARS-CoV-2 or immunoglobins)
• Sample type required for testing
• Whether CE marked and/or having emergency FDA approval
• Time required for sample preparation and to obtain diagnostic result
• Throughput (e.g. number of cartridges that could be processed at any one time)
• Storage requirements
• Diagnostic performance (e.g. sensitivity and specificity, and whether using laboratory or clinical samples)

We found six commercially available molecular POC tests, five antibody-based tests and no antigen tests at the time of conducting the search. A comparative summary is shown in Tables 1 and 2.

Molecular POC diagnostics

Most of the six molecular POC tests have either gained CE marking or emergency FDA approval. As at time of writing we could not find clinical evaluations of these assays in the literature, the information summarised below is extracted from the manufacturer package inserts or from their websites.

Almost all are portable, benchtop-sized analysers, apart from the MicrosensDx RapiPrep©COVID-19 test and the MesaBioTech Accula Test, which are smaller, handheld devices.

Typical validated sample types include nasal, throat, oral or nasopharyngeal swabs. The MicrosensDx also supports sputum samples.

All tests require sample preparation, which involves placing the swab sample into a viral transport media and pipetting a proportion of the sample into a single-use cartridge. This preparation step is typically quoted to take approximately two minutes but may take 5-10 minutes for some devices. The Abbot ID Now kit indicates a 1-2 minute preparation time, as the swab is mixed with the viral transport media within the cartridge in the analyser.

Most POC devices are single-access and operate with single-use cartridges. The Cepheid Xpert SARS-CoV-2 can run 2-4 samples per run in a random access manner, and the GenMark EPlex can run 3 samples per run in a random access manner.

Storage of most cartridges requires refrigeration plus some time to equilibriate to room temperature, apart from the Cepheid Xpert SARS-CoV-2, Mesa BioTech Accula SARS-CoV-2 and Abbott ID NOW COVID-19 tests, which can be stored at room temperature prior to use.

Time to result varies from 13 minutes (Abbott ID NOW) to 45 minutes (Cephied Xpert Xpress).

For these six devices there was no evidence of clinical diagnostic accuracy from prospective clinical evaluations. Preliminary evidence extracted from the package inserts of the cartridges showed validation data restricted to small numbers of spiked samples in a laboratory setting (typically 20-50 positive samples). Most compared positive agreement on a range of limits of detection and, where available, reported perfect diagnostic performance in this controlled setting. Validation information for each device is provided within Table 1, and a more concise summary appears in Table 3 for comparative purposes.

Antibody POC diagnostics
Of the five antibody-based tests, two are lateral flow immunoassays (BioMedomics rapid test and Surescreen rapid test cassette), one is a time-resolved fluorescence immunoassay (Goldsite diagnostics kit) and two are colloidal gold immunoassays (Assay Genie rapid POC kit and VivaDiag COVID-19 IgG-IgM test).

All assays detect the presence of IgG and IgM from whole blood, serum or plasma. They involve pipetting a few drops of blood from a fingerprick or vein onto the immunoassay, followed by a

couple of drops of buffer solution, with the result displayed (as lines similar to a pregnancy test) within 10-15 minutes. All use single-use disposable cartridges, and most can be stored at room temperature.

The reference standard used for comparison in these studies was RT-PCR testing. Some diagnostic accuracy data was collected from clinical, rather than laboratory testing, the largest such study being the evaluation of the BioMedomics IgM-IgG rapid test (Li et al (2020) (7)), which estimates 89% sensitivity and 91% specificity among 525 patient samples (Table 3). Being based on published clinical data, this evaluation constitutes stronger evidence than the other evaluations reported in Table 3. We also found a registered clinical trial protocol for VivaDiag and anticipate that further clinical accuracy data will become available as the COVID-19 pandemic progresses.

CONCLUSIONS

An increasing number of diagnostic devices that are potentially suitable for the diagnosis of COVID-19 at point-of-care are in development. Different devices may be more suitable for diagnosing new cases on infection, while others, especially those that test for the presence of antibodies, are better suited to determining whether an individual has previously been infected. This latter scenario is likely to be of paramount importance in identifying healthcare workers who may have recovered from initial infection, to ascertain suitability to return to frontline health services. It may also help to inform public health strategies at the end of periods of lockdown or as social distancing restrictions are relaxed.

Importantly, we have found relatively little current information reporting the diagnostic performance of these POC devices using clinical samples taken from community settings. Relevant data may still be under collection in ongoing studies, or may not be published publically. Typically, diagnostic performance might be expected to be lower in clinical settings than when using spiked samples in a controlled laboratory environment.

It should also be noted that the laboratory rRt-PCR reference standard is subject to some misclassification error, and in particular false negative results may arise. This has relevance to the conduct of clinical evaluations as misclassification in the reference standard may affect the apparent diagnostic performance of the POC tests being evaluated. Other considerations that may influence performance include pre-analytical factors such as the quality of the respiratory sample collected, the time point during infection when the sample is collected, and the handling and storage of the sample prior to analysis.

In the event of large-scale rollout in the community, any decline in diagnostic performance is likely to have serious consequences, either in providing false reassurance to infected cases, or by overdiagnosing disease-negative individuals. There is also little evidence as to the psychological and behavioural consequences of knowing immunity status, whether or not correctly diagnosed. Sufficient clinical testing is therefore vital in determining suitability.

Disclaimer: The article has not been peer-reviewed; it should not replace individual clinical judgement and the sources cited should be checked. While this article contains information about the performance of diagnostic devices available online on the search date, this information is subject to change and may be superseded as new data become available, and should not be interpreted as an endorsement of any particular device. The views expressed in this commentary represent the views of the authors and not necessarily those of the host institution, the NHS, the NIHR, or the Department of Health and Social Care. The views are not a substitute for professional medical advice.

SEARCH STRATEGY

We accessed the following websites on 26/03/2020 and extracted the list of POC tests available:
https://www.360dx.com/coronavirus-test-tracker-launched-covid-19-tests
https://www.bioworld.com/COVID19diagnostics
https://www.finddx.org/covid-19-backup/
https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations

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3. Corman VM et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 2020; 25(3): 2000045.

4. Xiao SY et al. Evolving status of the 2019 novel coronavirus infection: Proposal of conventional serologic assays for disease diagnosis and infection monitoring. Journal of Medical Virology 2020; 92(5): 464-7.

5. Khan S et al. Analysis of serologic cross-reactivity between common human coronaviruses and SARS-CoV-2 using coronavirus antigen microarray. bioRxiv 2020. https://doi.org/10.1101/2020.03.24.006544.

6. Loeffelholz MJ & Tang YW. Laboratory diagnosis of emerging human coronavirus infections – the state of the art. Emerging Microbes & Infections 2020; 9(1): 747-5

7. Li Z et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. Journal of Medical Virology 2020. https://doi.org/10.1002/jmv.25727.

Table 1. Manufacturer-quoted information on molecular point-of-care diagnostics.

Product	Manufacturer/Location	Sample type	CE marked?	Emergency FDA approval ?	Hands-on prep time	Time to result	Throughput	Storage temperature	Type	Target	Performance evidence
Xpert SARS-CoV-2	Cepheid (US/Worldwide distribution)	Nasopharyngeal swab, nasal aspirate	?	Yes	5 mins	45mins - 1 hour	2-4 cartridges PoC, 4-16 laboratory	2-28*C	RT-PCR	SARS-CoV-2 RNA	Contrived nasopharyngeal swabs. 2xLoD 20/20 agreement. 3xLoD 5/5, 5xLoD 5/5. Negative 35/35 agreement.
VitaPCR COVID-19 assay	Credo (Singapore)	Nasopharyngeal or oropharyngeal swabs	Yes	"pending"	2 mins	20mins	1 sample per cartridge at a time	15-30*C	RT-PCR	SARS-CoV-2	Full length SARS-CoV-2 RNA (N gene) at known titre spiked into sample collection buffer. 2.73X10^0 found as LoD. 1xLoD 20/20 agreement, 2xLoD 20/20, 20xLoD 20/20. Additional 60 spiked oro/nasopharyngeal samples had 100% positive agreement and 100% negative agreement at 1.5xLoD, 3xLoD and 5xLoD. Zero cross-reactivity with influenza, coronavirus 229E and some other targets.
RapiPrep COVID-19	Microsens Dx (London)	Sputum or swabs	"pending"	April	8-10 mins	30mins	1 sample per cartridge per run	?	LAMP amplification technology	SARS-CoV-2	"Assessed for clinical performance using 12 patient samples from London care home"
ePlex SARS-CoV-2	GenMark Diagnostics (United States)	Nasopharyngeal swab	?	Yes	<2 mins	?	3 test bays. 36/day near-patient up to 288/day ePlex tower	2-8*C	RT-PCR	SARS-CoV-2 RNA	65 samples from symptomatic US patients validated against SARS-CoV-2 RT-PCR Diagnostic Panel for EUA
Accula SARS-CoV-2	Mesa Biotech (United States)	Throat and nasal swabs (in same collection tube)	?	Yes	5 mins	30 mins	1 cassette per sample	15-30*C	RT-PCR + lateral flow	SARS-CoV-2 RNA	LoD determined as 200 copies / reaction in human clinical matrices, used as testing LoD. In Accula SARS-CoV-2 buffer LoD was determined at 100 copies / reaction. 1xLoD 20/20 agreement. Clinical evaluation from contrived, spiked throat

											and nasal swabs: 2xLoD 20/20, 5xLoD 7/7, 10xLoD 2/2, 50x LoD 1/1, Negative 30/30 (100%). Testing also performed on interfering substances likely to be found in respiratory or throat samples: none found to interfere at concentrations tested.
ID NOW COVID-19	Abbott Diagnostics (Worldwide)	Throat, nasal, nasopharyngeal and oropharyngeal swabs	?	Yes	1-2 mins	13 mins	1 cartridge per run	15-30*C	Isothermal nucleic acid amplification	SARS-CoV-2 nucleic acid	Performance evaluated using contrived nasopharyngeal swabs from individuals with symptoms of respiratory illness. Swabs spiked with purified viral RNA at 2x and 5x LoD. 2xLoD 20/20 agreement, 5xLoD 10/10, Negative 30/30. LoD rated at 125 Genome Equivalents/mL (19/20 positive replicates).

LoD: limit of detection

Table 2. Manufacturer-quoted information on antibody point-of-care diagnostics.

Product	Manufacturer/Location	Sample type	CE marked?	Emergency FDA approval?	Hands-on prep time	Time to result	Throughput	Storage temperature	Type	Target	Performance evidence
GT-100 SARS-CoV-2 IgG/IgM kit	Goldsite Diagnostics Inc. (China)	Human serum and plasma (20uL)	Yes	?	4-12 mins	12mins	1 sample per cartridge	?	Time-resolved fluorescence immunoassay	IgG / IgM	"Test validated by labs in Europe and China"
rapid POC kit	Assay Genie (Acro Biotech, Inc) (Ireland)	Blood, serum and plasma	Yes	?	2 mins	15mins	1 sample per test	2-30*C	Colloidal gold immunochromatography	IgG / IgM	Tested directly against PCR: IgG 20/20 positive, 49/50 negative, IgM 17/20 positive, 48/50 negative. No cross-reactivity with influenza A, B, RSV, Adenovirus, HBsAg, Syphilis, H.Pylori, HIV and HCV.
COVID-19 IgM-IgG Rapid Test	BioMedomics, BD (United States)	Finger prick / venous blood	Yes	?	1-2 mins	15mins	1 sample per test	Room temp	Lateral flow immunoassay	IgG / IgM	Validated using venous blood samples from COVID-19 patients, multiple hospital sites, China/Chinese CDC. 525 patient samples (397 positive clinically confirmed (including PCR test) SARS-CoV-2 infected, 128 negative). 352/397 tested positive and 116/128 tested negative. Information on disease stage not available. Limited case-control comparison (7 positive patients, 3 healthy controls) using sample types including fingerstick whole blood, serum and plasma, reported 100% consistency by sample type.
COVID-19 Rapid Test Cassette	SureScreen Diagnostics (England)	Finger prick	Yes	?	1-2mins	10-15mins	1 sample per test	2-30*C	Lateral flow immunoassay	IgG / IgM	Performance evaluated in Wuhan, China. Comparisons made against conventional laboratory assay which detected the presence of IgG and IgM in 902 blood samples. Quoted

											sensitivity >91% and specificity >99%.
VivaDiag COVID-19 IgG - IgM test	VivaChek (China)	10uL volume - finger prick / venous blood, plasma or serum	Yes	?	1-2mins	15mins	1 sample per test	2-30*C	Colloidal gold immunochromatography	IgG / IgM	Validated against 200 PCR samples. 81% agreement with PCR at 4-10 days infection. 100% coincidence after 11 days infection and 100% coincidence in healthy controls.

Table 3. Comparative diagnostic accuracy information.

	True positive	Total positive	% Sensitivity (95% CI)	True negative	Total negative	% Specificity (95% CI)
Data from clinical samples						
COVID-19 IgM-IgG Rapid Test	352	397	89% (85%, 92%)	116	128	91% (84%, 95%)
Data from laboratory samples						
Xpert SARS-CoV-2	30	30	100% (86%, 100%)	35	35	100% (88%, 100%)
VitaPCR COVID-19 assay	120	120	100% (96%, 100%)	60	60	100% (93%, 100%)
Accula SARS-CoV-2	50	50	100% (91%, 100%)	30	30	100% (86%, 100%)
ID NOW COVID-19	30	30	100% (86%, 100%)	30	30	100% (86%, 100%)
GT-100 SARS-CoV-2 IgG/IgM kit : using IgG	20	20	100% (80%, 100%)	49	50	98% (88%, 100%)
GT-100 SARS-CoV-2 IgG/IgM kit : using IgM	17	20	85% (61%, 96%)	48	50	96% (85%, 99%)

Only devices reporting absolute numbers suitable for estimating sensitivity and specificity are reported. For devices reporting laboratory samples at a range of limits of detection, data have been pooled. CI=95% confidence interval. For full details, refer to Tables 1 and 2.