



**Health
Information
and Quality
Authority**

An tÚdarás Um Fhaisnéis
agus Cáilíocht Sláinte

Rapid health technology assessment (HTA) of alternatives to laboratory- based real-time RT-PCR to diagnose current infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

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Version history

Version	Date	Specific updates
V1.0	2 October 2020	First draft of the rapid health technology assessment (HTA) circulated to the Expert Advisory Group (EAG) for review.
V2.0	7 October 2020	Rapid HTA submitted as advice to the National Public Health Emergency Team (NPHE).
V3.0	20 October 2020	Updated the rapid review of international guidance and use of tests and testing methods for the detection of SARS-CoV-2. Included minor wording amendments to the rapid HTA.

About the Health Information and Quality Authority

The Health Information and Quality Authority (HIQA) is an independent statutory authority established to promote safety and quality in the provision of health and social care services for the benefit of the health and welfare of the public.

HIQA's mandate to date extends across a wide range of public, private and voluntary sector services. Reporting to the Minister for Health and engaging with the Minister for Children and Youth Affairs, HIQA has responsibility for the following:

- **Setting standards for health and social care services** — Developing person-centred standards and guidance, based on evidence and international best practice, for health and social care services in Ireland.
- **Regulating social care services** — The Chief Inspector within HIQA is responsible for registering and inspecting residential services for older people and people with a disability, and children's special care units.
- **Regulating health services** — Regulating medical exposure to ionising radiation.
- **Monitoring services** — Monitoring the safety and quality of health services and children's social services, and investigating as necessary serious concerns about the health and welfare of people who use these services.
- **Health technology assessment** — Evaluating the clinical and cost-effectiveness of health programmes, policies, medicines, medical equipment, diagnostic and surgical techniques, health promotion and protection activities, and providing advice to enable the best use of resources and the best outcomes for people who use our health service.
- **Health information** — Advising on the efficient and secure collection and sharing of health information, setting standards, evaluating information resources and publishing information on the delivery and performance of Ireland's health and social care services.
- **National Care Experience Programme** — Carrying out national service-user experience surveys across a range of health services, in conjunction with the Department of Health and the HSE.

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Foreword

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly infectious virus which has caused tens of millions of cases of COVID-19 since its emergence in 2019, with a considerable level of associated mortality. In the context of the ongoing COVID-19 pandemic, SARS-CoV-2 constitutes a significant public health concern due to its high basic reproduction rate, the absence of immunity in the human population, and the current lack of an effective vaccination or treatment approaches.

The National Public Health Emergency Team (NPHE) oversees and provides national direction, guidance, support and expert advice on the development and implementation of strategies to contain COVID-19 in Ireland. Since March 2020, HIQA's COVID-19 Evidence Synthesis Team has provided research evidence to support the work of NPHE and associated groups and inform the development of national public health guidance. The COVID-19 Evidence Synthesis Team, drawn from the Health Technology Assessment Directorate in HIQA, conducts evidence synthesis incorporating the scientific literature, international public health recommendations and existing data sources, as appropriate.

From September 2020, as part of the move towards a sustainable response to the public health emergency, HIQA provides evidence-based advice in response to requests from NPHE. The advice provided to NPHE is informed by research evidence developed by HIQA's COVID-19 Evidence Synthesis Team and with expert input from HIQA's COVID-19 Expert Advisory Group. Topics for consideration are outlined and prioritised by NPHE. This process helps to ensure rapid access to the best available evidence relevant to the SARS-CoV-2 outbreak to inform decision-making at each stage of the pandemic.

The purpose of this report is to outline the advice provided to NPHE by HIQA, with consideration of the scientific literature, international recommendations and input from the COVID-19 Expert Advisory Group regarding the alternatives to laboratory-based real-time RT-PCR that could potentially be implemented in Ireland to detect current infection SARS-CoV-2.

HIQA would like to thank its COVID-19 Evidence Synthesis Team, the members of the COVID-19 Expert Advisory Group and all who contributed to the preparation of this report.

A handwritten signature in black ink, appearing to read 'Mairín Ryan', with a stylized flourish at the end.

Dr Máirín Ryan

Deputy CEO & Director of Health Technology Assessment

Health Information and Quality Authority

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The advice is developed by the HIQA Evidence Synthesis Team with support from the Expert Advisory Group. Not all members of the Expert Advisory Group and Evidence Synthesis Team are involved in the response to each research question. The findings set out in the advice represent the interpretation by HIQA of the available evidence and do not necessarily reflect the opinion of all members of the Expert Advisory Group.

Conflicts of interest

Professor Paddy Mallon recused himself from involvement in the development and review of this report in recognition of potential perceived conflict of interest associated with his research work.

List of abbreviations used in this report

BAL	bronchoalveolar lavage
CDC	Centers for Disease Control and Prevention
cDNA	complementary DNA
CE	Conformité Européene
CEA	cost-effectiveness analysis
CLIA	chemiluminescent immunoassay
COG-UK	COVID-19 Genomics UK Consortium
COVID-19	Coronavirus disease 2019
CRISPR	clustered regularly interspaced short palindromic repeats
CT	computed tomography
ddPCR	digital droplet polymerase chain reaction
DETECTR	DNA endonuclease-targeted CRISPR trans reporter
DNA	deoxyribose nucleic acid
DTA	diagnostic test accuracy
EAG	expert advisory group
ECDC	European Centre for Disease Prevention and Control
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EQA	external quality assurance
EUA	Emergency Use Authorization
EUnetHTA	European Network for Health Technology Assessment
FDA	Food and Drug Administration
FET	fluorescence energy transfer
FIA	fluorescent immunoassay
FOPH	Federal Office of Public Health
GDPR	General Data Protection Regulation
HIQA	Health Information and Quality Authority
HPRA	Health Products Regulatory Authority
HPSC	Health Protection Surveillance Centre
HSE	Health Service Executive

HTA	health technology assessment
ICT	information and communications technology
Ig	immunoglobulin
IQC	internal quality control
LAMP	loop-mediated isothermal amplification
LFIA	lateral flow immunoassay
LSRC	long stay residential centre
MedLIS	Medical Laboratory Information System
MOH	Medical Officer of Health
NAAT	nucleic acid amplification test
NCCP	National Cancer Control Programme
NEAR	nicking enzyme-assisted reaction
NGS	next-generation sequencing
NHS	National Health Service (UK)
NIPH	Norwegian Institute of Public Health
NPHE	National Public Health Emergency Team
NPT	near-patient testing
ONT	Oxford Nanopore Technologies
POC(T)	point-of-care (testing)
PPE	personal protective equipment
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RdRp	RNA-dependent RNA polymerase gene
RKI	Robert Koch-Institute
RNA	ribonucleic acid
RPA	recombinase polymerase amplification
rRT-PCR	real-time reverse transcription polymerase chain reaction
RSV	respiratory syncytial virus
RT-PCR	reverse transcription polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCOPI	Study to investigate COVID-19 Infection in People Living in Ireland

Sherlock™	Specific High Sensitivity Enzymatic Reporter UnLOCKing
SMRT	single molecule real time
SNOMED CT	Systematized Nomenclature of Medicine - Clinical Terms
SNP	single-nucleotide polymorphisms
SOP	standard operating procedure
STOP	SHERLOCK Testing in One Pot
TGS	third generation sequencing
WHO	World Health Organization

Advice to the National Public Health Emergency Team

The World Health Organization (WHO) has identified that diagnostic testing for SARS-CoV-2 infection is critical to tracking viral spread, understanding epidemiology, informing case management, and reducing transmission. The Health Information and Quality Authority (HIQA) was requested by the National Public Health Emergency Team (NPHEM) to undertake a rapid health technology assessment (HTA) of alternative diagnostic testing methods to laboratory-based real-time RT-PCR for the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The purpose of the HTA is to inform the work of the NPHEM in its response to the COVID-19 pandemic.

The assessment was undertaken as a rapid HTA within very restricted timelines and in the context of an evolving global pandemic of a new pathogen in humans. It therefore differs from a standard HTA in its scope and the approaches adopted to synthesising the available evidence. The rapid HTA considered the scientific literature, international recommendations and input from HIQA's COVID-19 Expert Advisory Group to generate advice to NPHEM regarding the alternatives to laboratory-based real-time RT-PCR that could potentially be implemented in Ireland to detect current infection with SARS-CoV-2.

The key findings of this rapid HTA, which informed HIQA's advice, are:

- Efficient and higher throughput testing is needed to fully meet the operational needs for COVID-19 prevention and control. Accurate rapid tests that can be readily deployed could facilitate timely clinical management and further support public health measures.
- Laboratory-based real-time reverse transcription polymerase chain reaction (rRT-PCR) to detect viral RNA is the current gold standard diagnostic test. Other molecular detection methods based on isothermal amplification (for example, RT-LAMP) offer faster run-times and typically require fewer sample manipulations than rRT-PCR and may therefore be suitable for use in near-patient settings. However, such methods typically have reduced throughput and sensitivity. Isothermal amplification methods can be combined with other detection methods (such as sequencing or CRISPR) to increase test sensitivity and throughput.
- Antigen detection tests can be laboratory-based (such as ELISA) or intended for use in near-patient settings (such as rapid antigen detection tests (RADTs)). RADTs, directed against SARS-CoV-2 proteins, facilitate fast delivery of results outside of the laboratory setting. They can be read visually, or using a specific reader device. The use of RADTs could expedite local

clinical management and public health interventions in the near-patient or point-of-care setting. However, tests to date show reduced diagnostic accuracy compared with rRT-PCR. They are most likely to perform well in individuals with high viral loads, which is typically observed in the early disease course of symptomatic patients.

- In interpreting test findings, a negative result should be interpreted as 'SARS-CoV-2 not detected' rather than 'not infected with SARS-CoV-2'. Conversely, positive results do not exclude concomitant bacterial infection or co-infection with other viruses. The interpretation of test results should take into consideration the pre-test probability of infection, which is dependent on factors such as local transmission rates, exposure history and symptoms.
- Flexible diagnostic platforms that can identify multiple pathogens in a single sample (multiplex testing) may be required in certain settings to distinguish between infection with other circulating viral respiratory pathogens — for example, respiratory syncytial virus (RSV) or influenza viruses — and SARS-CoV-2.
- Sample pooling strategies may be used to safely and effectively increase testing capacity where tests have been appropriately validated for this purpose. However, there are significant logistical issues in the preparation of pools and the associated data analysis. Pools with positive results require retesting in order to identify the individual(s) infected, which reduces the utility of pooling where disease prevalence is high or uncertain and can delay reporting of results.
- Broadly, rRT-PCR remains the recommended test for the detection of SARS-CoV-2 internationally and is recognised as the reference standard.
- In some countries, including Australia, Canada, Germany, Spain, and the US, approved rapid antigen detection tests (RADTs) may be used in certain clinical contexts for the detection of SARS-CoV-2; this usage depends on factors such as the performance and availability of the test, and time since symptom onset.
- While there appears to be increasing interest in the use of RADTs in near-patient settings both for diagnostic and screening purposes, to date these tests have largely been validated in symptomatic individuals. Due to reduced sensitivity associated with RADTs, the WHO suggests that RADTs should only be used when rRT-PCR is unavailable, or where prolonged turnaround times preclude clinical utility. Where RADTs are used, the WHO suggests a desirable minimum performance of $\geq 90\%$ sensitivity and $\geq 99\%$ specificity.

- The WHO currently advises against the use of RADTs in a number of situations, including for the purposes of screening in airports or other border points of entry; this is due to the highly uncertain prevalence of disease and unknown predictive value of the test.
- Diagnostic accuracy of tests for the detection of SARS-CoV-2 was examined within this rapid HTA through a scoping review of the literature. This review aimed to identify relevant systematic reviews, rapid reviews or evidence summaries which reported measures of sensitivity and specificity for alternatives to laboratory-based RT-PCR.
 - Sensitivity of tests in the real-world setting depends on various factors, including the timing and type of specimen obtained, the sampling technique, and the quality of particular test kits used to perform assays.
 - The diagnostic accuracy of commercial rRT-PCR platforms, for example, high-throughput assays and automated assays, was generally found to be high; in one meta-analysis, the average sensitivity pooled across multiple studies for several of these tests was found to be $\geq 99\%$, and specificity was $\geq 96\%$.
 - Results for the diagnostic accuracy of isothermal amplification based method and test platforms included the study of platforms using proprietary methods, and assays using RT-LAMP, CRISPR, and several other isothermal amplification methods (iAMP, RT-iiPCR, RT-RPA, RCA, RT-RAA).
 - In one review, the diagnostic accuracy of LAMP-based molecular methods appeared to depend on whether crude samples, such as nasopharyngeal or saliva (sensitivity ranging from 40% to 88%), or purified RNA samples (sensitivity $>90\%$) were analysed. Alternative isothermal amplification methods similarly showed sensitivity values above 90% in the majority of cases, although proprietary platforms varied significantly in sensitivity. Overall, specificity was high among isothermal amplification methods.
 - For RADTs, only one systematic review of diagnostic accuracy was identified. Sensitivity of such tests was found to vary significantly across test brands; estimates ranged from 0% to 94%, with an average sensitivity of 56.2% (95% CI 29.5% to 79.8%). Average specificity was 99.5% (95% CI 98.1% to 99.9%).
- Current estimates of diagnostic accuracy for alternative tests to rRT-PCR are limited by significant flaws in the design, execution and reporting of primary

diagnostic accuracy studies; it is not possible to ascertain whether these limitations have led to overestimation or underestimation of test accuracy estimates.

- There is a lack of information on the diagnostic accuracy of alternatives to rRT-PCR in important patient sub-populations and settings. This includes asymptomatic individuals, specific at-risk populations such as healthcare workers, and the validation of diagnostic performance of tests outside of the hospital setting.
- As the research landscape is rapidly-evolving, recent results will not have been captured in published reviews of diagnostic accuracy. Furthermore, there is a lack of data on emerging technologies. It is noteworthy that iterative development of diagnostic tests over time may result in improved sensitivity and specificity among technologies considered in this review.
- Effective testing strategies rely on a portfolio of tests, based on different technologies, that can be used in different settings and situations. Real-time RT-PCR relies on laboratory infrastructure and highly skilled staff. Rapid molecular tests have the potential to be deployed in both laboratory and near-patient settings, while RADT are intended for use in near-patient settings. Both rapid molecular tests and RADT can potentially expedite clinical decision-making.
- The benefits of rapid testing are dependent on the accuracy of the test and how the results of the test will affect patient treatment or the initiation of public health interventions. Near-patient testing (also known as point-of-care testing) provides the opportunity to improve clinical and public health outcomes in circumstances where laboratory test turnaround times preclude clinical utility.
- Near-patient testing eliminates the need for sample transportation to centralised laboratories for processing, allowing test turnaround times (from sample taking to test reporting) to be reduced. However, manufacturer-reported turnaround times (which may be based on pre-test handling and device run-time undertaken by trained laboratory staff), may not reflect turnaround times under real-world conditions.
- To enable monitoring of infection trends and tracing and isolation of close contacts, results from SARS-CoV-2 tests carried out in near-patient and laboratory settings should be recorded in the individual's test record (or patient health record if in clinical settings). As COVID-19 is a notifiable disease, confirmed cases must be reported to the Medical Officer of

Health/Director of Public Health with onward notification to the Health Protection Surveillance Centre (HPSC).

- Revisions to the national testing programme may be required as the evidence on the diagnostic accuracy and suitability of different tests and test methods evolves. In time, this should include consideration of the cost-effectiveness, resource considerations and budget impact of alternative approaches to ensure the best outcomes for the resources available.
- A cohesive national strategy is needed to ensure the right tests are undertaken in the right people at the right time for the right purpose. This is necessary to ensure appropriate governance of SARS-CoV-2 testing and should include clear criteria for the administration and reporting of tests. Planning now to support delivery of the strategy will facilitate rapid deployment of tests that meet the requisite standards once available and validated for use.
- All testing should be undertaken in the context of an ongoing quality assurance programme to provide confidence in the test results for both the physician and the patient.

Arising from the findings above, HIQA's advice to the NPHE is as follows:

- Alternative approaches for the detection of current infection with SARS-CoV-2 should be considered to enhance COVID-19 prevention and control; efficient processes with accurate and reliable rapid tests would facilitate timely clinical management and public health measures.
- In high-throughput laboratory settings, sample pooling strategies could be expanded to increase rRT-PCR testing capacity within existing resources. The following circumstances have been identified as potentially suitable for pooling of samples:
 - specimens collected for the purpose of serial testing of asymptomatic individuals in at-risk settings (for example, nursing homes, healthcare workers, food processing facilities, vulnerable communities).
 - specimens collected from patients as part of pre-admission precautions prior to elective procedures.
- Adoption of alternative approaches to testing requires consideration of factors including clinical performance (sensitivity and specificity), turnaround time, and ease of use. Similar to the guidance issued by the WHO, exact specifications

should be outlined for what constitutes a suitable test for each relevant purpose in the Irish setting.

- Variability in performance within individual technologies and devices precludes a class-based endorsement of specific technologies such as RT-LAMP and rapid antigen detection tests (RADTs).
- Hospital-based laboratories have validated and adopted a range of simplified rapid RT-PCR tests. These tests offer comparable accuracy and facilitate prompt clinical decision-making. However, these devices typically have limited throughput and currently can be subject to supply chain shortages. Therefore, they should be reserved for high priority clinical circumstances.
- Near-patient testing, including the use of RADTs, has the potential to expand test capacity, reduce test turnaround times and improve access. However, RADTs which are available or currently in development show lower sensitivity than that observed with rRT-PCR. Reported sensitivity varies significantly across brands, and there is a lack of performance data in asymptomatic populations. Investment is therefore required to perform clinical validation studies in the Irish setting. Potential opportunities identified for validation include:
 - supplementing the capacity of the high-throughput laboratories for the diagnosis of symptomatic patients early in the course of infection.
 - serial testing for the prevention of outbreaks in at-risk settings (for example, nursing homes, healthcare workers, food processing facilities, vulnerable communities).
 - testing for the investigation and management of outbreaks (for example, in university settings).
- Tests which demonstrate satisfactory performance, following clinical validation in the Irish setting, will support the ongoing development of a cohesive national strategy that ensures the right tests are undertaken in the right people at the right time for the right purpose.
- The introduction of near-patient testing must be within the context of a supporting quality management system. Such a system would support the quality assurance, governance, training and reporting requirements essential to delivering a safe and effective service.
- A coordinated multi-agency response is needed to mitigate potential risks associated with testing performed outside of the publicly-funded national Test and Trace programme. This should include multilateral communication with stakeholders, including members of the public and private providers.

Summary table of the characteristics of alternative strategies / technologies to laboratory-based real time RT-PCR to diagnose current infection with SARS-CoV-2 considered in this rapid HTA

Strategy	Type	Example tests	Intended setting	Characteristics	Potential clinical utility	International use	Accuracy
Sample pooling	rRT-PCR	Current gold standard rRT-PCR	High-throughput laboratory	<ul style="list-style-type: none"> • Increase testing coverage when prevalence is low and access to testing is limited. • Requires increased technical skill. • Only increases testing efficiency in low prevalence populations • Delays in the reporting of results from positive pools • Pooling procedures should be reviewed as prevalence changes. • Can be carried out within existing resources. 	Diagnostic testing in low-prevalence populations: <ul style="list-style-type: none"> – Planned hospital admissions e.g., elective surgery, chemotherapy – Serial testing in at-risk populations with low disease prevalence e.g., Health care workers, nursing homes (not suitable for outbreak settings). 	Recommended in low prevalence populations: <ul style="list-style-type: none"> – ECDC – CDC – WHO – Public Health Ontario – England – New Zealand – Spain 	Low risk of false negative results.
Technology	Type	Example tests	Intended setting	Characteristics	Potential clinical utility	International use	Accuracy
Simplified rRT-PCR	rRT-PCR	Cepheid 'Xpert Xpress'	Laboratory NPT	<ul style="list-style-type: none"> • Multiplex capacity (some tests) • Automated to reduce manual handling • Some tests require prior sample purification (not suitable for NPT) • Throughput is instrument-dependent: less than rRT-PCR, but generally higher than isothermal amplification tests • Possibility of connectivity for centralised reporting. • Requires substantial additional investment. • Turnaround time: <2 hours. 	Inform rapid clinical decision-making: <ul style="list-style-type: none"> – Testing high priority specimens. – Inform patient flow for unplanned hospital admissions. 	Inform rapid clinical decision-making: <ul style="list-style-type: none"> – Scotland (laboratory) – England (hospital) – Ireland (hospital) – Norway (hospital/laboratory) – Netherlands (hospital laboratory) Geographically difficult-to-reach populations: <ul style="list-style-type: none"> – Australia – Canada. 	Validated platforms largely have comparable accuracy to laboratory-based rRT-PCR.
		Luminex 'Aries'	Laboratory				
		DnaNudge 'COVID Nudge'	NPT				
Isothermal amplification	RT-NEAR	Abbott 'ID NOW COVID-19'	NPT	<ul style="list-style-type: none"> • Multiplex capacity (some tests) • Automated to reduce manual handling • Many available tests can only process a single sample per run • Some tests require prior sample purification (not suitable for NPT) • Possibility of connectivity for centralised reporting. 	Inform rapid clinical decision-making: <ul style="list-style-type: none"> – Testing high priority specimens. – Inform allocation of unplanned hospital admissions. 	Inform rapid clinical decision-making: <ul style="list-style-type: none"> – England (hospital) – Wales (laboratory; not yet implemented) At-risk populations:	Variable, dependent on the assay or device.
	RT-LAMP	HiberGene 'HG COVID-19'	Laboratory				Generally less sensitive than rRT-PCR.

		LampPORE 'GridION'	Laboratory	<ul style="list-style-type: none"> • Confirmatory testing may be required. • Requires substantial additional investment. • Turnaround time: <2 hours. 		<ul style="list-style-type: none"> – Canada (congregated living setting; pending authorisation) <p>NPT (care homes, ED)</p> <ul style="list-style-type: none"> – Wales (under consideration) 	
		LampPORE 'minION'	Low-complexity laboratory				
	SAMBA	Diagnostics for the Real World 'SAMBA II SARS-CoV-2 Test'	NPT				
RADT	Lateral flow assay	SD Biosensor 'Standard Q COVID-19 Ag'	NPT	<ul style="list-style-type: none"> • Typically low complexity; ease-of-use device-dependent • LFAs with a reader device have increased sensitivity and reproducibility. • Specimen requirements (swab, viral transport medium) for some LFA are compatible with those for rRT-PCR allowing potential reflex testing • LFAs without a reader may require manual reporting. • Confirmatory testing may be required. • Requires substantial additional investment. • Turnaround time: <30 minutes. 	<p>Expand access to testing:</p> <ul style="list-style-type: none"> – Near-patient testing in setting with high levels of community transmission and high disease prevalence (e.g. outbreak management). <p>Diagnosis:</p> <ul style="list-style-type: none"> – Testing of symptomatic patients early in the course of infection or close contacts. <p>Serial testing:</p> <ul style="list-style-type: none"> – Repeat testing in at-risk settings. <p>Maintenance of essential services:</p> <ul style="list-style-type: none"> – Determine suitability of HCW to return to work post-infection.* 	<ul style="list-style-type: none"> – Australia (symptomatic individuals and screening) – Canada (symptomatic individuals and serial testing) – Germany (symptomatic individuals and close contacts) – Spain (symptomatic individuals) – United States (laboratory and NPT) <p>Diagnosis in settings with no or limited laboratory access:</p> <ul style="list-style-type: none"> – WHO (minimum performance criteria). <p>Under consideration:</p> <ul style="list-style-type: none"> – France - HAS (with minimum performance criteria) – Netherlands (pending validation) – Scotland (pending validation) 	<p>Variable, dependent on device. Typically high specificity, wide ranging sensitivity.</p> <p>Generally less sensitive than rRT-PCR.</p>
		Lateral flow assay with reader	Shenzhen Bioeasy '2019-nCoV' (fluorescent)				

Key: CDC - Centers for Disease Control and Prevention; ECDC - European Centre for Disease Prevention and Control; ED – emergency department; HAS - Haute Autorité de santé; HCW – healthcare worker; LFA – lateral flow assay; NPT – near patient testing; RADT – rapid antigen detection test; RIVM - The National Institute for Public Health and Environment;; rRT-PCR – real-time Reverse Transcription Polymerase Chain Reaction; RT-LAMP - Reverse Transcription Loop-mediated isothermal amplification; RT-NEAR – Reverse Transcription Nicking Enzyme Amplification Reaction; SAMBA - Simple Amplification-Based Assay; VIDRL - Victorian Infectious Diseases Reference Laboratory WHO – World Health Organization.

* Failure to detect SARS-CoV-2 antigens using an RADT may indicate that a confirmed COVID-19 case is no longer infectious. RADT could be used to determine the suitability of HCW to return to work post-infection following resolution of symptoms in order to maintain an adequate workforce. The application of RADT for this purpose is contingent on the availability of accurate tests, validated for this purpose.

1 Background

The Health Information and Quality Authority (HIQA) was asked by the National Public Health Emergency Team (NPHE) to undertake a rapid health technology assessment (HTA) of the alternatives to laboratory-based real-time reverse-transcription polymerase chain reaction (rRT-PCR) to diagnose current infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The assessment will inform the work of the NPHE in their response to the coronavirus disease 2019 (COVID-19) pandemic.

Since the beginning of the pandemic, testing for the detection of current infection with SARS-CoV-2 has relied principally on laboratory-based techniques, such as real-time reverse transcription polymerase chain reaction (rRT-PCR). The highly sensitive and specific test is considered the gold standard test for detection of SARS-CoV-2, the causative agent of COVID-19. However, rRT-PCR is a time-consuming and resource-intensive technique; it requires highly skilled staff and specialised equipment as well as primers and reagents. Prolonged test turnaround times, particularly during periods of widespread community transmission, can preclude clinical utility in terms of rapidly isolating infected individuals, tracing close contacts and limiting the spread of the virus. However, a broad range of tests and testing methods, or strategies, could be used to rapidly detect SARS-CoV-2, increase laboratory capacity, and optimise resource use.

In this context, the request to undertake this rapid HTA was made by the NPHE in Ireland. In particular, the following questions were posed which inform the scope of the report:

1. What are the potential tests or testing methods that can detect SARS-CoV-2?
2. What testing methods are currently being used internationally for the detection of SARS-CoV-2?
3. What is the diagnostic accuracy of alternatives to laboratory-based real-time RT-PCR testing, for the purpose of diagnosis of current infection with SARS-CoV-2?
4. What are the potential organisational considerations and resource implications that might arise from the use of alternative tests for direct detection of SARS-CoV-2 infection in Ireland?

These questions are addressed in sequence in Chapters 2 to 5. A general discussion of the key issues arising from each section is provided in Chapter 6.

The assessment was undertaken as a rapid assessment within very restricted timelines and in the context of a global pandemic involving a new pathogen. It therefore differs from a standard HTA in its scope and the approaches adopted to synthesising the available evidence. For example, there are over 850 individual tests for COVID-19, as indicated on FIND, an online repository for commercially available or in development COVID-19 tests, as of 1 October 2020.⁽¹⁾ The goal of this assessment was not to investigate the clinical effectiveness of these tests, the accuracy of which is subject to a wide variety of clinical considerations (such as disease severity, disease prevalence, participant selection, specimen handling, timing and or location of the test, and so on), but instead to broadly summarise, where possible, the features and clinical performance of different alternatives to laboratory-based rRT-PCR. Leveraging off the experience and guidance of other international public health bodies, the assessment additionally considered ways in which alternatives to rRT-PCR could be deployed in Ireland. Where individual tests are mentioned in this report, this is for illustrative purposes only.

An agreed protocol for this assessment was developed (see Appendix A), in line with detailed discussions and feedback from a number of expert stakeholders in this area.

2 Description of technology

Key points

- Efficient and higher throughput testing is needed to fully meet the operational needs for COVID-19 prevention and control. Accurate and reliable rapid tests could facilitate timely clinical management and public health measures.
- Tests that detect fragments of the virus, either the viral RNA (ribonucleic acid) or antigens, are used to diagnose acute infection.
- Each type of SARS-CoV-2 test has inherent advantages and disadvantages related to the underlying technology. When considering the deployment of testing to a particular setting, it is important to consider the goal of testing, and the logistics of testing in a given environment, to inform the selection of the appropriate technology.
- Laboratory-based real-time reverse transcription polymerase chain reaction (rRT-PCR) to detect viral RNA is the current gold standard diagnostic test. Other molecular detection methods based on isothermal amplification are faster with fewer sample manipulations than rRT-PCR and may therefore be suitable for use in near-patient settings. However, such methods typically have reduced throughput or sensitivity. Isothermal amplification methods can be combined with other detection methods (such as sequencing or CRISPR) to increase the sensitivity and throughput.
- Antigen detection tests can be laboratory-based (such as ELISA) or intended for use in near-patient settings (such as rapid antigen detection tests [RADTs]). RADTs, normally directed against the nucleoprotein of SARS-CoV-2, involving lateral flow assays facilitate fast delivery of results outside of the laboratory setting. They can be read visually, or by the utilisation of a specific reader. This could expedite local clinical management and public health interventions in the case of a positive test. However, tests have reduced diagnostic accuracy compared with rRT-PCR.
- A negative test for SARS-CoV-2 in a symptomatic patient cannot rule out infection with one or more respiratory pathogens. Flexible diagnostic platforms that can identify multiple pathogens in a single sample (multiplex

testing) may be required in certain settings to distinguish between infection with other viral respiratory pathogens and SARS-CoV-2.

- Improvements to the overall testing process can enhance the efficiency and or capacity of testing. Such initiatives include deployment of testing to non-laboratory settings, altered sample collection and processing procedures, sample pooling, and centralised record linkage.
- Sample pooling strategies may be used to safely and effectively increase testing capacity when tests have been properly validated for this purpose. However, there are significant logistical issues in the preparation of pools, data analysis and the confirmation of positive results within the pools.
- Prospective effectiveness studies are required to assess operational challenges including access to equipment, utility of the assays in a given environment, the impact of testing on clinical and public health decision-making, and the cost-effectiveness of different technologies. Such studies will facilitate comparison across technologies.

2.1 Background

Coronavirus disease 2019 (COVID-19), which is caused by the pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a highly contagious disease which can give rise to severe medical complications in those infected. Efficient and effective diagnosis of infection is crucial for both the clinical management of patients and the implementation of disease control strategies such as self-isolation (isolation) of infected patients and restricted movements (quarantine) of close contacts.

Diagnosis of COVID-19 is complicated by the fact that the disease shares signs and symptoms with other respiratory pathogens, for example, influenza.⁽²⁾ Similarly, diagnostic imaging findings from tests such as pulmonary computed tomography (CT) scans may be indistinguishable from findings for other viral pneumonias. People infected with SARS-CoV-2 often do not display signs or symptoms of infection despite being infectious to others. As such, diagnosis of COVID-19 is accomplished by analysis of patient samples (for example, respiratory specimens) in order to detect the presence of the SARS-CoV-2 virus.

Analysis of patient samples for the purpose of detecting SARS-CoV-2 infection is generally referred to as 'laboratory-based testing' or simply 'testing'. However, recent developments in the landscape of testing methods have enabled near-patient or point-of-care testing (that is, testing outside of a central laboratory, hospital diagnostic laboratory or other designated laboratory setting). For the purposes of this review, the term 'testing' or 'test' will refer to analytical approaches, techniques or devices for the detection of SARS-CoV-2 infection based on patient samples in both laboratory and near-patient settings.

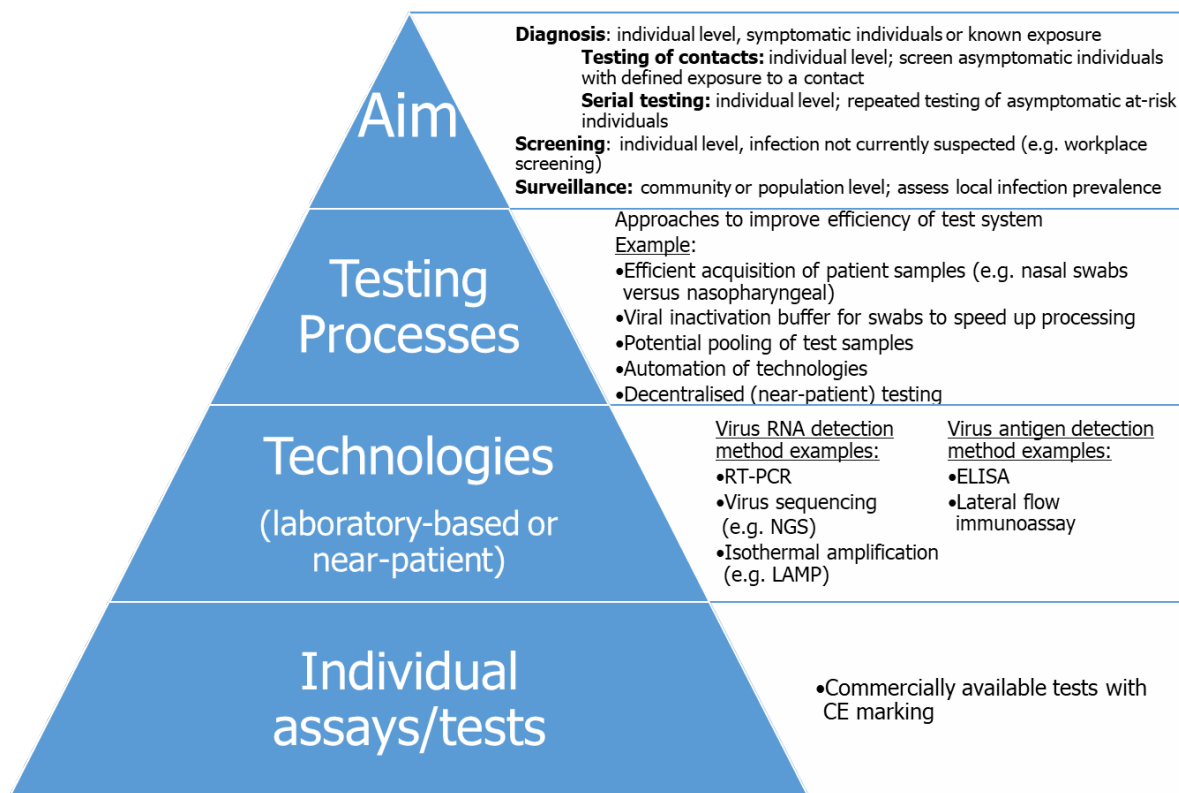
Testing for SARS-CoV-2 may be considered in terms of the:

- aims of testing (that is, diagnosis versus screening)
- testing processes (for example, near-patient or laboratory settings)
- specific technologies that permit detection of SARS-CoV-2 in the given setting (for example, laboratory-based real-time reverse-transcriptase polymerase chain reaction [rRT-PCR] or near-patient rapid antigen detection tests [RADTs])
- individual devices that may be safely and effectively deployed in a given setting to conduct testing.

These considerations are depicted in Figure 1, 'Framework for consideration of SARS-CoV-2 testing'. Selection of the appropriate technology for testing in a given setting will depend on numerous factors, including, but not limited to, the:

- prevalence of SARS-CoV-2
- specific aim of testing
- sensitivity and specificity of test
- turnaround time required
- necessary throughput required
- resource requirements of the testing method (for example, facility-related requirements, availability of accompanying reagents, reagent consumption, availability of appropriately trained staff, appropriate information management infrastructure, cost of testing method).

Figure 2.1: Framework for consideration of SARS-CoV-2 testing



2.1.1 Aims of testing

Testing may be performed for the purposes of diagnosis, screening or surveillance of SARS-CoV-2 infection.

Diagnostic testing for SARS-CoV-2 is intended to identify occurrence of infection at the individual level.⁽³⁾ It is performed when there is a reason to suspect that an individual may be infected, for example, where individuals are symptomatic.

Testing of contacts occurs where there is a reason to suspect that an individual may be infected despite them being asymptomatic. This occurs where an individual has a suspected recent exposure, for example, as identified through contact tracing efforts.

In Ireland, testing currently takes place among those people who meet the case definition for COVID-19 (that is, those with symptoms suggestive of COVID-19) and their identified close contacts.⁽⁴⁾

Serial testing is carried out in certain settings where, despite implementation of infection prevention and control measures, infection is more likely to occur due to the essential working arrangements (for example, food processing factories, healthcare workers) or increased risk of exposure in certain settings (such as residential care facilities, direct provision centres). Regular repeat testing of individuals in these settings is intended to support early identification of SARS-CoV-2 infection in order to prevent or manage outbreaks.

Screening tests for SARS-CoV-2 are intended to identify occurrence of infection at the individual level even if there is no reason to suspect infection, for example, where there is no known exposure.⁽³⁾ Screening tests are intended to identify infected individuals who may be contagious, but who are without, or prior to development of, symptoms. This is performed so that infection prevention and control measures can be taken to prevent further transmission, for example, in a workplace, educational or healthcare setting. Screening may also be conducted as part of pre-admission protocols to ensure patients who are infected with SARS-CoV-2 are identified prior to admission. In the context of unscheduled care (for example, patients presenting to the emergency department), rapid turnaround of test results, if available, can facilitate patient flow whereby patient groups may be separated with different patient management pathways applied for SARS-CoV-2 positive and negative patients. For scheduled care (for example, planned elective surgery), screening may inform a decision to defer admission in those with a positive test result. This is recommended in order to maximise patient safety among those undergoing surgery, due to possible adverse outcomes where surgery is performed on a patient with underlying COVID-19, and to protect staff and patients within the hospital setting.⁽⁵⁾

In Ireland, screening is conducted as part of pre-admission protocols for both scheduled and unscheduled care. For example, the National Cancer Control Programme (NCCP) recommends rRT-PCR testing for SARS-COV-2 up to three days prior to scheduled cancer surgery testing of otherwise healthy asymptomatic patients who are undergoing elective cancer surgery.

Surveillance for SARS-CoV-2 includes ongoing systematic activities, including collection, analysis and interpretation of health-related data that are essential to planning, implementing and evaluating public health practice.⁽³⁾ Surveillance testing is generally used to monitor for community or population-level infection, for example, an infectious disease outbreak, or to look back at the level of incidence and prevalence of infection that has already occurred. Surveillance testing is used to gain information at a population level rather than an individual level - for example, to evaluate the effect on the population of public health interventions such as social distancing - and usually involves testing a representative group of the population.

In Ireland, the HSE Health Protection Surveillance Centre and the UCD National Virus Reference Laboratory have studied a sample of the Irish population in order to estimate the proportion of people in Ireland who have been exposed to SARS-CoV-2, based upon the development of antibodies to SARS-CoV-2.⁽⁶⁾ Also, ongoing surveillance of current infection rates is based on the numbers of people with confirmed SARS-CoV-2 infection as per RT-PCR tests performed for diagnostic and screening purposes. As such, testing for the purposes of diagnosis and surveillance are intricately linked for this disease.

2.1.2 Testing processes

Testing processes involve the:

- acquisition of patient samples (for example, through swabbing)
- preparation of samples for analysis (for example, viral inactivation of the sample prior to testing, use of reagents to release viral antigens or nucleic acid)
- analysis of samples using particular technologies (for example, rRT-PCR, antigen detection technologies).

Efficient acquisition of patient samples may include:

- the use of more easily acquired samples, such as throat and or nasal swabs, or oral fluid including saliva
- increasing workload capacity in teams involved in sample collection
- or patient self-sampling.⁽⁷⁾

Following acquisition of samples, preparation and analysis of samples may occur in a central laboratory facility, or may occur in a 'near-patient' or point-of-care setting (for example, in a community health centre); in some testing approaches, minimal manual preparation of samples is required. Preparation and analysis of samples may furthermore be automated or semi-automated to improve efficiency.

In certain circumstances, in the interests of maximising resources, pooling of samples from multiple patients may be performed for testing, with retesting of individual samples from positive pools to confirm the positive result.^(8, 9) However, large scale testing using pooling has logistical issues and requires a significant level of automation.⁽¹⁰⁾

Testing processes may involve analysing a single sample at a time or may be high-throughput. Laboratory-based testing for SARS-CoV-2 RNA allows for high-throughput batch processing of clinical specimens; for example, rRT-PCR tests may be automated using robotic molecular platforms to allow large scale testing to take place.⁽¹¹⁾ In contrast, point-of-care or rapid diagnostic test devices typically involve the analysis of a single clinical specimen, or low numbers of specimens, at one time.

Testing may be limited to the detection of only SARS-CoV-2 RNA, or may be multiplex, that is, multiple pathogens (for example, influenza) may be tested for simultaneously. Analysis may be qualitative, whereby the result of the test for SARS-CoV-2 is either detected, not detected or indeterminate. Alternatively testing may be quantitative, where the quantity of virus present is determined using specific controls within the assay.

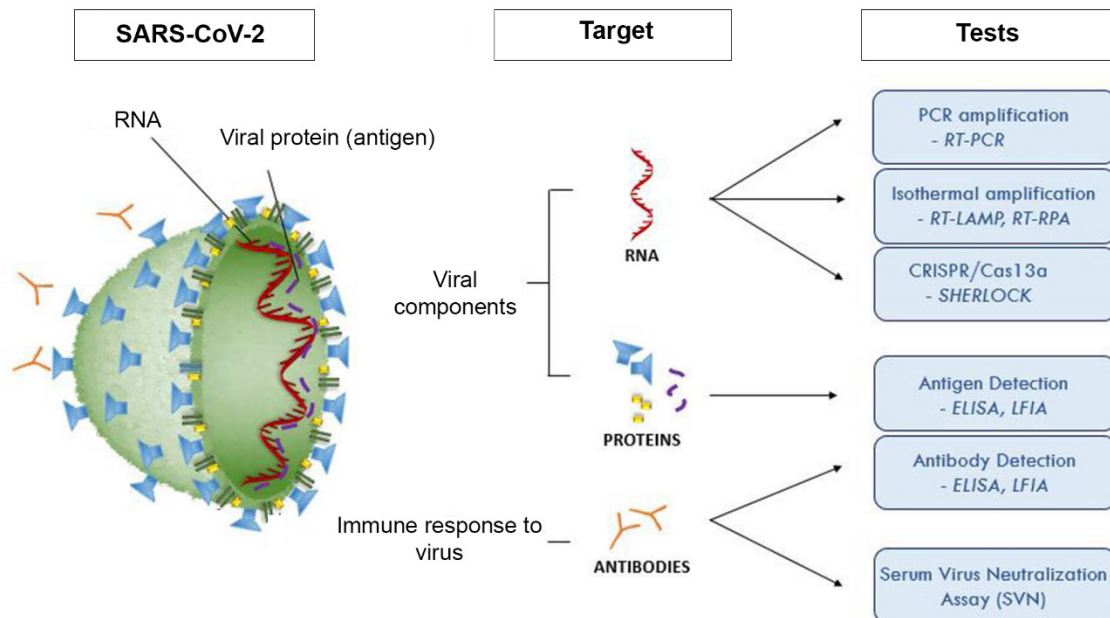
One particular subset of tests includes 'rapid tests', which specifically relates to speed of processing.⁽¹²⁾ These are defined under the European Commission In-Vitro Diagnostic Medical Devices Directive as 'qualitative or semi-quantitative devices, used singly or in a small series, which involve non-automated procedures and have been designed to give a fast result'. Such rapid tests are relatively simple to perform and interpret and therefore require limited test operator training, and may be intended either for use in hospital laboratories or near-patient settings (otherwise known as point-of-care).⁽¹³⁾

2.1.3 Technologies for detection of SARS-CoV-2

Understanding of the technologies used to specifically identify SARS-CoV-2 first requires understanding of the characteristics of the virus. Coronaviruses, generally, are enveloped viruses with a single-stranded, positive-sense ribonucleic acid (RNA) genome. The ~30 k base pair genome of the coronavirus SARS-CoV-2 encodes structural, replication and non-structural accessory proteins.⁽¹⁴⁾ COVID-19 tests target various SARS-CoV-2 specific markers at either the genetic level (RNA) or protein level (antigens or antibodies) (Figure 2). Diagnosis of acute SARS-CoV-2 infection is based on the detection of specific genetic sequences of SARS-CoV-2 (RdRP, N and S genes) and may also include the E gene, using nucleic acid amplification tests (NAATs) such as real-time reverse-transcriptase polymerase chain reaction (rRT-PCR).⁽¹⁰⁾

Figure 2.2: Molecular, antigen and antibody targets of available laboratory and near-patient tests for SARS-CoV-2

Adapted from D'Cruz et al 2020.⁽¹⁴⁾



2.1.4 Viral RNA detection

Detection of genetic material, otherwise known as molecular testing, commonly requires amplification of the SARS-CoV-2 genetic material suspected to be present. Molecular detection methods can produce a large volume of nucleic acid through the amplification of trace quantities of genetic material found in the original clinical samples. NAATs, which amplify RNA molecules, include those using rRT-PCR technology, the current gold standard for the molecular diagnosis of SARS-CoV-2 infection. This technology requires thermal cycling (repeated heating and cooling) to operate. Alternatively, isothermal NAAT technologies have been developed; these include technologies such as reverse transcription loop-mediated isothermal amplification (RT-LAMP) which do not require sequential heating and cooling, and therefore are more suitable for near-patient testing.

Additional methods of detecting SARS-CoV-2 RNA include the use of gene sequencing technologies such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) or next-generation sequencing (NGS). These approaches may require combination with amplification techniques.

2.1.5 Virus antigen detection

Detection of viral proteins (antigens) is performed using immunoassays or immunochromatographic tests, which detect the presence of proteins in clinical samples. Immunoassays are available in a wide range of different formats. Where

they are used to detect an antigen, they essentially consist of an antibody, immobilised on a surface, which is capable of binding to virus-specific antigens. Tests using viral antigen technologies are typically less analytically sensitive than NAATs; however, in individuals with a high viral load the antigen tests are usually positive.⁽¹⁵⁾

2.1.6 Antibody detection

Detection of human antibodies to SARS-CoV-2 is similarly performed using immunoassay technologies. The presence of antibodies (IgM, IgG and IgA) against SARS-CoV-2 in serum, plasma or venous blood samples demonstrates recent or previous SARS-CoV-2 infection. However, due to the window between infection and antibody detection they are not recommended for use as a primary diagnostic tool to diagnose acute infection. However, antibody status may be used to aid the clinical diagnosis of suspected non-critical cases that present a minimum of seven days after the onset of symptoms, or to diagnose COVID-19 in those with a negative rRT-PCR result; sensitivity is highest 15 days or more after the onset of symptoms.^(16, 17)

However, historically, serum antibody-based investigations of respiratory infection are compromised as the infection occurs in the respiratory tract and there is a locally derived mucosal immune response. Therefore, using a serum sample to detect an antibody response is not optimal, especially in those with mild symptoms. It has been observed that anti-SARS-CoV-2 antibodies are detectable more frequently in those patients with more severe systemic infection. It is possible that individuals who had milder disease or were asymptomatic may be less likely to develop a detectable antibody response in serum. Therefore, some previous mild infections may not have been detected. Even among those with a clear history of symptomatic infection, a small percentage do not have serological evidence for SARS-CoV-2 infection.⁽¹⁸⁾ Emerging evidence suggests a variable antibody response to SARS-CoV-2 both in level of antibody produced and to which viral protein target, nucleoprotein or spike, the antibodies are directed.

Antibody testing may also be used for surveillance, vaccine development and convalescent plasma therapy, which is currently under investigation as a potential therapy for COVID-19. The requirement for rapid turnaround times and on-site processing are less relevant to antibody detection.

Due to the limited capabilities of antibody testing in detecting acute infection, it will not be considered with respect to the description of technologies for detection of SARS-CoV-2 during acute infection. However, the types and applications of both laboratory-based and rapid antibody testing are described in the review of international guidance to ensure that the overall testing approach is in line with

international best practice. Methods for the detection of the antibody response to SARS-CoV-2 are described in detail in a previous rapid HTA published by HIQA in May 2020.⁽¹⁹⁾

2.2 Consideration of alternatives to standard technology

Laboratory testing to diagnose SARS-CoV-2 infection has evolved over the course of the pandemic as more has become known about the virus. Since the outbreak of SARS-CoV-2 in Ireland in February 2020, diagnostic testing capacity has significantly expanded to include use of high-throughput automated rRT-PCR-based diagnostic testing platforms in the National Virus Reference Laboratory (NVRL) and other designated laboratories, as well as expanded availability of rRT-PCR testing in hospital laboratories. However, typically there is still in excess of a 24-hour turnaround time (that is, the time from sample collection to delivery of results) for swabs taken in the community. For swabs taken in hospitals, the turnaround time is considerably shorter due to the on-site processing capacity. Prolonged turnaround times could negatively impact on the immediate management of an infection. Additional capacity for larger scale testing will most likely be necessary to fully meet the urgent clinical and public health needs in the coming months. This may include the use of alternative technologies for detection of SARS-CoV-2, and or the use of alternative devices, including rapid tests that have been clinically validated for use at, or near the point of care.⁽¹¹⁾

Two approaches to meeting the increasing demand for testing have specifically been proposed:

1. the use of alternative test technologies in laboratory or near-patient settings in addition to or to replace existing rRT-PCR-based tests
2. improvements in the overall testing process including sample collection and processing, sample pooling strategies and centralised record linkage.

The purpose of this assessment is to consider both of these approaches, that is, all practical laboratory-based or near-patient alternatives to rRT-PCR and improvements in the efficiency of rRT-PCR as currently employed in Irish laboratories. Figure 3 presents a summary schematic of the landscape of approaches (settings, detection technologies and examples of commercial tests) for potential consideration. These technologies are then described in detail in Sections 1.3 to 1.6. Given the speed at which new and emerging technologies for the detection of SARS-CoV-2 are becoming available, the review includes descriptions of both commercially available tests and tests that are in development to facilitate an awareness of the rapidly changing landscape of COVID-19 testing. At present, the FIND (Foundation for

Innovative New Diagnostics) SARS-CoV-2 diagnostic pipeline database includes over 800 tests, either in development or commercialised, for the detection of SARS-CoV-2 or the immune response to SARS-CoV-2. These include at least 377 molecular tests and 69 antigen tests. It is not possible to assess the characteristics of each of these devices in the course of a rapid HTA. Where particular tests are noted in the following sections these are used as examples and may not be representative of all devices included in a technology category. A brief summary of possible improvements in the overall testing process is provided in Section 1.8.

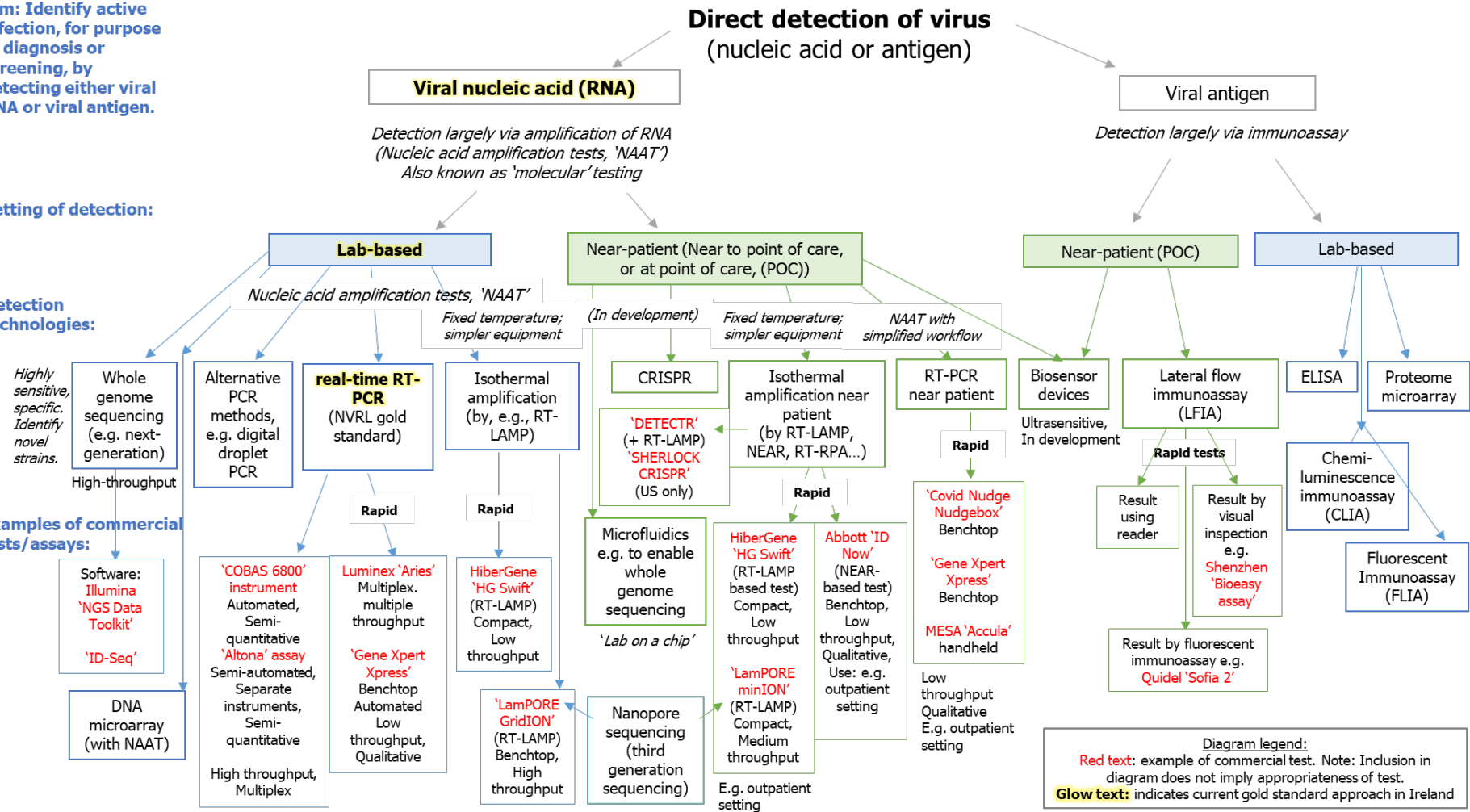
Figure 2.3: Landscape of SARS-CoV-2 testing approaches; settings, technologies, and commercial test examples

Aim: Identify active infection, for purpose of diagnosis or screening, by detecting either viral RNA or viral antigen.

Setting of detection:

Detection technologies:

Examples of commercial tests/assays:



The technology underpinning a given test has a significant impact on the test's performance (including its sensitivity, specificity, dynamic range, reproducibility and ease-of-use) in addition to safety issues regarding the initial loading of the sample onto the device and resource considerations such as reagent consumption, equipment requirements, cost and throughput.⁽²⁰⁾ Although rRT-PCR is considered the gold standard for the diagnosis of COVID-19, there are several limitations associated with its use⁽²⁰⁾ - in particular its ability to detect remnants of non-infectious or non-viable SARS-CoV-2 RNA potentially for weeks following resolution of infection. This makes it difficult to distinguish on the basis of the test result between COVID-19 patients who are infectious to others and those recovering from infection that are no longer infectious. Furthermore, an alternative and comparable technology to rRT-PCR may be required to facilitate timely detection of SARS-CoV-2, particularly in near-patient settings where the infrastructure and expertise required for rRT-PCR is not available. Newer and emerging diagnostic technologies may offer potential advantages over rRT-PCR in terms of shorter time to generate results, and portability, thereby expanding access to testing and accelerating clinical and public health decision-making.

Alternatives to laboratory-based RT-PCR include other viral RNA detection tests and antigen detection tests. A number of the technologies identified in this list may better be considered as future developments where it is unclear that they have been deployed or are ready to be widely deployed in clinical settings. Tests that have been identified internationally as having been deployed as a 'rapid test' are identified with an asterisk. Given the exceptional international demand for testing, it must be noted that in addition to the widely acknowledged restricted availability of testing consumables (test cartridges, reagents and so on) there may also be restricted availability of certain testing platforms.

Viral RNA detection tests include:

- isothermal amplification:
 - reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) for RNA detection*
 - reverse transcription nicking enzyme-assisted reaction (RT-NEAR)*
 - reverse transcription recombinase polymerase amplification (RT-RPA)
- isothermal amplification in combination with other detection methods:
 - isothermal amplification and CRISPR/Cas13a
 - isothermal amplification and nanosequencing*
- DNA sequencing

- next generation sequencing (NGS)
- third generation sequencing (TGS)
- DNA microarray

Antigen detection tests include:

- enzyme- immunoassays (EIA) – also termed ELISA
- chemiluminescence immunoassay (CLIA) and fluorescent (FIA) immunoassays
- lateral flow immunoassays (LFIA) and lateral flow fluorescent immunoassays*
- protein microarrays.

Only significant technological advances or variations to improve rRT-PCR efficiency reduction in assay time, and or throughput, independent of reaction conditions and parameters, will be considered within the scope of this assessment.

2.2.1 Rapid testing

For the purposes of this assessment, the term 'rapid tests' will be used to describe both rapid antigen detection tests (RADTs) and rapid molecular tests intended for use in near patient (point-of-care) settings. The time to obtain a result with these rapid tests is significantly reduced compared with laboratory-based tests, which may be impacted by transport time to the laboratory and the requirement for sample inactivation and nucleic acid extraction. Any rapid antigen or automated test intended to improve the efficiency of and or access to timely SARS-CoV-2 testing suitable for use outside of the traditional laboratory setting is considered in this report.

As described briefly above, rapid tests' are officially defined within the European Commission In-Vitro Diagnostic Medical Devices Directive as 'qualitative or semi-quantitative devices, used singly or in a small series, which involve non-automated procedures and have been designed to give a fast result', compared with current gold standard diagnostic tests.⁽¹³⁾ In the context of COVID-19, rapid test results are available in less than two hours, require minimal or no additional equipment and negate the need for transport of clinical samples to centralised laboratories for processing. These rapid tests are relatively easy to perform and interpret (although some of the devices require visual interpretation which can be subjective), and therefore require less operator training. While they can still be deployed in hospital laboratories, they are also suitable for deployment in near-patient (point-of-care) settings, including for example clinical settings, or community or 'pop-up' testing centres.⁽²¹⁾

Automated dedicated molecular platforms to investigate SARS-CoV-2 facilitate a reduction in the number of repetitive tasks that must be carried out manually, thereby improving efficiency, reducing costs and the potential for human error. Automated molecular tests for use with small portable or table-top analysers can also be fast and portable, but do not fall under the above definition of rapid tests.⁽¹³⁾ Rapid molecular tests can facilitate on-site processing of results within a number of hours of sample collection, reducing the time to test result.⁽²²⁾ Depending on the level of technical skill required, these tests are intended for use in laboratory or near-patient settings.

A note on sampling:

Appropriate specimen collection is a key step in the laboratory diagnosis of COVID-19. Clinical samples accepted for SARS-CoV-2 diagnostic testing in Ireland were originally limited to a combined nasopharyngeal and oropharyngeal swab in ambulatory patients OR a choice of bronchoalveolar lavage (BAL), endotracheal aspirate or sputum specimen in cases of serious illness.⁽²³⁾ However, the list has now been expanded to include nasal swabs as an acceptable alternative for use in children in community settings.⁽²⁴⁾ Consideration may also be given to oral fluid or salivary samples for selected populations or settings to improve the acceptability and feasibility of SARS-CoV-2 diagnostic testing, particularly in paediatric populations, pending independent clinical validation studies.^(7, 25, 26) When considering alternative sampling methods or sites, it is important that the tests have been validated for the method or specific type of clinical sample used.

2.2.2 Viral RNA detection

Molecular assays significantly amplify small amounts of specific genetic material in a sample to enable detection.⁽²⁷⁾ With the advancement of technologies, a number of alternative nucleic acid amplification tests (NAATs) have been described that remove the need for thermal cyclers that are necessary for laboratory-based rRT-PCR; these include loop mediated isothermal amplification (LAMP) and transcription-mediated amplification (TMA).^(28, 29) At present, LAMP is the most widely available alternative NAAT technology for the detection of SARS-CoV-2 RNA.

Two general methods are available for NAATs: one-step and two-step tests. In both methods, reverse transcription (RT) is used to generate a complementary DNA (cDNA) copy of the viral RNA. With one-step tests, the RT step occurs within the same tube as the amplification reaction for the specific viral target. With two-step tests, the cDNA is first generated by means of a separate reverse transcription reaction, then the cDNA is added to the amplification reaction tube. There are advantages and disadvantages to both methods that should be considered before choosing the optimal method for a particular application, ranging from the ease of

use and cost of reagents to the resulting yield and sequence representation (Table 2.1).^(30, 31) Due to the increased complexity and associated limitations of two-step tests, they are unsuitable for near-patient settings. Test systems that integrate and automate all steps necessary for molecular analysis, such as sample preparation (extraction and purification of nucleic acid) and detection based on nucleic acid amplification technology (that is, one step protocols), may be used in near-patient settings.⁽³²⁾

Some of the available tests are limited by the small number of samples which can be processed at a time. This limitation may be offset by the rapidity of some one-step assays, due to the lack of nucleic acid extraction.⁽³³⁾ In addition, detection kits that eliminate the RNA extraction and purification steps will be resistant to reagent shortages and supply chain irregularities associated with RNA extraction kits that are crucial for two-step NAAT.⁽³⁴⁾ However, such tests kits remain critically dependent on the availability of sample collection tools and transport media.

Table 2.1. Comparison of one-step and two-step nucleic acid amplification tests^(30, 31)

Method	Advantages	Disadvantages
One-step	<ul style="list-style-type: none"> ▪ Accurate representation of target copy number. ▪ Less technically complex. ▪ Faster. ▪ Fewer sample handling steps (reducing the risk of human error and contamination). ▪ High-throughput. ▪ Best method when only a few assays are run repeatedly. ▪ Use liquid handling robotics amenable to high-throughput applications. ▪ May be suitable for near-patient use. 	<ul style="list-style-type: none"> ▪ Usually less sensitive. ▪ Reaction conditions needed to support both the RT and amplification may not be optimal for either reaction. ▪ Difficult to identify the source of errors (troubleshooting). ▪ cDNA cannot be stored after one-step tests. ▪ Sub-optimal reaction conditions may impact efficiency and yield.
Two-step	<ul style="list-style-type: none"> ▪ Optimised for independent steps (RT and amplification). ▪ Potentially more efficient (flexible and controlled reaction optimisation). ▪ High sensitivity. ▪ Recommended when the reaction is performed with a limited amount of RNA. ▪ Extraction and precipitation steps can be used to concentrate and/or further purify the cDNA. ▪ cDNA produced during the first step is more stable than the initial 	<ul style="list-style-type: none"> ▪ Time consuming. ▪ Increased complexity and sample handling (increases the risk of human error and contamination). ▪ Requires optimisation of individual reactions (time consuming at outset). ▪ Two-tube protocol cannot be as easily adapted to automated workflows. Less amenable to high-throughput applications. ▪ Limited to laboratory settings.

	RNA sample and can be stored for future use.	
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Key: cDNA - complementary deoxyribose nucleic acid; RNA – ribonucleic acid; RT – reverse transcription.

2.2.3 Current laboratory-based methods

2.2.3.1 Real-time reverse transcription polymerase chain reaction (rRT-PCR)

Real-time RT-PCR detects SARS-CoV-2 ribonucleic acid (RNA) using primers that target different regions of the virus genetic sequence, such as the RNA-dependent RNA polymerase gene (RdRp), the nucleocapsid gene (N)⁽¹⁴⁾ and, in some cases, the envelope gene (E) gene. However, the E gene is highly conserved among coronaviruses and requires confirmation with a second SARS-CoV-2-specific gene.⁽¹⁴⁾ Real-time RT-PCR tests comprising primer and probe sets directed at different regions in the SARS-CoV-2 genome can improve test sensitivity, in the event of viral mutations in one of the gene targets.^(14, 35)

RNA is extracted from the clinical samples (such as nasopharyngeal and oropharyngeal secretions), and reverse transcribed to convert RNA into complementary DNA (cDNA). The final cDNA is then amplified by PCR using thermocycling equipment.⁽¹⁴⁾ While RT-PCR techniques (end-point or traditional RT-PCR) only provide results at the end of the PCR amplification process, real-time RT-PCR (rRT-PCR or quantitative [q]RT-PCR) measures the DNA product as it is being amplified (that is, in 'real-time').⁽³⁶⁾ With rRT-PCR, an increase in reporter fluorescence during the exponential growth phase of the reaction (log phase) is directly proportional to the amount of DNA generated, facilitating semi-quantification of the amount of SARS-CoV-2 present in the clinical sample. For this reason, rRT-PCR is widely used as the gold standard test to diagnose COVID-19.⁽¹⁰⁾

Multiplex rRT-PCR methods can facilitate differential diagnosis by distinguishing between SARS-CoV-2 and other common respiratory pathogens in circulation, and identify co-infections (if present), thus facilitating optimal clinical management.⁽³⁷⁾ The assay includes primer and probes to a range of respiratory pathogens.

Real-time RT-PCR allows accurate and high-throughput batch processing of clinical specimens;⁽¹¹⁾ however, the longer turnaround times resulting from transportation, sample processing and testing compared with other diagnostic methods and the requirement for large quantities of high-grade reagents present challenges for the exclusive use of rRT-PCR in COVID-19 diagnostic testing.⁽²⁰⁾

2.2.3.2 Simplified rRT-PCR methods

Rapid rRT-PCR tests with simplified workflows have been developed that can be used outside of the laboratory environment; these include for example, the Cepheid 'Xpert Xpress' and the 'COVID Nudge' test developed by DnaNudge Ltd.⁽³⁸⁾ The 'Xpert Xpress' has multiplex capacity with the ability to detect influenza A, influenza B and respiratory syncytial virus (RSV) in addition to SARS-CoV-2. The 'Xpert Xpress' is currently used in a number of Irish hospital laboratories to test high priority specimens in order to inform rapid clinical decision-making. The COVID Nudge test has been implemented in the NHS patient care and elective surgery settings, in addition to out-of-hospital locations, and is currently being rolled out on a national level in the UK.^(38, 39) The test is reported to be easy to use with minimal manual handling necessary for sample preparation.⁽⁴⁰⁾ Depending on the experience of the operator, test results can be available in 30 minutes.⁽⁴⁰⁾ The platform comprises two components: the DnaCartridge, an integrated lab-on-chip device reaction (negating the need for prior sample processing), which drives the thermal cycling conditions required for the PCR reaction, and a processing unit which measures the fluorescent readout (the NudgeBox).⁽³⁸⁾ The device can be linked to a secure cloud-based database, allowing results to be delivered directly to clinical information systems, which could facilitate linking records from multiple healthcare facilities or testing sites.⁽³⁸⁾ However, high throughput processing cannot be achieved with the CovidNudge platform at the current time (due to a single-use cartridge). To achieve adequate processing capacity, multiple processing units might be required depending on the clinical setting.⁽³⁸⁾

2.2.4 Commercially available near-patient isothermal amplification methods

2.2.4.1 Isothermal nucleic acid amplification

Molecular diagnostic tools using isothermal nucleic acid amplification technology has been developed including reverse transcription-loop-mediated isothermal amplification (RT-LAMP), nicking enzyme-assisted reaction (NEAR) and recombinase polymerase amplification (RPA) that enable the amplification of nucleic acids at a constant temperature (ranging from 60-65°C) in a single tube, negating the need for thermal cycling required for PCR reactions and reducing the risk of cross-contamination of samples, respectively.⁽³⁵⁾ Isothermal nucleic acid amplification offers a fast and easy-to-use alternative to rRT-PCR for the detection of SARS-CoV-2. It has been suggested that isothermal amplification methods have comparable accuracy to rRT-PCR.⁽³⁵⁾ Isothermal nucleic acid amplification has been used in several commercially available COVID-19 molecular platforms, such as the Abbott ID NOW which uses the NEAR technique in a compact, integrated diagnostic system.⁽⁴¹⁾

Diagnostic platforms using isothermal NAAT are suitable for use in near-patient settings. As outlined in Table 2.1, the number of steps involved in sample processing can influence the sensitivity of the test. The sensitivity of some isothermal amplification methods can be improved by initial purification of RNA.

2.2.4.2 Isothermal amplification and nanosequencing

Isothermal nucleic acid amplification can be combined with other detection methods to increase sensitivity. Successful amplification of the target sequence is often inferred from a proxy measurement (such as, changes in turbidity, colour or fluorescence). However, these proxy measurements can be affected by substances present in biological samples, leading to spurious results.⁽⁴²⁾ The use of sequencing as a read-out can result in increased sensitivity and multiplex capacity facilitates the detection of multiple targets in a single sample (for example, SARS-CoV-2, Influenza A and B and respiratory syncytial virus [RSV]).^(42, 43) The LamPORE assay developed by Oxford Nanopore technologies combines LAMP with real-time nanopore sequencing.⁽⁴⁴⁾ Available sequencing devices have variable processing capacity, suitable for small or large-scale processing.⁽⁴⁴⁾ Results can be delivered in less than two hours for between 1-96 samples (high throughput), facilitating rapid turnaround of results.⁽⁴³⁾ The LamPORE assay is currently in development for use with saliva samples. Early diagnostic test accuracy studies have primarily been conducted in symptomatic patients. The utility of the test in the general population is as yet unknown.⁽⁴⁵⁾

2.2.5 Near-patient methods currently in development

2.2.5.1 Isothermal amplification and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)

CRISPR-based diagnostic platforms have been developed, such as specific high sensitivity enzymatic reporter unlocking (SHERLOCK) or DNA endonuclease-targeted CRISPR trans reporter (DETECTR), that use RT-LAMP or RT-RPA isothermal amplification methods to amplify the target sequences in a clinical sample, in addition to the CRISPR-Cas12/13 system for detection.⁽⁴⁶⁻⁴⁸⁾ RNA-guided CRISPR-associated 12/13 (Cas12/13) nucleases are programmed to recognise and bind target sequences of the amplified RNA product, resulting in cleavage of nucleic acid reporters and qualitative detection of SARS-CoV-2 by fluorescence detection or the lateral-flow strip method.⁽¹⁴⁾ One-step or two-step variants of CRISPR diagnostic protocols have been developed. While one-step protocols simplify the operational procedure and decrease the risk of contamination, they are less sensitive than the two-step variant.⁽⁴⁸⁾ CRISPR SARS-CoV-2 kits require a heat block and fluorescence detection instrumentation for some test kits, in addition to standard diagnostic laboratory equipment, and have thus only been

authorised for use by qualified clinical laboratory personnel specifically trained in the techniques of molecular in vitro diagnostic procedures.⁽⁴⁹⁾

CRISPR SARS-CoV-2 kits have been shown to demonstrate high concordance with laboratory-based PCR and other rapid diagnostic tests.⁽⁴⁸⁾ Readouts can be performed with either fluorescent or lateral-flow readout, depending on the setting.⁽⁴⁸⁾ The less sensitive lateral-flow readouts may have acceptable sensitivity for the purpose of triage outside of the diagnostic laboratory setting, while fluorescence detection methods may be more amenable to higher-throughput assessment in diagnostic laboratories.⁽⁴⁸⁾ The simplified SHERLOCK testing in one pot (STOP) test is said to be suitable for use in near-patient settings.⁽⁵⁰⁾ A commercially available test is currently in development, however, it is unclear when this might be available for deployment.

2.2.6 Other laboratory methods

2.2.6.1 Digital droplet PCR (ddPCR)

Digital droplet PCR is an optimised PCR method which is reported to have a lower limit of detection compared with rRT-PCR, thereby reducing the risk of false negative results. The reaction mixture is divided into tens of thousands of nanodroplets. Each of these small PCR reactions is then run individually.⁽⁵¹⁾ Some portion of these reactions contain the target RNA (positive) while others do not (negative). Following the PCR reaction, the fraction of negative answers is used to determine the exact number of target molecules in the sample, without reference to standards or endogenous controls.⁽³⁶⁾ Digital droplet PCR can be used to determine the absolute quantification of viral RNA in low viral load samples. An important advantage of ddPCR is that the quantification is independent of variations in the amplification efficiency and is robust to the presence of PCR inhibitors (that is, reagents that come into contact with clinical sample during processing or cell-derived inhibitors such as proteins).⁽²⁰⁾ While both rRT-PCR and ddPCR provide sensitive detection and precise quantitation, it is suggested that their distinct characteristics provide different advantages for different applications. Real-time RT-PCR is recommended for pathogen detection due to its economical running costs and broad dynamic range. While ddPCR may be more suited to detection of mutations and single-nucleotide polymorphisms (SNPs), as well as allelic discrimination.⁽⁵²⁾

Available ddPCR test kits are intended for use by qualified clinical laboratory personnel specifically trained in ddPCR techniques.⁽⁵³⁾ However, ddPCR is currently more costly than rRT-PCR, due to the requirement for specialised instrumentation and consumables.⁽⁹⁾ While potentially a promising alternative to laboratory-based RT-PCR, it is unclear the extent to which ddPCR has been deployed, or is ready to be widely deployed, in clinical settings.

2.2.6.2 DNA sequencing

Next-generation sequencing (also known as NGS or short-read sequencing) is a sequencing technology that allows DNA sequencing to be carried out more quickly and cheaply than the previously used Sanger sequencing.⁽⁵⁴⁾ NGS technologies differ from the first-generation Sanger method in that they provide in-parallel, extremely high-throughput analysis from multiple samples. NGS is characterised by improved accuracy and speed, as well as reduced resource use and cost. Although NGS has mostly superseded conventional Sanger sequencing, it has typically not been used in routine diagnostic testing.⁽⁵⁴⁾ Although it has not been widely adopted as a diagnostic technique, NGS is important for genomic surveillance to identify the rate and degree of mutational variability in SARS-CoV-2. NGS therefore facilitates confirmation of re-infection with a different strain of SARS-CoV-2 in the case of patients who have previously been infected with a different strain of SARS-CoV-2.

Third-generation sequencing (long-read sequencing) technology, including Pacific Biosciences (PacBio) Single Molecule Real Time (SMRT) sequencing, and the Oxford Nanopore Technologies (ONT) nanosequencing platform, increases the efficiency of sequencing. As previously described, it is suggested that the Oxford Nanopore Technologies LamPORE assay will allow rapid, low-cost and scalable detection of SARS-CoV-2 using RT-LAMP and downstream nanosequencing technology.⁽⁵⁵⁾

Ongoing genomic surveillance will be necessary to ensure the target sequences used in SARS-CoV-2 detection tests are genetically stable. Viral mutations in target sequences could render some tests ineffective or less sensitive leading to false-negative test results. Genomic sequencing for SARS-CoV-2 can also be used to investigate the dynamics of an outbreak (locally or nationally), including changes in the size of an epidemic over time, its spatiotemporal spread and testing hypotheses about routes of transmission.⁽¹⁰⁾ Whole virus genome sequencing (WvGS) using next generation sequencing has been applied in an outbreak investigation in a tertiary referral centre in Ireland to analyse route of transmission in hospital-acquired COVID-19.⁽⁵⁶⁾

2.2.6.3 DNA microarray

DNA microarrays are made up of DNA spots containing a known DNA sequence (probe). Labelled cDNA molecules (target) bind to the DNA probes on the slide through a process called hybridisation. Probe-target hybridisation can be detected and quantified by detection of the labelled targets to determine the relative abundance of nucleic acid in the sample.⁽⁵⁷⁾ It is suggested that the combination of nucleic acid amplification with a DNA microarray incorporating probes for multiple

pathogens improves test specificity through multiplex testing. It also facilitates concurrent testing for a range of respiratory viruses (such as, influenza A or B).

2.3 Antigen detection tests

Antigen-detection diagnostic tests are designed to directly detect SARS-CoV-2 proteins (antigens), mostly nucleoprotein (NP) which is the most abundant protein in SARS-CoV-2.⁽⁵⁸⁾ A number of immunoassays have received approval for use in both laboratory and near-patient settings, using formats such as enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassays (LFIA). The types of sample (for example, nasopharyngeal, nasal, or lower respiratory tract specimens) for which a test is initially approved is contingent on the validation studies undertaken by individual manufacturers and may change subject to completion of further validation studies.

At present, data on the accuracy of currently available antigen detection tests for SARS-CoV-2 is highly variable.⁽⁵⁸⁾ While data on the analytical sensitivity and specificity of tests are reported, evidence of clinical (or diagnostic) sensitivity and specificity are more limited. However, there are concerns regarding the occurrence of a negative (SARS-CoV-2 not detected) antigen result in individuals with a low or variable viral load; the potential for sampling variability may further compound the problem in cases with low viral loads, increasing the risk of false-negative results.⁽⁵⁹⁾ The diagnostic accuracy of antigen detection tests is discussed in detail in Chapter 3. Research is ongoing to improve the reliability and clinical utility of these tests. Developments to increase test sensitivity have included addition of a fluorescent label that is read in a dedicated instrument to amplify the detection signal and changes to the antibodies coating the reaction strips. Despite potential limitation of the first generation tests, it is suggested that there is a role for their use to quickly detect infectious individuals for urgent management in parallel with molecular assays.^(59, 60)

A potential limitation common to all antigen detection testing is that the targets may demonstrate cross-reactivity with other viruses, in particular, the other human coronaviruses in circulation, in particular the β -coronaviruses.⁽⁶¹⁾ False-positive results may occur with ELISA or LFIA tests targeting the N protein, as this protein is said to be highly conserved among human-infecting β -coronaviruses.⁽⁶²⁾ Within the spike (S) protein, the S1 subunit is less conserved and more highly specific to SARS-CoV-2, and is therefore said to be a better target for COVID-19 serologic detection at the present time.⁽⁶³⁾ However, it is uncertain how many currently available assays are spike antigen assays. Clarification of test specificity for SARS-CoV-2 will be necessary prior to widespread use, given the potential for cross-reaction with other human coronaviruses;⁽⁵⁹⁾ this will include requirement for clinical validation studies.

Despite potential limitations in performance, the World Health Organization (WHO) has reported that if antigen detection tests with adequate performance are performed and interpreted correctly, they could play a significant role in guiding patient management, public health decision-making and disease surveillance.⁽⁵⁸⁾ While laboratory-based antigen detection tests and molecular-based tests are more reliable and robust, they do not offer the same advantages as RADTs (that, is lateral flow immunoassays) in terms of rapidity (<30 minutes compared with hours for rRT-PCR testing) or ease of use, and have greater requirements in terms of technical skill and infrastructure compared with their RADT equivalents.⁽⁵⁹⁾

2.3.1 Immunoassays

2.3.1.1 *Enzyme immunoassay (EIA) or Enzyme-linked immunosorbent assay (ELISA)*

ELISA is a common qualitative, quantitative or semi-quantitative laboratory-based enzyme immunoassay that can be used to detect the presence of SARS-CoV-2 antigens.⁽²⁷⁾ There are several formats used for ELISAs including:

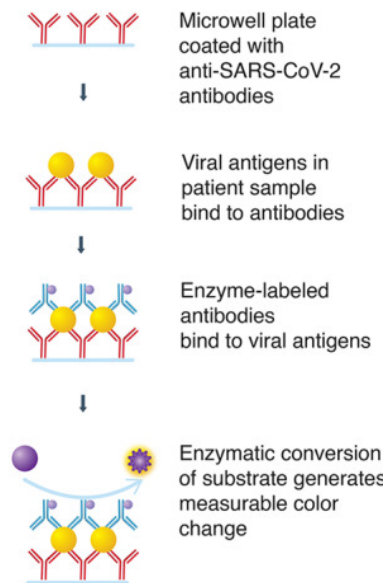
- direct ELISA
- indirect ELISA
- sandwich ELISA (Figure 2.3)
- competitive or inhibition ELISA.

The key step is immobilisation of the antigen of interest which can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (such as labeled secondary antibody).⁽⁶⁴⁾

The most widely used format is the sandwich ELISA assay or capture assay, which immobilises and detects the presence of the target antigen (Figure 2.3). The capture antibody is coated to the surface of the multi-well plate and is used to facilitate immobilisation of the antigen of interest (a specific SARS-CoV-2 protein). The sample solution containing the SARS-CoV-2 antigen (if present in the clinical sample) is added to the plate and binds to the capture antibody. The antigen is then detected by complexing with a 'detection antibody' linked to a reporter-enzyme, either directly (labelled primary antibody) or indirectly (labelled secondary antibody). In this way, the SARS-CoV-2 antigen of interest is bound or 'sandwiched' between the capture antibody and the detection antibody.⁽⁶⁵⁾ Detection is accomplished by measuring the activity of the reporter enzyme by incubation with an appropriate substrate to produce a product (for example, a colour change) that can be measured using

specialised laboratory-based instrumentation.^(20, 66) Up to 96 samples (including positive and negative controls) can be processed at the same time, allowing batch testing of large numbers of patient samples.⁽⁶⁶⁾ Sandwich ELISAs are suitable for processing of crude or impure clinical samples.⁽⁶⁷⁾ A number of ELISA kits are in development for the detection of SARS-CoV-2-specific antigens.⁽⁶⁸⁾

Figure 2.3. Sandwich ELISA for the detection of SARS-CoV-2 antigens



Carter et al. 2020.⁽²⁷⁾

2.3.1.2 Chemiluminescence and fluorescence assays

Fluorescent (FIA) or chemiluminescence immunoassay (CLIA) are a variant of ELISA. An enzyme converts a substrate to a reaction product that emits a fluorescent and luminescent signal, instead of developing a visible colour change as with a typical ELISA.⁽⁶⁹⁾ FIA and CLIA have a greater diagnostic range than EIAs as they do not reach an absorbance optical density value that occurs with EIAs.⁽⁷⁰⁾

2.3.1.3 Lateral flow immunoassays or immunochromatographic tests

Lateral flow assays (LFA) or immunochromatographic tests consist of a simple, portable diagnostic strip or dipstick containing immobilised test reagents, enclosed in a cassette to measure SARS-CoV-2 antigen, such as nucleoprotein (N).⁽¹⁴⁾

There are two types of lateral flow devices:

- LFIA with visual read-out (qualitative; no requirement for additional instrumentation)

- LFIA with an associated reader device (quantitative or semi-quantitative; removes subjectivity from the interpretation of results).

The sample is applied at one end of the strip (sample pad), which contains buffer salts and surfactants that make the sample suitable for interaction with the detection system. The sample migrates through the conjugate release pad, which contains antibodies that are specific to the target SARS-CoV-2 antigen, and are conjugated to coloured or fluorescent particles (most commonly colloidal gold and latex microspheres).⁽⁷¹⁾ The original sample, together with the conjugated antibody bound to the target antigen (if present), migrates along the strip into the detection zone - a porous membrane (usually composed of nitrocellulose) with specific antibodies immobilised in lines which react with the antigen bound to the conjugated antibody. If the antigen is detected, a response will appear on the test line.⁽⁷¹⁾ The read-out can be assessed visually or using a device reader depending on the design of the test.^(58, 61) The use of a reader standardises the interpretation of test results, reducing inter-operator variance in assay interpretation, but requires ancillary equipment.⁽⁵⁸⁾ LFIAs cannot quantify the amount of antibodies present in the sample to indicate the adequacy of the immune response to the virus.⁽⁵¹⁾ LFIAs are easy to use and facilitate fast delivery of results in near-patient settings, which could expedite public health interventions in the case of a positive antigen test.⁽²⁰⁾ LFIA tests may be an attractive option due to their portability and rapid turnaround times. However, they are less sensitive, which should be taken into consideration in the interpretation of results using LFIAs.⁽⁷²⁾

Lateral flow fluorescent immunoassays with associated reader device offer the most sensitive detection using the lateral flow method. Readers can provide a semi-quantitative or quantitative result, thus removing the subjectivity in the interpretation of results. CE-marked lateral flow fluorescent immunoassays for the detection of SARS-CoV-2 in nasopharyngeal samples are available.⁽⁷³⁾ In general, available tests are easier to use than rRT-PCR, however there are still a number of steps involved including sample collection, preliminary operations (for example, pre-treating the sample application pad for some tests), sample application, measurement and evaluation of results.⁽⁷⁴⁾ Indicative times for test performance reported by manufacturers (typically 10 to 15 minutes) may not reflect the time needed to carry out testing by unskilled operators in near-patient settings.

2.3.2 Protein microarrays

A protein microarray is a miniaturised and parallel assay for high-throughput diagnostics, typically consisting of proteins embedded on a solid surface in a high-density format.⁽⁷⁵⁾ Microarrays have multiplexing capabilities facilitating simultaneous determination of multiple target antigens from small amounts of samples within a

single experiment, allowing clinicians to quickly and efficiently differentiate between SARS-CoV-2 and other respiratory pathogens. At present, the majority of available protein microarrays are aimed at the detection of SARS-CoV-2-specific antibodies in patient sera. While potentially a promising alternative to laboratory-based RT-PCR, it is unclear the extent to which protein microarrays have been deployed, or are ready to be widely deployed, for diagnostic testing in clinical settings.

2.4 Biosensors

Biosensors can be developed to target a range of SARS-CoV-2 targets including antibodies, antigens or nucleic acids. Sensors consist of chemical or biological receptors coupled with transducers. The receptor interacts specifically with a target analyte and the transducer converts the recognition process into a quantitative signal.⁽⁷⁶⁾

Based on technology employed, biosensors can be grouped into four categories, or combinations of these groupings; these include optical biosensors, electrochemical biosensors, piezoelectric biosensors, and thermal biosensors.⁽⁷⁶⁾ A number of biosensor platforms have been used for the detection of other SARS-CoV-2. A graphene-based biosensor comprising SARS-CoV-2 spike antibody (COVID-19 FET sensor) immobilised onto a graphene sheet (sensing area) for the detection of SARS-CoV-2 antigens in clinical samples without the need for sample pre-treatment or labelling has been developed.⁽⁷⁷⁾ It is unclear if any tests using biosensor technology have been deployed, or are ready to be widely deployed, for diagnostic testing in clinical settings.

2.5 Microfluidic technology

Microfluidic technology is not a laboratory test, but refers to the design of test devices to facilitate manipulation and control of fluids in networks of channels with dimensions from tens to hundreds of micrometers to enable testing to be performed at greatly reduced spatial scales.⁽⁷⁸⁾ Microfluidic devices have the ability to miniaturise, automate and integrate sample preparation, reactions and detection on a micron-scale chip ('Lab-on-a-chip' technologies).⁽⁷⁹⁾ It is suggested that microfluidics-based platforms offer many advantages including rapid and sensitive detection, portability, high throughput, multiplex capabilities and a reduction in the volume of reagents used (thus reducing the cost of testing).⁽⁸⁰⁾ Microfluidic devices to enable COVID-19 testing to occur in near-patient settings are currently in development and while potentially a promising alternative to laboratory-based RT-PCR, it is unclear when they will be ready for widespread deployment in clinical settings.

2.6 Testing approaches

The COVID-19 testing process involves a number of steps including clinical consultation, safe sample collection, safe processing of sample, analysis, interpretation of results (some steps may be carried out individually or as a single step depending on the technology) and delivery of results, together with interpretation in the clinical context. Improvements at any stage can lead to improvements in the overall testing process. As described in the previous sections, some alternative tests can achieve a reduction in complexity and turnaround times through technological advancements. A second approach involves improvements at stages of the testing process independent of the testing technology to improve test efficiency and or capacity, including sample collection and processing procedures, sample pooling and centralised record linkage.

2.6.1 Sample collection and processing methods

Sample collection methods impact the yield and quality of viral RNA in clinical samples. If a poor sample is collected (for example, only a few cells collected on the swab), the result will be compromised, irrespective of the adherence to quality assurance processes thereafter. Therefore, some of the more recent rRT-PCR assays incorporate a primer/probe set to detect a human gene, and in the event that this gene is not detected, the sample is rejected as inadequate. Problems with sample transport and initial processing can impact on the final result.⁽⁸¹⁾ Swab sampling and fluid transfer are also relevant to LFIAs intended for non-laboratory settings. In addition, methods that reduce or eliminate the manual handling steps associated with RNA extraction and purification can increase the speed of sample processing; however, test sensitivity may be reduced.⁽⁸²⁾

2.6.2 Sample pooling approaches

The ongoing COVID-19 pandemic is placing a substantial burden on healthcare systems and their infrastructure. Clinical laboratories may use a sample pooling strategy as an alternative to individual testing to expand SARS-CoV-2 RNA testing capacity when using a validated testing protocol for such a purpose in circumstances where the disease prevalence is low.

The cost of testing may be reduced and accuracy may be maintained by pooling of samples from multiple patients, with retesting of individual samples from positive pools.^(8, 9) If a pooled test result is negative, then all specimens are considered negative.⁽³⁾ If the test result is positive or indeterminate, then all specimens in the pooled sample are retested individually, and the subsequent individual diagnostic results are reported.⁽³⁾ However, the time taken to prepare the pools and the logistics regarding pool testing, result breakdown and subpool positive confirmation

are complex and require significant automation (for example, robotic systems, software supporting the algorithms to identify positive samples, laboratory information systems) to avoid errors.⁽¹⁰⁾ In addition, poor quality samples that would ordinarily be captured through the use of a primer/probe set to detect a human gene to check for sample quality, will no longer be identified when only tested as part of a pooled test.

As an alternative to simple pooling approaches, a number of matrix-based methods have been proposed that aim to exploit the positional information of samples in order to further improve testing efficiency.⁽⁸³⁾ Matrix pooling strategies allow laboratories to test samples as pools while preventing the need to retest individual samples by identification of the positive specimen based on its position in the matrix if the prevalence is sufficiently low.⁽¹⁰⁾ Depending on how robust the matrix testing method is in the specific context, and the available resources, it might still be advisable to retest the identified positive samples for confirmation.⁽¹⁰⁾

The advantages of sample pooling include:

- conservation of limited testing reagents and other laboratory consumables
- potential reduction in the amount of time required to test large numbers of samples (that is, increased throughput)
- reducing the cost of testing.⁽³⁾

The potential limitations include:

- pooling preparation time takes longer compared with rRT-PCR, and due to the requirement for sub-pooling to confirm positive results of pooled samples, there could be an increase in the time to generate a positive result
- the diagnostic integrity of individual samples cannot be ensured because they are combined with other samples
- pooled sampling techniques are technically more challenging than individual level testing⁽⁸⁴⁾
- capacity increases only apply to the sample processing stage. Resource constraints associated with sample collection or reporting of results are unchanged⁽⁸⁴⁾
- high prevalence of SARS-COV-2 infection may negate any efficiency gained through pooling, due to an increased likelihood of a positive pool.

The aim of sample pooling is to determine a pool size that allows the greatest conservation of resources while maintaining reliable test performance.⁽⁹⁾ While cycle

threshold (Ct) values may be higher for some genes using rRT-PCR in pooled samples, sample pooling can increase test capacity with existing resources and detects positive samples with sufficient diagnostic accuracy.^(8, 84) The dilution of samples is unlikely to result in a clinically significant reduction in viral load. Borderline positive samples might not be detected in large pools, increasing the risk of false-negative results, particularly if the test has not been properly validated for this purpose.^(8, 85) However, borderline positive rRT-PCR results may not be clinically significant and may not be infectious to others; they have been reported in convalescent patients 14 to 21 days after symptomatic infection.⁽⁸⁾ In this way, sample pooling could potentially improve the clinical utility of testing. Given that sample pooling greatly increases the number of individuals that can be tested using existing resources, a small reduction in sensitivity may be acceptable.⁽⁸⁵⁾ Furthermore, as the prevalence of the infection increases, the efficiency of a pooled testing technique decreases; if the prevalence is high, most of the pools will be positive, necessitating follow-up testing of individual samples from pooled samples. These limitations mean that monitoring the prevalence of COVID-19 and properly validating the test for a pooled testing strategy are important to limit the potential for false-negative results and reporting delays.

When considering the implementation of a sample pooling strategy, a standardised methodology should be used that takes the disease prevalence, the diagnostic test accuracy of the test and the cost of testing into consideration to determine when the positivity rate is low enough to justify the implementation of a pooling strategy.⁽³⁾ Any laboratory that wishes to use a sample pooling strategy with a SARS-CoV-2 nucleic acid test assay should validate the test for a pooling strategy to determine appropriate parameters for use, in accordance with the principles of good laboratory practices and quality assurance programmes for clinical laboratory testing. It may therefore be appropriate for consideration to be given to development of a national approach to pool testing. During the rapidly changing pandemic, testing strategies will need to adapt to potential changes in the test positivity rate. Ongoing monitoring of the positivity rate and test performance with a pooling strategy should be undertaken as the positivity rate fluctuates.^(9, 85) If a laboratory modifies the testing procedure by using alternative test components, such as nucleic acid extraction kits or instrumentation, the laboratory should evaluate and validate the performance of such changes.⁽³⁾

2.6.3 Record linkage

Any delays in returning results to the patient potentially leads to delays in therapeutic and infection control interventions (for example, isolation and contact tracing), increasing the risk of onward transmission. Development of effective record linkage approaches for healthcare and other testing locations (in particular near-

patient settings) is essential to ensure all tests are accounted for and to provide timely access to results.

2.7 Considerations for use

Validated and accurate laboratory and near-patient testing for SARS-CoV-2 are a crucial part of the timely management of COVID-19, supporting the clinical decision-making process and infection prevention and control within the community. Due to the limited laboratory-based molecular testing capacities, scalable rapid diagnostic tests have been developed to allow COVID-19 diagnostics to be conducted outside of laboratory settings.⁽⁸⁶⁾ The major challenges faced in developing a diagnostic test for SARS-CoV-2 are the ability to measure low concentrations of viral components for early detection, to provide low or no cross-reactivity with other viral strains, and to deliver results rapidly.

At present, the majority of SARS-CoV-2 diagnostic testing carried out in Ireland uses the rRT-PCR technologies. By diversifying testing platforms and using innovative technologies, less reliance is placed on a single manufacturer or supply chain. This could help ensure there is enough testing capacity to adequately respond to the COVID-19 pandemic in the case of supply shortages.^(14, 87) The current standard, rRT-PCR, is principally performed using high-throughput instruments in a clinical laboratory, whereby samples are processed in large batches (up to 96 samples with some test kits, including positive and negative controls) and typically taking six hours to complete, but can take a number of days to deliver results due to the requirement for sample shipment to a specialist laboratory.^(51, 87) Other rapid or point-of-care tests can only process a single or limited number of samples at once; however, the processing time per test is faster (<2 hours) than with laboratory-based high-throughput instrumentation. Table 2.2 summarises information on commonly proposed test alternatives for detection of SARS-CoV-2. Each type of test has its own distinct advantages and disadvantages related to the underlying technology. Therefore, when considering the potential application of a test to a particular context or setting, it is important to consider the goal of testing; whether this is the ability to process large numbers of samples simultaneously or to inform rapid clinical decision-making. Many of the current or proposed approaches to scale up testing require substantial additional resources. Automated, high-throughput solutions are needed that will increase both testing volumes and test turnaround times in both laboratory and near-patient settings.

In some circumstances SARS-CoV-2 may not be detected by rRT-PCR or other comparable alternative tests due to pre-analytical or analytical errors such as insufficient viral components at the anatomical site sampled, incorrect sample collection methods, the timing during the course of infection, errors during sample

transport, storage and or processing or errors in the reporting of results.⁽¹⁵⁾ The ability of any diagnostic test to achieve acceptable clinical performance is contingent on it being performed within the appropriate time frame with due consideration of the principles of good pre-analytical and analytical testing practice. It is therefore important for there to be a clear understanding that a negative result should be interpreted as 'no virus detected' rather than 'not infected with SARS-CoV-2'.⁽¹⁵⁾ The interpretation of test results should take potential uncertainties into consideration, and should also consider the probability that an individual is infected based on clinical data and epidemiological history to increase the probability of correct diagnosis.⁽¹⁵⁾ In addition, positive results do not exclude concomitant bacterial infection or co-infection with other viruses. In order to ensure safe, reliable and effective diagnostic testing, clinical samples should be processed in an environment with adequate quality assurance and safety precautions in place to ensure accurate diagnosis and the protection of operators from contracting SARS-CoV-2 infection, particularly when considering rollout in near-patient settings.

Generally, the ease of use and rapid turnaround time of antigen detection tests offer the potential to expand access to testing and accelerate the identification of those individuals with a high SARS-CoV-2 viral load, and facilitate timely cross-infection protocols. Due to their reduced sensitivity compared with rRT-PCR, RADTs are most likely to perform well in individuals with high viral loads (that is, Ct values ≤ 25) — high viral loads are usually detectable in the pre-symptomatic (1-3 days before symptom onset) and early symptomatic phases of the illness (within the first 5-7 days of illness)⁽⁵⁸⁾ when the patient is most infectious. It is suggested that their greatest clinical utility may be in symptomatic patients, when the viral load is likely to be highest, to enable accurate triage.⁽⁵⁹⁾ The risk of false-negative results will be greater for those presenting later in the course of infection, therefore, it has been suggested that RADTs should only be used in circumstances where molecular-based methods are unavailable or where prolonged turnaround times preclude clinical utility.⁽⁵⁸⁾ Due to the risk of false negative results, it has been suggested that repeat testing or preferably confirmatory testing using molecular methods should be performed on negative rapid antigen detection test results, particularly in symptomatic patients.⁽⁵⁸⁾ Where clinical performance is found to be acceptable, it has been suggested that RADTs could be implemented in a diagnostic algorithm to reduce the number of molecular tests needed and to support rapid identification and management of COVID-19 cases.⁽¹⁰⁾ The ability to incorporate antigen detection into a testing algorithm would be dependent on the sensitivity and specificity of a given antigen test and the prevalence of SARS-CoV-2 infection in the population for whom the test is intended.

Contingent on the availability of accurate and reliable tests, near-patient testing may have a greater impact on public health than RT-PCR as it does not require the same technical expertise (although some training of operators is necessary) and does not require sample transport to centralised testing facilities, potentially reducing turnaround times. As these are rapid tests, results can be returned within the same clinical encounter, facilitating timely decisions concerning the need for isolation and contact tracing.⁽²²⁾ Such approaches would be dependent on the accuracy of particular tests and the ability to feed results into national surveillance platforms. Considerations of the organisational issues associated with deployment of near-patient testing are discussed in detail in Chapter 4.

Table 2.2. SARS-CoV-2 testing methods; summary information on commonly proposed test types

Technology	Type	Target	Setting	Accuracy†	Additional equipment and supplies *	Training	Processing time	Additional considerations
DNA sequencing	NGS	RNA	Laboratory	+++	Sequencing device.	Technically challenging. Requires skilled staff. Variable dependent on technology.	Variable dependent on product (WGS v targeted sequencing) 4-10 hours ⁽⁸⁸⁾	NGS is more accurate than TGS.
	TGS	RNA	Laboratory or near-patient	Further clinical validation required.	Sequencing device.	Variable dependent on technology. Requires some training.	2 hours ⁽⁴²⁾	High-throughput.
Nucleic Acid Amplification Tests (NAAT)	rRT-PCR	RNA	Laboratory	Gold standard +++	Thermocycler (heats and cools samples in repetitive, pre-programmed cycles).	Technically challenging. Requires skilled staff.	5-6 hours	Multiplex capacity. High throughput. Already in widespread use.
	ddPCR	RNA	Laboratory	+++	Thermocycler, droplet reader.	Technically challenging. Requires skilled staff.	5-6 hours	Multiplex capacity. High throughput.
	Isothermal amplification (e.g. RT-LAMP, RT-RPA, RT-NEAR)	RNA	Laboratory or near-patient	++	Isothermal amplification device (maintain reaction temperature 60-65°C).	Training required.	15-60 minutes ⁽⁴¹⁾	Throughput dependent on device.
NAAT in combination with	Isothermal amplification and	RNA	Laboratory or near-patient	Further clinical validation required.	Thermocycler or isothermal amplification device.	Training required.	30-60 minutes ⁽⁸⁹⁾	Throughput dependent on protocol.

other technology	CRISPR/Cas13a							
	Isothermal amplification and nano-sequencing	RNA	Laboratory or near-patient	Further clinical validation required.	Sequencing device.	Training required.	90 minutes ⁽⁹⁰⁾	Throughput dependent on device. Multiplex capacity.
Immunoassays	ELISA	Antibodies/antigens	Laboratory	Further clinical validation required.	No additional instrumentation.	Training required.	3-5 hours	Multiplex capacity. High throughput.
	CLIA/FIA	Antibodies/antigens	Laboratory	Further clinical validation required.	Chemiluminescent or fluorescent plate reader)	Training required.	3-5 hours	Multiplex capacity. High throughput.
	LFIA	Antibodies/antigens	Near-patient	+	No additional instrumentation.	Lowest training requirements.	<15 minutes	Single-use only.
Microarray	DNA or proteome microarrays	Nucleic acid/Antibodies/antigens	Laboratory	Further clinical validation required.	RNA amplification device, microarray scanner.	Technically challenging. Requires skilled staff.	6-8 hours ⁽⁹¹⁾	High throughput. Multiplex capacity.

Key: CLIA – Chemiluminescence assay; CRISPR - Clustered Regularly Interspaced Short Palindromic Repeats; DNA – deoxyribose nucleic acid; DTA – diagnostic test accuracy; EIA – enzyme-linked immunosorbent assay; LFIA - lateral flow immunoassays; NAAT - Nucleic Acid Amplification Tests; NGS- next generation sequencing; RPA - recombinase polymerase amplification; RNA – ribonucleic acid; RT-LAMP- reverse-transcription loop-mediated isothermal amplification; RT-NEAR - Reverse Transcription Nicking Enzyme Amplification Reaction; rRT-PCR - real-time reverse transcription polymerase chain reaction; RT-RPA – Reverse Transcription Recombinase Polymerase Amplification; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; TGS – third generation sequencing; WGS – whole genome sequencing.

* Additional instrumentation refers to any additional reagents or instrumentation for sample preparation, analysis and/or detection not provided in the test kit, and not already in widespread use in clinical laboratories (e.g. microfuge, microcentrifuge tubes, pipette, pipette tips). Requirements for additional equipment and consumables will vary dependent on the particular device under consideration, and the platforms already in use in a particular setting. Some devices may integrate several functions into a single step.

3 Rapid review of international testing methods

Key points

- Across the 18 countries included in the review, the broadly recommended primary test for the detection of SARS-CoV-2 is nucleic acid amplification test (NAAT), such as real-time reverse transcription polymerase chain reaction (rRT-PCR). In some countries, including Australia, Canada, Germany, Spain, and the US, approved rapid antigen detection tests (RADTs) may be used in certain clinical contexts for the detection of SARS-CoV-2.
- Antibody or serological testing remains broadly focused on the serosurveillance of defined target cohorts or populations — carried out for the purpose of understanding the spread of the disease and potential level of immunity within countries.
- None of the included countries appear to be using whole genome sequencing for the purposes of primary detection of SARS-CoV-2. However, the method is being used in some countries to investigate outbreaks and study routes of transmission, as well as host response and evolution of the virus.
- A number of the included countries have yet to recommend the use of near-patient testing for the detection of SARS-CoV-2 infection, including Belgium, Denmark, Ireland, New Zealand, Sweden, Switzerland, and Wales.
- Where near-patient molecular tests (non-RADTs) have been introduced, the primary purpose for using these tests is either to ensure rapid diagnosis of SARS-CoV-2 infection in clinical settings (for example, Norway and the Netherlands); or to ensure testing is accessible to difficult-to-reach populations due to their geographical or physical location (for example, Australia and Canada).
- The World Health Organization (WHO) has suggested that RADTs that meet the minimum performance requirements of $\geq 80\%$ sensitivity and $\geq 97\%$ specificity may be used to diagnose SARS-CoV-2 where NAAT is unavailable or where prolonged turnaround times preclude clinical utility. RADTs are recommended for use in symptomatic individuals, but can be used in asymptomatic individuals with known or suspected exposure.
- The WHO has also detailed a number of situations in which RADTs should not be used, including for the purposes of screening in airports or other border

points of entry due to the highly uncertain prevalence of disease and hence unknown predictive value of the test in these populations.

3.1 Background

Since the beginning of the coronavirus disease 2019 (COVID-19) pandemic, countries around the world have predominantly been using laboratory-based real-time reverse-transcription polymerase chain reaction (rRT-PCR) to detect severe acute respiratory syndrome 2 (SARS-CoV-2), the virus that causes COVID-19. A wide range of alternatives to rRT-PCR is now commercially available for the purposes of detecting current SARS-CoV-2 infection, including alternatives such as rapid molecular-based tests as well as rapid antigen detection tests (RADTs). The extent that these alternatives are being used internationally is unclear. The purpose of this chapter is to conduct a rapid review of guidance and international use of tests and testing methods for the detection of SARS-CoV-2.

3.2 Methods

This review was first undertaken on 15 September 2020, in line with HIQA's protocol for the rapid HTA. It was subsequently updated on 30 September 2020, before the rapid HTA was submitted as advice to the National Public Health Emergency Team (NPHET), and again on 19 October 2020, in advance of publication on the HIQA website.

The methods for the rapid review are detailed in protocol (see Appendix A). Briefly, a range of relevant international resources was identified as per the protocol. In the first instance, a database of related guidance from international organisations, ministries of health, public health agencies, and associated national governmental departments was collated. Information on the recommended primary test for the detection of SARS-CoV-2 was extracted from relevant guidance for each organisation and country (including information on the type, sample, and specimen). Information on any other tests that have been recommended for use or are currently in use in any country was also extracted. Where alternative tests were recommended, or were in use for the detection of acute SARS-CoV-2 infection, the following information was extracted:

- type of test
- location / specialty required
- setting(s)
- criteria for testing
- testing strategy

- quality assurance processes
- centralised reporting or data linkage
- out-of-pocket expenses.

3.3 Results

Seventy-four public health guidance documents related to testing for SARS-CoV-2 infection were identified from 18 countries (10 European, two North American, two Australasian, and the UK), including four guidance documents from international public health bodies (the World Health Organization [WHO] and the European Centre for Disease Prevention and Control [ECDC]). Information on testing methods from all countries identified in the protocol was obtained, with the exception of Austria, Iceland and Singapore. Sufficient information on testing in these countries could not be ascertained from the respective Ministries of Health or related governmental departments.

Guidance from the following national or international public health bodies, ministries of health and associated national governmental departments were included in this review:

International public health bodies

- WHO^(92, 93)
- ECDC^(94, 95)

United Kingdom

- England (Public Health England (PHE) and Department of Health and Social Care)^(90, 96-100)
- Scotland (Health Protection Scotland; Scottish Government)⁽¹⁰¹⁻¹⁰⁵⁾
- Northern Ireland (Public Health Agency)^(106, 107)
- Wales (Welsh Government)⁽¹⁰⁸⁻¹¹⁰⁾

Other European countries

- Belgium (Belgian Government)⁽¹¹¹⁾
- Denmark (Statens Serum Institute and Danish Health and Medicines Authority)⁽¹¹²⁻¹¹⁴⁾
- France (Ministry of Solidarity and Health and la Haute Autorité de Santé)⁽¹¹⁵⁻¹¹⁸⁾
- Germany (Federal Ministry of Health, Robert Koch-Institut (RKI))^(119, 120)
- Ireland (Health Protection Surveillance Centre, Health Service Executive, and Health Products Regulatory Authority) ^(6, 121, 122)

- Norway (Norwegian Institute of Public Health (NIPH))⁽¹²³⁻¹²⁷⁾
- Spain (Ministry of Health)^(128, 129)
- Sweden (The Public Health Agency of Sweden)⁽¹³⁰⁻¹³³⁾
- Switzerland (Federal Office of Public Health (FOPH))^(134, 135)
- The Netherlands (Ministry of Health, Wellbeing and Sports)⁽¹³⁶⁻¹³⁸⁾

North America

- Canada (Health Canada, Ministry of Health, Public Health Ontario, British Columbia Center for Disease Control)⁽¹³⁹⁻¹⁴⁴⁾
- United States (US) (Centers for Disease Control and Prevention (CDC))^(3, 145-147)

Australasia

- Australia (Department of Health and Therapeutic Goods Administration)⁽¹⁴⁸⁻¹⁵⁶⁾
- New Zealand (Ministry of Health).⁽¹⁵⁷⁻¹⁶¹⁾

The information summarised in this rapid review was correct on 19 October 2020, but is subject to change. A summary of the guidance on testing for SARS-CoV-2 is provided below. Table 3.1 provides an overview of international testing practices for the detection of SARS-CoV-2, while Table 3.2 provides examples of the international use of rapid tests for the detection of SARS-CoV-2. As illustrated in Chapter 2, for the purposes of this assessment, rapid tests describe both rapid antigen detection tests and rapid molecular tests. Table 3.3 summarises international guidance from the WHO and the CDC in the US on the use of rapid antigen tests.

3.3.1 Primary test for the detection of SARS-CoV-2

The WHO recommends that nucleic acid amplification tests (NAATs), such as rRT-PCR, should be used wherever possible to detect suspected, active SARS-CoV-2 infections.⁽⁹²⁾ The NAAT assay should target the SARS-CoV-2 genome and include regions on the RdRP, N and S genes. In addition some assays also detect the Sarbeco group (which includes SARS-CoV-1) E gene. Where possible, two independent targets should be used for optimal diagnostics. A single discriminatory target can be adopted in areas with widespread community transmission, but a strategy should be in place to monitor for mutations that might affect the assay's performance, although there is little evidence to suggest that this is currently an issue. In line with the WHO, the ECDC also recommends the use of NAATs for the detection of SARS-CoV-2.⁽⁹⁵⁾ A single discriminatory target is also recommended, but only in the event of severe shortages of reagents, such as primers. Both the WHO and ECDC suggest that SARS-CoV-2 antigen tests, which detect SARS-CoV-2 viral proteins, can also be used to detect current infection.

Across many of the included countries in the review, NAAT is routinely used as the primary test for the detection of SARS-CoV-2. Countries typically report using RT-PCR, rather than rRT-PCR; however, it is assumed the latter, which allows for the detection of PCR amplification during the early phases of the reaction, is being used since this is the gold standard. Some countries also report using antigen detection tests, such as England, Wales and the US. In Wales, antigen detection tests are reportedly available for all education and childcare workers, as well as critical workers that require testing for SARS-CoV-2.⁽¹¹⁰⁾ The extent that antigen tests are used in England and Wales is unclear as the term is used interchangeably with RT-PCR in some documents.⁽¹⁶²⁾

In Australia,⁽¹⁵³⁾ Canada,⁽¹⁴⁴⁾ Germany,⁽¹²⁰⁾ Spain⁽¹²⁸⁾ and the US,⁽¹⁴⁶⁾ rapid antigen detection tests (RADTs) can be used to detect SARS-CoV-2 in symptomatic individuals. A number of these countries have also approved the use of RADTs to detect SARS-CoV-2 in asymptomatic individuals in a limited number of scenarios. For instance, in Germany, RADTs can be used to detect SARS-CoV-2 in contacts of confirmed cases, but only in exceptional circumstances, such as when rRT-PCR capacity is limited. In Canada, RADTs can be used for the purposes of serial (that is, repeated) testing of workers in remote work areas to prevent introduction or minimise the chance of spread within a work site. It can also be used to test workers in high-risk settings such as those working in meat plants, long-term care facilities, and offshore/marine industries. In Australia, RADTs can be used for screening purposes, as determined by individual public health authorities, as a complement to, and not a replacement for, rRT-PCR testing.⁽¹⁵⁶⁾

In France, the national health body, la Haute Autorité de Santé (HAS), has indicated in a formal opinion that it is in favour of the use of RADTs in symptomatic individuals, but only within four days of symptom onset. Acknowledging the high specificity observed in clinical validation studies, it has noted that positive tests do not need to be confirmed by rRT-PCR. However, for symptomatic individuals over the age of 65 years and or those who have been identified as being at high risk of serious complications from COVID-19, negative tests must be confirmed with rRT-PCR.⁽¹¹⁷⁾ HAS has also approved use for asymptomatic individuals who are not close contacts in order to identify clusters. This specifically applies to testing of target populations, such as those who live, study, or work in confined places, where the risk of infection is greater than in the general population.⁽¹¹⁸⁾ Pending data from clinical validation studies, the HAS has specified that there is currently insufficient data to inform the use of RADTs for asymptomatic contacts who have been identified via contact tracing (individually or within a cluster); rRT-PCR should continue to be used in this scenario.

Table 3.1 International testing recommendations for the detection of SARS-CoV-2 as of 19 October 2020

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
INTERNATIONAL					
WHO	Nucleic acid amplification test (rRT-PCR)	<p>Upper: 2 individual swabs can be combined in 1 collection tube or a combined nasopharyngeal and oropharyngeal swab can be taken</p> <p>Lower (if collected later in the course of the COVID-19 disease or in patients with a negative URT sampling and there is a strong clinical suspicion of COVID-19): - sputum, if spontaneously produced. In patients with more severe respiratory disease: - endotracheal aspirate - bronchoalveolar lavage.</p> <p>Faecal specimens may be considered from the second week after symptom onset. In deceased persons, a post-mortem swab, needle biopsy or tissue specimens from the autopsy can be considered</p>	<p>If negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected. One specimen taken in the acute phase and one in the convalescent phase 2-4 weeks later can be used to look for seroconversion or a rise in antibody titres. These 2 samples can be used retrospectively to determine whether the individual has had COVID-19, especially when the infection could not be detected using NAAT.</p> <p>Genomic sequencing for SARS-CoV-2 can be used to investigate the dynamics of the outbreak, including changes in the size of an epidemic over time, its spatiotemporal spread, and testing hypotheses about transmission routes</p>	Pooling of samples from multiple individuals can be used to increase the diagnostic capacity for detecting SARS-CoV-2 when the rate of testing does not meet the demand in some settings	When performance is acceptable (i.e., when sensitivity is $\geq 80\%$ and specificity is $\geq 97\%$), antigen detecting rapid diagnostic tests could be implemented in a diagnostic algorithm to reduce the number of molecular tests that need to be performed and to support rapid identification and management of COVID-19 cases

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
ECDC	Nucleic acid amplification test (RT-PCR)	<p>Upper:</p> <ul style="list-style-type: none"> - nasopharyngeal swab - oropharyngeal swab - nasopharyngeal wash/aspirate. <p>Lower (if patient is hospitalised or in intensive care):</p> <ul style="list-style-type: none"> - bronchoalveolar - sputum - aspirate 	Serum (to be stored pending serology availability), acute and convalescent (possibly 2 to 4 weeks after acute phase), along with faeces	In the event of severe shortages of reagents and to speed up testing, ECDC recommends (1) pooling of low-risk samples from different individuals in one testing run (group testing); this can be used in prevalence studies or to enhance testing of mild or asymptomatic patients. This should not be used in cases where diagnosis is critical, due to the possibility of error. For diagnosis, the samples will need to be retested separately if there is a positive result in the pooled sample; (2) performing a sample preheating step, instead of RNA extraction	Rapid testing is not currently recommended; however, they could play a role within a testing algorithm with confirmatory testing provided when there is a substantial probability that a negative result is a false negative, for example. All positive results should also be confirmed by RT-PCR.
UNITED KINGDOM					
England	Viral (nucleic acid or antigen) test	<p>Upper:</p> <ul style="list-style-type: none"> - nose swab in 1 collection tube OR - single swab used for throat then nose in 1 collection tube OR - nasopharyngeal aspirate in a universal transport pot <p>Lower (if obtainable):</p> <ul style="list-style-type: none"> - sputum, in a universal container <p>Neck breathers:</p> <ul style="list-style-type: none"> -should have their stoma swabbed if it is accessible and not contraindicated 	Antibody tests are available to the public through the UK Antibody Testing Programme. Tests are prioritised to those living in England or Wales, aged 18 or over, working in a care home, cannot travel to an NHS test site to take the test	The NHS has developed a standard operating procedure for the use of pooling specimens from asymptomatic individuals, suggesting the strategy is being used in the country	PHE advises against the use of rapid POC tests in community pharmacies or at home; however, two new rapid tests (RT-LAMP (LAMPore) and RT-PCR (DnaNudge)) are being made available in select settings across the country

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
Northern Ireland	Nucleic acid amplification test (RT-PCR)	Upper: - nasopharyngeal and throat swab Lower (if obtainable): - sputum	Unclear. Northern Ireland is participating the UK-wide study, the COVID-19 Infection Survey (CIS), which will invite a sample of people living in around 13,000 households to complete a questionnaire, take a swab test for viral infection and, in 10-20% of households, 1 individual will also be asked to provide a blood sample for antibody testing. This study will follow up participants over 12 months.	Unclear	Unclear
Scotland	Nucleic acid amplification test (RT-PCR)	Upper: - nose swab in one collection tube OR - single swab used for throat then nose in one collection tube OR - nasopharyngeal aspirate in a universal transport pot Lower (if obtainable): - sputum, in a universal container Neck breathers: -should have their stoma swabbed if it is accessible and not contraindicated	Antibody tests are not currently being offered on demand to health and social care workers or NHS patients as it is not believed that the evidence supports such a measure in Scotland. However, antibody tests will be made available for clinicians to use in their management of NHS patients when clinically appropriate	Unclear	Rapid automated tests (such as the Cepheid SARS-CoV-2 assay) are recommended for use in laboratory settings as a confirmatory test to allow rapid clarification of results (e.g., in the event that only 1 virus region is positive following PCR test that detects multiple virus regions). A number of RADTs (LumiraDx) are being purchased for use in communities in Scotland, pending validation of the tests
Wales	Viral (nucleic acid or antigen) test	Upper: - 'two wet swab' sample from the nose and throat Lower: - 'single dry swab' taken from the back of the throat	Antibody testing is currently focused on the serosurveillance of defined target cohorts for the purpose of understanding the cumulative level of historical infection. National priority areas for antibody testing include school staff, healthcare workers and the social care sector	Unclear	Rapid tests are not currently recommended; however, the Welsh government is working towards introducing RT-LAMP (which can be used in labs and or the POC) pending validation of the test
EUROPE					

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
Belgium	Nucleic acid amplification test (RT-PCR)	Upper: - nasopharyngeal - oropharyngeal Lower: - bronchoalveolar lavage - endotracheal aspirates - expectorated sputum	Serological antibody tests are not currently recommended for use as they are subject to considerable scientific uncertainty and constraints. On a GP prescription, an antibody test is available for certain target groups if laboratory-approved test equipment is used. If testing is undertaken outside these target groups, a charge or fee is applied for the identification of the antibodies	Unclear	Not recommended
Denmark	Nucleic acid amplification test (RT-PCR)	Upper: - oropharyngeal Lower: - sputum, if the individual has signs of pneumonia	Antibody tests only available to those willing to take part in a random sampling survey of the prevalence of COVID-19 in the Danish population, or research project as part of government monitoring	Statens Serum Institute developed a new simplified workflow for molecular detection of SARS-CoV-2, without NA extraction, which could serve as an alternative in diagnostic laboratories to overcome chemical based kit-shortage. Heating of the oropharyngeal swabs for 5 min. at 98°C followed by cooling for 2 min. at 4°C prior to a SARS-CoV-2 RT-qPCR reaction would detect 97.4% of the COVID-19 positive patients with no false positives	Not recommended
France	Nucleic acid amplification test (RT-PCR)	Upper: - nasopharyngeal swab - salivary swab (symptomatic individuals) - oropharyngeal swab (asymptomatic individuals), if nasopharyngeal is contraindicated Lower: - unclear	Serological antibody tests are available and reimbursed under social security	Unclear	Following the approval by the HAS RADTs may soon be used for the diagnosis of COVID-19 in symptomatic patients, as well as asymptomatic individuals who are not close contacts, provided the device meets a minimum performance criteria of ≥80% sensitivity and ≥99% specificity (to ensure that positive cases are cases of COVID-19 and not other seasonal respiratory viruses)

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
Germany	Nucleic acid amplification test (RT-PCR) or rapid antigen test	Upper: - nasopharyngeal swab or lavage - oropharyngeal smear Lower (deep airway): - bronchoalveolar lavage - sputum (produced or induced) - tracheal secretions	Antibody testing outside of studies may be considered to clarify suspicions in patients under medical treatment with persistent symptoms of COVID-19 who cannot be detected by a throat swab	Unclear	RADTs may be used in exceptional cases in symptomatic persons (for example, when there is limited PCR capacity or when a test result must be returned quickly) and asymptomatic individuals in certain contexts
Ireland	Nucleic acid amplification test (RT-PCR)	Upper: - combined swab for Oropharyngeal and nasopharyngeal samples (1 swab to test both is sufficient) in ambulatory patients - nasal swabs are an acceptable specimen type for use in children in the community Lower: - bronchoalveolar lavage OR - endotracheal aspirate OR - sputum (if produced); preferred in cases of severe illness	Antibody testing was provided as part of a national study on the spread of COVID-19 in the country (SCOPI)	Unclear	Rapid molecular testing, using RT-PCR, has been widely deployed in hospital laboratory settings. Adoption of such tests has been contingent on completion of validation testing. Alternative molecular testing approaches and RADT have not as yet been deployed as part of the publicly funded national testing strategy.
Netherlands	Nucleic acid amplification test (RT-PCR)	Upper: - nasopharyngeal AND - oropharyngeal smears Lower (if possible): - bronchoalveolar lavage - sputum	Serology tests are only used for research purposes at a population level by selected laboratories to investigate if people are producing antibodies against COVID-19	The National Institute for Public Health and Environment (RIVM) is working with national and international laboratories to find different testing methods that require other laboratory materials. Besides RT-PCR tests, which detect the genetic material of the virus (RNA), other types of tests including tests that detect the presence of virus proteins (antigen tests) are being explored	Rapid RT-PCR tests are being used in laboratories for situations where rapid diagnosis is absolutely vital (e.g., if someone has to be treated in hospital for heart failure or organ transplant). Rapid tests are not recommended for use in any other situation, including in test lanes (or centres) for general COVID-19 diagnostics. However, research on the accuracy of rapid tests in the Netherlands is underway. A decision on the use of RADTs is expected, pending validation of the tests

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
Norway	Nucleic acid amplification test (RT-PCR)	<p>Upper (recommended):</p> <ul style="list-style-type: none"> - nasopharyngeal (deep) <p>Acceptable alternatives:</p> <ul style="list-style-type: none"> - nasopharyngeal AND oropharyngeal - nasopharyngeal - oropharyngeal <p>Lower (only relevant in specialist settings):</p> <ul style="list-style-type: none"> - bronchoalveolar lavage OR - tracheal aspirate 	Antibody tests should not be used as the only diagnostic test in a case of suspected current infection, but can supplement other diagnostics. Possible uses for serological laboratory analyses include, for example: clinical suspicion of COVID-19 and negative or inconclusive PCR, seroepidemiological studies, identification of blood donors as potential donors to convalescent plasma.	Unclear	Rapid RT-PCR tests can be used for diagnostic test purposes in hospitals or laboratories to support urgent diagnosis of COVID-19. RADTs are not currently recommended
Spain	Nucleic acid amplification test (RT-PCR), other molecular diagnostic technique, or rapid antigen test	<p>Upper:</p> <ul style="list-style-type: none"> - nasopharyngeal and oropharyngeal exudate (preferred) - nasopharyngeal exudate - saliva (exceptional circumstances) <p>Lower:</p> <ul style="list-style-type: none"> - bronchoalveolar lavage - aspirate - sputum (if possible) - endotracheal aspirate, especially in patients with severe respiratory disease 	<p>A serological test may be considered if 2 RT-PCR tests (48 hours apart) return as negative and there is a high clinical suspicion of COVID-19.</p> <p>Screening studies through serological tests may be carried out in vulnerable populations or those with greater exposure, such as health or social-health personnel or residents of nursing homes or other health centers, as determined by individual health authorities</p>	Pooling is reportedly being used for screening purposes to optimise RT-PCR resources	RADTs may be routinely used for the detection of SARS-CoV-2 in symptomatic patients
Sweden	Nucleic acid amplification test (RT-PCR)	<p>Upper:</p> <ul style="list-style-type: none"> - nasopharyngeal <p>Lower:</p> <ul style="list-style-type: none"> - bronchoalveolar lavage - sputum <p>Other (if relevant):</p> <ul style="list-style-type: none"> - serum and EDTA blood - urine and faeces 	Antibody testing is recommended where the results may be of value, such as in situations where RT-PCR returns a negative result and there is still a clinical suspicion of COVID-19. It may also be of value in high risk groups where the result may provide reassurance to patients that they have some protection against the virus. It may also be of value to workplaces so they can safely plan and allocate resources	Unclear	Not recommended

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
Switzerland	Nucleic acid amplification test (RT-PCR)	<p>Upper (outpatients):</p> <ul style="list-style-type: none"> - nasopharyngeal OR - oropharyngeal smear <p>Lower (if suspicion remains after negative RT-PCR test and condition permits):</p> <ul style="list-style-type: none"> - bronchoalveolar lavage - endotracheal aspirate 	<p>Serological tests are currently not recommended for routine use. A special situation in which serology may be justified includes in hospitalised patients: serology can be used as an additional diagnostic method if the PCR test is negative but the clinical picture and the radiological image indicate an infection (high pre-test probability). A positive serology in such a situation makes it possible to make the diagnosis of COVID-19 with a high probability and to take the appropriate measures (put in isolation, consider certain treatments, etc.)</p>	Unclear	RADTs or other POC tests have not yet been fully validated or monitored and are currently not suitable for individual diagnosis
NORTH AMERICA					

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
Canada	Nucleic acid amplification test (RT-PCR) or rapid antigen test	<p>Upper:</p> <ul style="list-style-type: none"> - nasopharyngeal (preferred) - combined swab of throat and both nares (when nasopharyngeal cannot be collected) - deep nasal swab <p>Acceptable alternatives:</p> <ul style="list-style-type: none"> - anterior nares swab (both sides) - oropharyngeal swab <p>Lower (in hospitalised patients with respiratory symptoms):</p> <ul style="list-style-type: none"> - bronchoalveolar lavage - sputum - bronch wash - pleural fluid - lung tissue - tracheal aspirate. <p>Public Health Ontario suggest saliva specimens are acceptable in cases where another specimen may not be possible/tolerated. In British Columbia, children may provide a mouth rinse and gargle sample</p>	<p>When clinically relevant, indeterminate results should be investigated further by testing for an alternate gene target using a validated real-time PCR or nucleic acid sequencing assay at the community, hospital or reference laboratory. Serological tests have not been authorised by Health Canada for diagnosis or self-testing. However, decision-making on testing methods is made by the health authorities of the different provinces.</p> <p>In British Columbia, serology tests are recommended for a limited number of clinical scenarios: (1) in patients who present with atypical clinical manifestations such as inflammatory syndromes (i.e., multisystem inflammatory syndrome in children (MIS-C)); (2) to help diagnose patients who are SARS-CoV-2 RNA negative, but present with a compatible syndrome, or who present later during their disease course; (3) case-by-case testing after consultation with a Clinical/Medical microbiologist; (4) at the direction of Medical Health Officers as part of public health investigations; (5) epidemiologic and research studies</p>	<p>To increase throughput and increase testing, Public Health Ontario recommends pooling specimens. A portion of three individual specimens are combined into a single pool and run on the SARS-CoV-2 PCR assay as a single test. Reflex testing is done based on the intermediate result of the pool, as follows: i) if the intermediate pool result is not detected, all three specimens are individually reported as not detected; ii) if the intermediate pool result is detected, indeterminate or invalid, each individual specimen is then tested individually and reported according to the result obtained for the individual specimen</p>	<p>Health Canada suggests that POCT can be implemented in situations where testing needs to be on-site and rapid, such as before admission to a high-risk congregate living setting. The allocation of limited POC resources to locations where such devices will have the most beneficial impact is recommended. Health Canada published interim guidance on the use of RADTs. The tests can be used for diagnostic and screening purposes.</p> <p>The Government of Canada has signed an agreement to purchase up to 7.9 million ID NOW rapid point-of-care tests and up to 3,800 analysers, pending Health Canada authorisation of the test</p>

Country / organisation	Primary test for the detection of SARS-CoV-2	Sample / specimen	Additional tests for SARS-CoV-2	Use of alternative approaches to testing, such as pooling	Use of rapid or near-patient (POC) tests for the detection of SARS-CoV-2
United States	Viral (nucleic acid or antigen) test	<p>Upper:</p> <ul style="list-style-type: none"> - nasopharyngeal swab - oropharyngeal swabs - nasal mid-turbinate (NMT) swab (using a flocked tapered swab) - anterior nares specimen (using a flocked or spun polyester swab) - nasopharyngeal wash/aspirate or nasal wash/aspirate - saliva <p>Lower (if available):</p> <ul style="list-style-type: none"> - sputum - aspirate OR bronchoalveolar lavage, when it is clinically indicated (e.g., those receiving invasive mechanical ventilation) 	<p>Does not currently recommend using antibody testing for diagnosis of any infection. Antibody tests may be used in conjunction with viral detection tests to support clinical assessment of persons who present late in their illnesses.</p> <p>CDC has launched a national viral genomics consortium to better map SARS-CoV-2 transmission</p>	CDC suggests pooling can be used to expand diagnostic or screening capacity but only in areas or situations where the number of positive test results is expected to be low (e.g., in areas with a low prevalence of SARS-CoV-2 infections)	<p>The FDA has granted EUA for six rapid antigen tests (CareStart COVID-19, Sofia 2 Flu + SARS Antigen FIA, BinaxNOW, LumiraDx, BD Veritor System, Sofia SARS Antigen FIA) that can identify SARS-CoV-2, as well as a wide range of rapid molecular tests.</p> <p>The US government has agreed to purchase 150 million RADTs (BinaxNOW; Abbott) worth \$760 million to expand strategic, evidence-based testing. Tests may be deployed to schools and to assist with serving other special needs populations</p>
AUSTRALASIA					
Australia	Nucleic acid amplification test (rRT-PCR) or rapid antigen test	<p>Upper:</p> <ul style="list-style-type: none"> - oropharyngeal and bilateral deep nasal swab - nasal wash/aspirate - nasopharyngeal swab <p>Lower (recommended where possible):</p> <ul style="list-style-type: none"> - bronchoalveolar lavage, tracheal aspirate, pleural fluid - sputum <p>Saliva</p>	<p>Serum: should be collected during the acute phase of the illness (preferably within the first 7 days of symptom onset). It should be stored and tested in parallel with convalescent sera collected 2 or more weeks after the onset of illness.</p> <p>The (CDGN), in collaboration with industry partners, will soon start sequencing the virus genomes of all positive COVID-19 tests and track the spread of the virus across the country</p>	Pooling was explored by The Victorian Infectious Diseases Reference Laboratory in Australia to investigate its value in increasing testing capacity. The study found pooling is a viable strategy for high-throughput testing of SARS-CoV-2 in low-prevalence settings only	POC or near-POC assays are recommended for use and can be performed on a bench without employing a biosafety cabinet, when the local risk assessment so dictates and proper precautions are in place. POCT devices include rapid RT-PCR (such as GeneXpert Xpress SARS-CoV-2 test) and RADTs (such as NowCheck COVID-19 Antigen Test), which can be used in the diagnosis of SARS-CoV-2 in symptomatic patients, and may play a role in screening asymptomatic individuals as determined by individual public health authorities
New Zealand	Nucleic acid amplification test (RT-PCR)	<p>Upper:</p> <ul style="list-style-type: none"> - nasopharyngeal and oropharyngeal swab <p>Lower (preferred):</p> <ul style="list-style-type: none"> - bronchoalveolar lavage - sputum - endotracheal excretions 	Whole genome sequencing is used to investigate outbreaks	Pooling is reportedly being used to test asymptomatic individuals during outbreaks	The importation of all rapid or POC COVID-19 test kits has been banned in New Zealand since 22 April 2020. There are currently no rapid or POC COVID-19 test kits that can be sold or supplied in New Zealand

Key: CDC – Centers for Disease Control and Prevention; CDGN – Communicable Disease Genomics Network; COVID-19 – coronavirus disease 2019; ECDC – European Centre for Disease Prevention and Control; EUA – Emergency Use Authorization; HAS - la Haute Autorité de Santé; LAMP - loop-mediated isothermal amplification; NHS – National Health Service; POC – point-of-care; RKI – Robert Koch Institute; RNA – Ribonucleic acid; rRT-PCR – real-time reverse transcription polymerase chain reaction; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; WHO – World Health Organization.

3.3.2 Sample / specimen

The recommended approach to testing is broadly consistent across countries. A sample from the upper and or lower respiratory tract is taken using a nasopharyngeal or oropharyngeal specimen (upper respiratory tract), often using the same swab, and or endotracheal, bronchoalveolar or sputum specimen (lower respiratory tract). A lower respiratory tract is often only recommended if a patient is severely ill or hospitalised with evidence of lower respiratory tract infection. The CDC in the US recently updated its list of acceptable specimens for testing to include saliva specimens (8 October 2020).⁽¹⁶³⁾ In France, saliva specimens can be used to test for SARS-CoV-2 in symptomatic patients.⁽¹¹⁶⁾ Salivary specimens may also be used in exceptional circumstances in Spain; for example, when other sample sites are contraindicated.⁽¹²⁸⁾ Saliva specimens may be routinely used in Australia.⁽¹⁵³⁾ In Canada, Public Health Ontario suggests that saliva specimens are acceptable in cases where other specimens may not be possible or tolerated.⁽¹⁶⁴⁾ In British Columbia, children may provide a mouth rinse and gargle sample.⁽¹⁴¹⁾ Nasal swabs are now an acceptable specimen type for use in children in the community in Ireland.⁽¹⁶⁵⁾ No other country has yet appeared to adopt this approach. In England⁽⁹⁸⁾ and Scotland,⁽¹⁰⁵⁾ individuals with a tracheostomy should have their stoma swabbed if it is accessible and not contraindicated.

Faecal specimens are rarely recommended. The WHO suggests that these can be considered from the second week after symptom onset in cases where upper and lower respiratory tract specimens are negative and there is still a clinical suspicion of a COVID-19 infection. In deceased persons, the WHO also suggests that a post-mortem swab, needle biopsy or tissue specimen from the autopsy may be considered.⁽⁹²⁾

3.3.3 Additional tests for the detection of SARS-CoV-2

3.3.3.1 Serology

In each of the included countries, antibody or serological testing has been broadly focused on the serosurveillance of defined target cohorts, or populations, for the purpose of understanding the spread of the disease and potential level of immunity in each country. However, serological testing has been recommended by a number of public health bodies for a limited number of clinical scenarios for the purpose of diagnosing current infection. For instance, the WHO and ECDC suggest that if negative NAAT results are obtained from a patient in whom SARS-CoV-2 infection is strongly suspected, a paired serum specimen could be collected.^(92, 95) The first sample should be taken during the acute phase of illness, with the second sample taken in the convalescent phase (two to four weeks later). The samples can be used

to investigate whether an individual has had COVID-19 by testing for antibodies, especially when the infection could not be detected using NAAT. In Spain, a serological test may be considered if two RT-PCR tests taken 48 hours apart return as negative and there is a high clinical suspicion of COVID-19.⁽¹²⁸⁾ In Sweden, antibody testing is recommended where the results may be of value, such as in situations where RT-PCR returns a negative result and there is still a clinical suspicion of COVID-19.⁽¹³⁰⁾ It may also be of value in high-risk groups where the result may provide reassurance to patients that they have some protection against the virus. Additionally, they note it may be of value to work places, so they can safely plan and allocate resources. In Switzerland, a serology test may be used as an additional diagnostic method in hospitalised patients if the RT-PCR test is negative, but the clinical picture and radiological image indicate an infection.⁽¹³⁴⁾ The guidance documents note that a positive serology in such a situation makes it possible to make the diagnosis of COVID-19 with a high probability and to put in place relevant measures, such as isolation. In the US, antibody tests may be used in conjunction with viral detection tests to support clinical assessment of persons who present late in their illnesses.⁽¹⁴⁵⁾

Historically, serum antibody-based investigations of respiratory infection are compromised as the infection occurs in the respiratory tract and there is a locally derived mucosal immune response. Therefore, using a serum sample to detect an antibody response is not optimal, especially in those with mild symptoms. It has been observed that anti-SARS-CoV-2 is detectable more frequently in those patients with more severe systemic infection. It is possible that individuals who had milder disease or were asymptomatic are less likely to develop a detectable antibody response in serum. Therefore, some previous mild infections may not have been detected. Even among those with clearly symptomatic infection, a small percentage do not have serological evidence for SARS-CoV-2 infection. Emerging evidence suggests a variable antibody response to SARS-CoV-2 both in level of antibody produced and to which viral protein target, nucleoprotein or spike, the antibodies are directed.

3.3.3.2 *Genome sequencing*

None of the included countries appear to be using whole genome sequencing (WGS) for the purposes of primary detection of SARS-CoV-2 infection. However, the method is being used in some countries to investigate outbreaks and study routes of transmission, as well as host response and evolution of the virus. The WHO recommends that genomic sequencing for SARS-CoV-2 can be used to investigate the dynamics of an outbreak, including changes in the size of an epidemic over time, its spatiotemporal spread, and testing hypotheses about transmission routes.⁽⁹²⁾

A number of national consortiums have been created to study the spread of SARS-CoV-2. In the US, the CDC has launched a national viral genomics consortium to better map SARS-CoV-2 transmission.⁽¹⁶⁶⁾ The national genomics consortium will:

- monitor important genetic changes in the virus
- support contact tracing
- aid in identifying diagnostic and therapeutic targets
- advance public health research in the areas of transmission dynamics, host response and evolution of the virus.

A similar consortium has been established in the UK, called COVID-19 Genomics UK Consortium (COG-UK).⁽¹⁶⁷⁾ The consortium is made up of a partnership of National Health Service (NHS) organisations, the four Public Health Agencies of the UK, the Wellcome Sanger Institute and over 12 academic partners. The goal of the COG-UK Consortium is to deliver large-scale SARS-CoV-2 genome sequencing capacity to hospitals, regional NHS centres and the government, as well as to:

- investigate outbreaks
- study genetic changes in the virus, as well as changes in severity
- target the development of treatments and vaccines.

In Ireland, the Irish Coronavirus Sequencing Consortium, funded by Science Foundation Ireland, has commenced sequencing 5,000 virus samples that were collected prior to 25 June 2020. The sequences and limited pseudonymised patient metadata will be deposited in open access genome data repositories to allow researchers to analyse the data for purposes including epidemiological or virus mutation studies. The completion date for the project is the end of October 2020.⁽¹⁶⁸⁾

In New Zealand, genome sequencing is being used to understand how the virus is spreading in the community and how outbreaks are occurring.⁽¹⁶⁹⁾ In Australia, the Communicable Disease Genomics Network (CDGN), in collaboration with industry partners, will soon start sequencing the virus genomes of all positive COVID-19 tests to track the spread of the virus across the country, rather than state by state.⁽¹⁷⁰⁾ The purpose of the initiative is to aid investigation of outbreaks; identify mutations of COVID-19 in the Australian population; and identify persistent infection versus reinfection or incurrent infection.

3.3.4 Use of alternative approaches to testing

3.3.4.1 Pooling

The WHO recommends that pooling of samples from multiple individuals can be used to increase the diagnostic capacity for detecting SARS-CoV-2 RNA, as well as

reduce reagent use, when the rate of testing does not meet the demand in some settings. The ECDC also suggests using this approach when the expected proportion of positive samples is very small (up to 5%), as a means to speed up testing and save resources.⁽¹⁷¹⁾ The ECDC has developed a methodology for estimating the point prevalence of SARS-CoV-2 infection from the results of a pooled RT-PCR test.⁽¹⁷²⁾

In the US, the CDC recommend pooling samples to expand diagnostic or screening capacity, but only in areas or situations where the number of positive test results is expected to be low (for example, in areas with a low prevalence of SARS-CoV-2 infections).⁽³⁾

The extent that pooling of specimens is undertaken in any of the included countries in this rapid review is somewhat unclear. While Health Canada has yet to provide guidance on pooling, the approach has been recommended by Public Health Ontario.⁽¹⁶⁴⁾ A portion of three individual specimens can be combined into a single pool, with reflex testing undertaken based on the intermediate result of the pool. The public health body recommends that if the intermediate pool result is not detected, all three specimens should be individually reported as not detected. However, if the intermediate pool result is detected, indeterminate or invalid, each individual specimen must be tested individually and reported according to the result obtained for the individual specimen.⁽¹⁶⁴⁾ In Australia, the approach was explored by the Victorian Infectious Diseases Reference Laboratory (VIDRL) to investigate its value in increasing testing capacity. The study found pooling is a viable strategy for high-throughput testing of SARS-CoV-2 in low-prevalence settings only.⁽¹⁷³⁾ Whether it is being widely used in Australia, however, is unclear. In England, the NHS has developed a standard operating procedure for the use of pooling specimens from asymptomatic populations (usually between six to 12 people), suggesting the strategy is being used to test for SARS-CoV-2 in the country.⁽¹⁷⁴⁾ It also reportedly played a significant role in testing asymptomatic individuals following the emergence of new cases in New Zealand in August 2020.⁽¹⁷⁵⁾

There was no evidence that any other alternative approach to testing was being used in any of the other countries included in this rapid review.

3.3.5 Use of rapid or near-patient (point-of-care) testing

The review of international practice identified inconsistencies in the wording around rapid and or near-patient testing. As discussed in Section 2.1, rapid tests are officially defined within the European Commission In-Vitro Diagnostic Medical Devices Directive as 'qualitative or semi-quantitative devices, used singly or in a small series, which involve non-automated procedures and have been designed to give a fast result', compared with current gold standard diagnostic test. They include

rapid antigen detection tests (RADTs) and rapid molecular tests. These are considered suitable for deployment in near-patient (point-of-care) settings, including for example clinical settings, or community or 'pop-up' testing centres. However, it is also possible to deploy rapid molecular tests (including simplified RT-PCR-based rapid tests) in, for example, hospital laboratories to support timely diagnosis in hospital settings. Where deployed in hospital laboratories, it is assumed that they are operated by trained laboratory staff under usual laboratory governance and quality assurance procedures.

From the review, it would appear that a number of the included countries have yet to recommend the use of rapid or near-patient tests for the detection of SARS-CoV-2, including in Belgium, Denmark, New Zealand, Sweden, Switzerland, and Wales. However, it is unclear if this relates to all rapid tests, irrespective of where they are used, or specifically to the use of these tests in near-patient (non-laboratory) settings. In Norway, rapid or near-patient tests are being used in some emergency departments and hospital-based laboratories to support urgent diagnosis of COVID-19 in these settings.⁽¹²⁷⁾ A similar use of rapid tests, namely laboratory-based rapid RT-PCR tests, is happening in Irish hospitals to support the clinical diagnosis of COVID-19 in these settings (personal correspondence). Adoption of such tests was contingent on satisfactory results from validation testing. A pilot scheme using a rapid test based on simplified RT-PCR has also been provided in Ireland as part of a scheme for vulnerable individuals (for example, people experiencing homelessness). As yet, near-patient testing has not otherwise been adopted as part of the Irish national Test and Trace programme. In New Zealand, the importation of rapid or near-patient COVID-19 test kits has been banned since 22 April 2020. There are currently no rapid or near-patient COVID-19 tests that can be sold or supplied in the country.⁽¹⁵⁸⁾

A number of public health agencies have recommended using rapid tests, but in a limited number of clinical scenarios. In Scotland, rapid automated tests (such as GeneXpert) are recommended for use in laboratory settings as a confirmatory test to allow timely clarification of results. For example, in the event that only one virus region is detected following an RT-PCR test that detects multiple virus genes, a rapid test is used to confirm diagnosis.⁽¹⁰⁵⁾ In Canada, near-patient testing can be implemented in situations where testing needs to be on site and rapid, such as before admission to a high-risk congregated living setting. Health Canada has recommended that near-patient devices should be allocated to locations where rapid testing is likely to have the most beneficial impact.⁽¹⁴⁰⁾ The government has signed an agreement to purchase up to 7.9 million rapid near-patient tests and up to 3,800 analysers (ID NOW; Abbott).⁽¹⁷⁶⁾ In Australia, near-patient devices are recommended for use and can be performed on a bench without employing a biosafety cabinet,

when the local risk assessment so dictates and proper precautions are in place.⁽¹⁵³⁾ A number of rural and remote communities in Australia have been given access to rapid RT-PCR tests for SARS-CoV-2 (using GeneXpert machines).

Although Public Health England has yet to update its guidance on the use of rapid or near-patient tests (previously it advised against their use in community pharmacies or at home),⁽⁹⁷⁾ two new rapid molecular tests (LAMPore and DnaNudge) are being made available in select settings in England.⁽⁹⁰⁾ LAMPore is a LAMP assay with combined nanopore analysis, while DnaNudge (or CovidNudge) is a portable, non-laboratory based rapid RT-PCR platform. Currently, the rapid RT-PCR device is operating in the cancer wards of eight London hospitals, while a further 5,000 platforms are due to be made available across NHS hospitals in the coming months. LAMPore is also expected to be made available across adult care settings, NHS laboratories and other (lighthouse) laboratories. The platform is currently being evaluated in community settings in Salford, England.⁽¹⁷⁷⁾

In Scotland, a number of RADTs (LumiraDx) are also being purchased for use in communities, pending successful validation of the tests.⁽¹⁰¹⁾ Scotland has reportedly agreed to purchase 300 devices upon validation of the test. In Wales, rapid tests are not currently recommended; however, the Welsh government is working towards introducing RT-LAMP, which can be used in laboratories and or in near-patient settings (such as care homes, airports, or accident and emergency departments), pending validation of the test.⁽¹⁰⁸⁾

In the US, The Food and Drug Administration (FDA) has granted emergency use authorisation (EUA) for four RADTs (BinaxNOW, LumiraDx, BD Veritor System, Sofia SARS Antigen FIA) that can identify SARS-CoV-2 antigen, as well as a wide range of rapid molecular tests.⁽¹⁷⁸⁾ The US government has also reportedly agreed to purchase 150 million rapid antigen tests (BinaxNOW; Abbott) worth \$760 million.⁽¹⁷⁹⁾

The tests are currently authorised for use in the following locations:

- laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 that meet requirements to perform high complexity tests;
- laboratories certified under the CLIA that meet requirements to perform moderate complexity tests;
- patient care settings operating under a CLIA Certificate of Waiver.

Examples of potential uses for point-of-care instruments, such as rapid antigen tests, for COVID-19 diagnostic purposes include:⁽¹⁸⁰⁾

- deployment to rural hospitals or other critical care sites that lack widely available testing

- use at public health department testing sites that perform CLIA-waived testing for other purposes
- deployment to long-term care facilities or correctional institutions
- rapid deployment to aid in the investigation of a newly identified case cluster
- placement in laboratories to test high-priority specimens that require a rapid result.

Further details on the settings that these tests are being deployed in is provided in Table 3.2 for each of the included countries, as well as the criteria and strategies that are being used for testing. For example, in Spain, the results of RADTs are presumed to be accurate if the test is used at the point of care (for example, in primary or hospital emergency care) and patients have been symptomatic for five or fewer days and do not need to be hospitalised.⁽¹²⁸⁾ This is because the estimated prevalence of infection in these settings may be between 10% and 30%, and the negative predictive value may be between 97.2% and 99.3%, while the positive predictive value may be between 94.5% and 98.5%.⁽¹²⁸⁾ In settings where the prevalence of infection may be as high as 50% and patients require hospitalisation, such as in nursing homes, negative test results should be confirmed by RT-PCR since the negative predictive value of the rapid antigen test falls to 93.7% in this scenario, while the positive predictive value rises to 99.4%, due to a higher rate of false negatives.⁽¹²⁸⁾ Similar recommendations have been issued by the WHO⁽⁹³⁾ and CDC,⁽¹⁴⁶⁾ as detailed in Section 3.3.5.

With the exception of the US (which is detailed below in section 3.3.5), the review found no information on how rapid or near-patient tests are being quality assured or whether a centralised data reporting system is being used. There was also no evidence that patients were being charged for the use of rapid or near-patient tests in any of the countries using these tests. In France, the HAS has indicated that RADTs should be covered by health insurance.⁽¹¹⁷⁾

While a number of countries have started to use rapid or near-patient tests, few public health bodies have issued guidance on the appropriate or effective use of rapid testing. Both the CDC⁽¹⁴⁶⁾ in the US and the WHO,⁽¹⁰⁸⁾ have published detailed guidance on the potential role of RADTs for the detection of SARS-CoV-2. A summary of the guidance is provided in Section 3.3.5; Table 3.3 presents an overview of the guidance.

Table 3.2 Examples of international use of rapid tests for the detection of SARS-CoV-2

Country	Test characteristics	Setting(s) deployed	Criteria for testing (e.g., prioritising of vulnerable groups)	Testing strategy (in the event of positive/negative result)
UNITED KINGDOM				
England	Rapid RT-LAMP (LAMPore) - Sample: swab and saliva - Location: laboratory - Skill: skilled lab technician Rapid RT-PCR (DnaNudge) - Sample: nasopharyngeal swab - Location: POC (non-laboratory) - Skill: trained personnel	LAMPore: soon to be made available in adult care settings, NHS labs and lighthouse labs DnaNudge: currently operating in the cancer wards of 8 London hospitals; soon to be made available across NHS hospitals, care homes and labs	Not reported	Not reported
Scotland	Rapid RT-PCR (e.g., GeneXpert) - Sample: not reported - Location: laboratory-based - Skill: skilled lab technician	Hospital laboratories	If one virus region is positive following RT-PCR which detects multiple virus regions, confirmatory testing should be carried out by a rapid test using an alternative assay of similar analytical sensitivity	If repeat test is positive in any target, the result is reported as positive. If repeat test is negative, result is reported as negative. Repeat testing is acceptable if there's a high clinical suspicion of current infection
EUROPE				
Germany	Rapid antigen test - Sample: unclear - Location: Laboratory or POC (non-laboratory) - Skill: trained medical personnel	POC settings include hospitals, rehabilitation facilities, inpatient care facilities, facilities for people with disabilities, facilities for outpatient operations, dialysis centers ambulant care	Symptomatic persons, as well as asymptomatic persons (with criteria of exposure or disposition)	A positive antigen test must always be confirmed by a PCR test
Netherlands	Rapid RT-PCR - Sample: unclear - Location: laboratory - Skill: unclear	Hospital laboratories	Patients who require a rapid diagnosis and emergency intervention due to heart failure or organ transplant, for example	Not reported
Norway	Rapid RT-PCR - Sample: unclear - Location: unclear - Skill: unclear	Hospitals (such as in emergency departments) and laboratories	To support rapid diagnosis of COVID-19	Not reported
Spain	Rapid antigen test - Sample: nasopharyngeal swab - Location: POC (non-laboratory) - Skill: trained personnel	POC (e.g., primary care ambulatory emergency services, hospital emergencies; in people without hospitalisation criteria) Health facilities (in people with hospitalisation criteria including health workers and people hospitalized for other causes)	Not reported	POC: If symptomatic for ≤5 days: - If the result is positive, the diagnosis will be confirmed - If the result is negative, current infection is considered ruled out* Health facilities and social health centres: Negative test results should be confirmed by RT-PCR if symptomatic for > 5 days and clinical suspicion remains ¥

Country	Test characteristics	Setting(s) deployed	Criteria for testing (e.g., prioritising of vulnerable groups)	Testing strategy (in the event of positive/negative result)
		Social health centres (elderly care homes including correctional institutions)		[Social health centres: RT-PCR is preferred if waiting time is <24 hours]
NORTH AMERICA				
Canada	Rapid RT-PCR / antigen detection tests - Sample: variable - Location: unclear - Skill: trained healthcare provider	POCT should be considered in the following settings: remote, rural, isolated and/or Indigenous communities, and situations where testing needs to be onsite and rapid such as before admission to a high-risk congregate living setting For the purposes of screening, RADTs may be deployed in high-risk work settings (e.g., meat processing plants, long-term care facilities, etc.), as well as correctional facilities	Health Canada hasn't specified any criteria for testing; however, Saskatchewan Health Authority prioritised POCT devices for certain groups, such as those living in remote/isolated communities with a declared outbreak; long-term care residents or staff in an outbreak investigation; patients for whom an immediate result is needed to determine triaging; among other groups that were requested by the Medical Health Officer. Public-facing service workers such as Mounted Police, firefighters, and food stores were also prioritised Health Canada identified a number of scenarios in which RADTs can be used including testing symptomatic individuals within five days of symptom onset; serial testing of asymptomatic individuals; prospective testing of asymptomatic individuals; and outbreak investigations	All positive RADTs must be confirmed by a PCR test. Confirmatory PCR testing is also recommended following negative RADTs if the pre-test probability is high
United States	Rapid molecular and antigen tests - Sample: variable - Location: laboratories certified under the CLIA that meet requirements to perform high or moderate complexity tests; and patient care (i.e., POC) settings operating under a CLIA Certificate of Waiver - Skill: depends on test setting	Examples of potential settings for deployment of POC instruments include: - Rural hospitals or other critical care sites that lack widely available testing - Public health department testing sites that perform CLIA-waived testing for other purposes - Long-term care facilities or correctional institutions - To aid in the investigation of a newly identified case cluster - Laboratories to test high-priority specimens that require a rapid result	Not reported	When used for diagnostic testing purposes, CDC recommends confirming negative antigen test results with an RT-PCR test when the pretest probability is relatively high, especially if the patient is symptomatic or has a known exposure to a person confirmed to have COVID-19 When used for screening testing in congregate settings, test results for SARS-CoV-2 should be considered presumptive. Confirmatory testing following a positive antigen test may not be necessary when the pretest probability is high, especially if the person is symptomatic or has a known exposure. When the pretest probability is low, those persons who receive a positive antigen test should isolate until they can be confirmed by RT-PCR
AUSTRALASIA				

Country	Test characteristics	Setting(s) deployed	Criteria for testing (e.g., prioritising of vulnerable groups)	Testing strategy (in the event of positive/negative result)
Australia	<p>Rapid RT-PCR / antigen detection tests</p> <ul style="list-style-type: none"> - Sample: nasopharyngeal swab - Location: POC (non-laboratory) - Skill: medical practitioner or suitably qualified person 	<p>Rapid RT-PCR: Some rural and remote communities were initially given access to rapid RT-PCR tests for SARS-CoV2 using GeneXpert machines.</p> <p>Rapid antigen tests may be used for diagnostic and screening purposes in certain contexts and settings, as determined by individual public health authorities, however, the RADTs must be considered as a complement to, and not a replacement for, RT-PCR tests</p>	<p>Rapid RT-PCR: A number of groups were prioritised:</p> <ul style="list-style-type: none"> - Individuals meeting the suspect or probable case definition for COVID-19 - Aboriginal and Torres Strait Islander people who meet the criteria for enhanced testing (e.g., respiratory symptoms, or unexplained fever or history of fever) and where delays in testing or longer periods of self-isolation places others at higher risk of transmission (e.g., due to overcrowded or inadequate housing). Within this category, the following could be considered as priorities: individuals who are likely to leave the community prior to a result becoming available, or have or have had multiple contacts, or major barriers to self-isolation - Non-Aboriginal and Torres Strait Islander residents and visitors to Aboriginal and Torres Strait Islander communities, who meet criteria for enhanced testing and have had direct contact with community members in the 14 days prior to onset of illness (that may have resulted in disease transmission), or will need to have direct contact with community members as part of their role, where waiting for a conventional PCR test 	Not reported

* Provided the prevalence of infection is between 10 and 30% (as may be the case in symptomatic individuals in these care settings), when the test is done in the first 5 days and the NPV is between 97.2% and 99.3%, and the PPV is between 94.5% and 98.5%

‡ In a population with a 50% prevalence of infection, the NPV falls to 93.7%, while the PPV rises to 99.4%

Key: COVID-19 – coronavirus disease 2019; NPV and PPV – negative and positive predictive value, respectively; POCT – point-of-care-testing; RT-PCR – reverse transcription polymerase chain reaction; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; WHO – World Health Organization.

3.3.6 International guidance on rapid antigen tests

On 11 September 2020, the WHO updated its recommendations on the use of RADTs (previously, the public health body had recommended against using any type of near-patient test until the evidence on their clinical performance was sufficient to justify their use in the context of COVID-19),⁽¹⁸¹⁾ and indicated that these types of tests could play a significant role in guiding patient management, public health decision-making and surveillance of SARS-CoV-2.⁽⁹³⁾ However, these current recommendations specify that RADTs must meet a recommended minimum performance requirement of $\geq 80\%$ sensitivity and $\geq 97\%$ specificity compared with a NAAT reference assay and should only be considered when NAAT is unavailable or where prolonged turnaround times preclude clinical utility. To ensure optimal performance of the RADT, the WHO recommends that testing should be conducted within the first five to seven days of symptom onset.⁽⁹³⁾

Following EUA of RADTs from the FDA in the US, the CDC issued guidance to support the effective use of these tests in different testing situations on 29 August 2020 (updated on 4 September 2020).⁽¹⁴⁶⁾ The rapid antigen tests are authorised for diagnostic testing within the first five to seven days of symptomatic individuals or individuals with known or suspected exposure to COVID-19.

3.3.6.1 *Setting(s) / scenarios for use*

The WHO outline five scenarios in which RADTs could be used. These include:

1. responding to suspected outbreaks in remote settings, institutions and semi-closed communities where NAAT is unavailable
2. supporting outbreak investigations (for example, in closed or semi-closed groups including schools, care homes, cruise ships, prisons, workplaces and dormitories, and so on)
3. monitoring trends in disease incidence in communities, and particularly among essential workers and health workers during outbreaks or in regions of widespread community transmission
4. early detection and isolation of positive cases in health facilities, COVID-19 testing centres or sites, care homes, prisons, schools, front-line and healthcare workers, and for contact tracing, when there is widespread community transmission
5. testing of asymptomatic contacts of cases.⁽⁹³⁾

The CDC recommends using RADTs for the purposes of diagnostic testing during the early stages of infection when the viral load is highest, or when a person has a known exposure to a confirmed case.⁽¹⁴⁶⁾ The CDC also recommends using RADTs for screening purposes in high-risk settings in which repeat testing could quickly

identify persons with a SARS-CoV-2 infection. Since RADTs have not been authorised for use in asymptomatic persons in the US, the CDC note that there are limited data to support their use in this group for diagnostic purposes.

Both the WHO and CDC outline criteria for interpreting RADT results in the different testing situations or scenarios.

3.3.6.2 *Criteria for interpreting results*

The results of RADTs must be interpreted within the epidemiological (for example, local prevalence of disease) and clinical (for example, presentation of symptoms or lack thereof) context that the test is conducted. This pre-test probability (the likelihood that the patient has COVID-19 before their results are known, based on epidemiological and clinical factors) will determine the positive or negative predictive values of the test. Both the CDC and WHO recommend that the pre-test probability should be determined by the test operator in advance of conducting the test.⁽⁹³⁾ The CDC suggest that prevalence should be estimated using a rolling average of the positivity rate over the previous seven to 10 days.⁽¹⁴⁶⁾ Where a specific site, such as a care home, has a positivity rate near zero, the prevalence of COVID-19 in the community (for example, cases per population) should be used to determine the pre-test probability. The pre-test probability of COVID-19 disease is typically high and positive test results have a high predictive value when there is widespread community transmission.⁽¹⁴⁶⁾ Similarly, the negative predictive value may be low in a setting of community transmission, even when there are strong indicators (epidemiological or clinical) of COVID-19 exposure or disease.

3.3.6.3 *Testing strategy*

The appropriate testing strategy typically depends on the scenario and or pre-test probability, as illustrated in Table 3.3. The WHO provides guidance for each of the five scenarios that are considered appropriate for rapid antigen testing, while the CDC provides guidance on testing for the purposes of diagnosis versus screening.

When responding to outbreaks in remote settings, institutions and semi-closed communities where NAAT is not immediately available, the WHO recommends that positive RADT results can be presumed to reflect an outbreak.⁽⁹³⁾ However, where possible, all positive test results, or at least a subset, should be confirmed by NAAT. Positive test results can also be presumed to reflect positive cases when rapid antigen testing is used to screen at-risk individuals during a NAAT-confirmed outbreak; confirmatory testing of positive samples is not necessary (due to the high pre-test probability). In this situation, test-negative individuals should be prioritised for sample collection for NAAT confirmation.⁽⁹³⁾ The CDC similarly recommends confirming negative test results when the pre-test probability is high, especially if the

patient is symptomatic or has a known exposure to a person confirmed to have COVID-19; conversely, negative test results can be considered presumptive when the pre-test probability is low (both for diagnostic and screening purposes).⁽¹⁴⁶⁾

When monitoring trends in disease incidence or attempting to quickly detect and isolate cases when there is widespread community transmission, the WHO suggests that the positive and negative predictive value of a RADT result should be used to enable effective infection control, or pre-test probability; that is, when the pre-test probability is high (due to a high prevalence of disease, for example), a negative test-result cannot completely exclude an active COVID-19 infection (low predictive value).⁽⁹³⁾ As a consequence, repeat rapid antigen testing, or preferably confirmatory testing, is recommended within 48 hours (see sample flowchart, adapted from the WHO guidance, in Figure 3.1). The same advice is provided by the CDC in this situation due to the increased likelihood of false negatives.⁽¹⁴⁶⁾

Finally, when testing asymptomatic contacts of cases, the WHO advises that a negative test result cannot be presumed to reflect an absence of infection. Therefore, test-negative individuals should not be removed from quarantine requirements since asymptomatic cases have been shown to have similar viral loads to symptomatic cases.⁽⁹³⁾

The WHO identify a number of scenarios where RADTs should not be used for different reasons.⁽⁹³⁾ These include:

- airport or border screening at points of entry, since the prevalence of disease will be highly variable and predictive values of the test unknown
- in individuals without symptoms unless the person is a contact of a confirmed case, due to the low pre-test probability
- where there are zero or only sporadic cases, due to the increased likelihood of false positives
- where appropriate biosafety and infection prevention and control measures are lacking, for the purposes of protecting health workers
- when the management of the patient does not change based on the result of the test because of unknown or low predictive value of the test. In this situation there is no benefit to testing
- screening prior to blood donation, since a positive test result may not correlate with the presence of viraemia.

3.3.6.4 *Quality assurance processes*

Both the CDC and WHO document the importance of quality control as this can affect test performance. The WHO recommends that all test operators must have

training in sample collection, relevant biosafety, performance of the test and interpretation and reporting of results.⁽⁹³⁾ Test operators must also be trained in waste management to ensure there is no further risk of transmission from poorly disposed of samples or specimens, for example. To ensure the device is functioning as it should, the WHO additionally recommends post-market surveillance, with regulatory oversight. Verifying the product's performance should be a requirement for the manufacturer, while the health system should ensure there are clear mechanisms for reporting problems, as well as adequate processes for monitoring and evaluating testing activities.⁽⁹³⁾ Similar biosafety advice is provided by the CDC. Additionally, laboratory and testing professionals must comply with CLIA regulations in the US; obtain a CLIA certificate; and meet all the requirements to perform the test.⁽¹⁴⁶⁾

3.3.6.5 Centralised reporting or data linkage

In the US, as with all other diagnostic tests, a CLIA-certified laboratory or testing site must report RADT results to the local, state, tribal, or territory health department.⁽¹⁴⁶⁾ The results must also be clearly distinguished from other COVID-19 tests. Additionally, the individual's healthcare provider should be informed of the results by the laboratory or testing site, in line with the instructions for use of the FDA-authorized device that was used to test for SARS-CoV-2. When reporting negative test results to patients, the laboratory or testing site may be required to report the results as 'presumptive negative', depending on the stipulations of the FDA authorisation.⁽¹⁴⁶⁾

The WHO do not provide guidance on reporting RADT results.

Table 3.3 Guidance on the use of rapid antigen detection tests for SARS-CoV-2

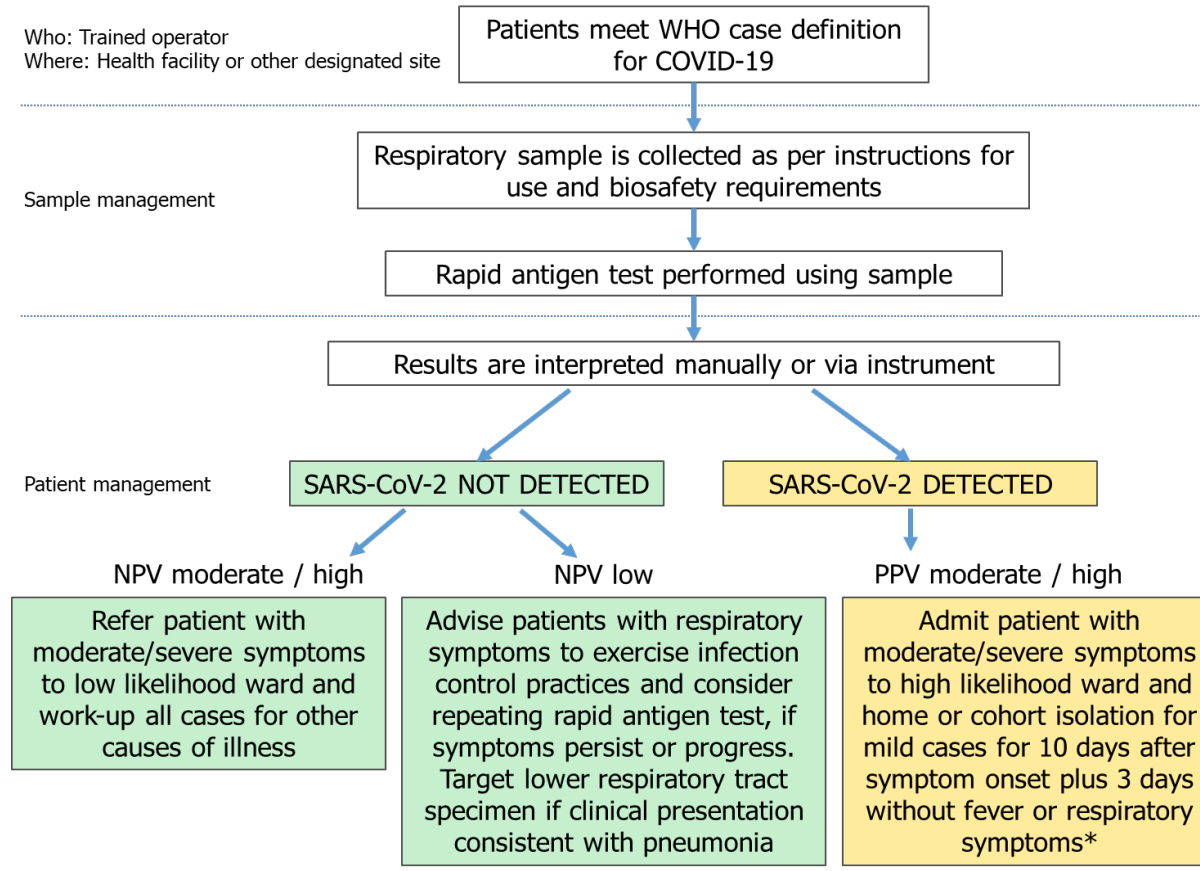
	WHO	CDC
Setting(s) / scenarios for use	<p>Appropriate scenarios for use of COVID-19 rapid antigen tests include the following:</p> <ol style="list-style-type: none"> 1. To respond to suspected outbreaks of COVID-19 in remote settings, institutions and semi-closed communities where NAAT is not immediately available 2. To support outbreak investigations (e.g. in closed or semi-closed groups including schools, care-homes, cruise ships, prisons, work-places and dormitories, etc.) 3. To monitor trends in disease incidence in communities, and particularly among essential workers and health workers during outbreaks or in regions of widespread community transmission 4. Where there is widespread community transmission, rapid tests may be used for early detection and isolation of positive cases in health facilities, COVID-19 testing centres/sites, care homes, prisons, schools, front-line and health-care workers and for contact tracing 5. Testing of asymptomatic contacts of cases may be considered even if the rapid antigen test is not specifically authorised for this use, since asymptomatic cases have been demonstrated to have viral loads similar to symptomatic cases. 	<p>The CDC suggests rapid antigen tests could be used for the following purposes:</p> <ul style="list-style-type: none"> - diagnostic testing during the early stages of infection with SARS-CoV-2 when viral load is generally highest; - diagnostic testing in which a person has a known exposure to a confirmed case of COVID-19; - screening in high-risk congregate settings in which repeat testing could quickly identify persons with a SARS-CoV-2 infection <p>There are limited data to guide the use of rapid antigen tests as screening tests on asymptomatic persons to detect or exclude COVID-19, or to determine whether a previously confirmed case is still infectious.</p>
Criteria for interpreting results	<p>To correctly interpret and act on the results of the rapid antigen test, the prevalence of disease (according to the reference standard) must be estimated based on surveillance, since this determines the positive and negative predictive values of the rapid test.</p> <p>For the purposes of case management when there is widespread community transmission, the pre-test probability of COVID-19 disease (the likelihood that the patient has COVID-19 before their results are known, based on epidemiologic and clinical factors) is relatively high, and positive test results have a high predictive value. Likewise, in a setting of community transmission, the predictive value of a negative</p>	<p>Positive and negative predictive values of all rapid antigen tests vary depending upon the pre-test probability of the patient being tested. Pre-test probability is impacted by the prevalence of the target infection in the community as well as the clinical context of the recipient of the test. CDC recommends that laboratory and testing professionals who perform rapid antigen testing should determine infection prevalence based on a rolling average of the positivity rate of their own SARS-CoV-2 testing over the previous 7–10 days. Infection prevalence at the time of testing, as well as the clinical context of the recipient of the test, impacts pre-test probability. If a specific testing site, such as a nursing home, has a positivity rate near zero, the prevalence of disease in the</p>

	WHO	CDC
	test result may be low, even when there are strong epidemiologic or clinical indicators of COVID-19 exposure or disease	community (e.g., cases per population) should instead be used to help determine pre-test probability
Testing strategy	<p>The appropriate testing strategy depends on the testing scenario:</p> <ol style="list-style-type: none"> Responding to suspected outbreaks: <ul style="list-style-type: none"> Positive rapid antigen test results from multiple suspects is highly suggestive of an outbreak and would allow for early implementation of infection control measures. Where possible, all samples giving positive rapid antigen test results (or at least a subset) should be transported to laboratories with NAAT capability for confirmatory testing. Supporting outbreak investigations: <ul style="list-style-type: none"> In NAAT-confirmed COVID-19 outbreaks, rapid antigen tests could be used to screen at-risk individuals and rapidly isolate positive cases (and initiate other contact tracing efforts) and prioritize sample collection from test-negative individuals for NAAT. Monitoring trends in disease incidence during outbreaks or in regions of widespread community transmission <ul style="list-style-type: none"> The positive predictive value and negative predictive value of a rapid antigen test result should be used to enable effective infection control. Early detection and isolation of cases during widespread community transmission: <ul style="list-style-type: none"> Negative samples will depend on the performance of the test and the community prevalence of COVID-19. A negative test result cannot completely exclude an active COVID-19 infection, and, therefore, repeat testing or preferably confirmatory testing (NAAT) should be performed whenever possible, particularly in symptomatic patients. Testing asymptomatic contacts of cases <ul style="list-style-type: none"> A negative test should not remove a contact from quarantine requirements. 	<p>The appropriate testing strategy depends on the testing scenario:</p> <p>Diagnostic testing (e.g., symptomatic individuals): In most cases, negative antigen diagnostic test results are considered presumptive. CDC recommends confirming negative antigen test results with an RT-PCR test when the pre-test probability is relatively high, especially if the patient is symptomatic or has a known exposure to a person confirmed to have COVID-19. Ideally, confirmatory RT-PCR testing should take place within two days of the initial antigen testing. If RT-PCR testing is not available, clinical discretion can be used in whether to recommend the patient isolate.</p> <p>Screening testing (e.g., asymptomatic individuals without known or suspected exposure): Test results for SARS-CoV-2 should be considered presumptive. Confirmatory nucleic acid testing following a positive antigen test may not be necessary when the pre-test probability is high, especially if the person is symptomatic or has a known exposure. When the pre-test probability is low, those persons who receive a positive antigen test should isolate until they can be confirmed by RT-PCR.</p> <p>Confirmatory nucleic acid testing following a negative antigen test used for screening testing may not be necessary if the pre-test probability is low, the person is asymptomatic, or has no known exposures, or is part of a cohort that will receive rapid antigen tests on a recurring basis. Nucleic acid testing is also considered presumptive when screening asymptomatic persons, the potential benefits of confirmatory testing should be carefully considered in the context of person's clinical presentation.</p>
Quality assurance processes	<p>All test operators must have training in sample collection, relevant biosafety, performance of the test and interpretation and reporting of results as well as in waste management. Quality control measures also need to be put in place.</p> <p>WHO advises that post-market surveillance, with regulatory oversight,</p>	<p>Laboratory and testing professionals who conduct <i>diagnostic or screening testing</i> for SARS-CoV-2 with rapid antigen tests must comply with CLIA regulations. Any laboratory or testing site that intends to report patient-specific test results must first obtain a CLIA certificate and meet all requirements to perform that testing. Laboratory and testing professionals who conduct <i>surveillance testing</i> for SARS-CoV-2 with rapid antigen tests are not obligated</p>

	WHO	CDC
	is critical to discover defects in product performance and is an important requirement for the manufacturer. The health system should ensure there is monitoring and evaluation of COVID-19 diagnostic testing activities and clear mechanisms for reporting problems	to comply with these FDA and CLIA requirements. Biosafety measures and instructions for collection and handling of clinical specimens should be followed precisely to ensure accurate testing and safety of those who perform the testing
Centralised reporting or data linkage	Not reported	A CLIA-certified laboratory or testing site must report rapid antigen diagnostic test results to the local, state, tribal, or territory health department. Antigen test results that are reported to public health departments must be clearly distinguished from other COVID-19 tests, such as RT-PCR tests and antibody tests. Laboratory and testing professionals should collect and report complete patient demographic information and ensure that they report antigen test results using the proper LOINC code for their particular FDA-authorized assay(s). A CLIA-certified laboratory or testing site must report antigen test results to the individual or the individual's healthcare provider according to the instructions for use of the FDA-authorized SARS-CoV-2 in vitro diagnostic device that was used. Depending on the stipulations of the FDA authorisation, the laboratory or testing site may be required to report negative test results to patients as "presumptive negative."

Key: CDC – Centers for Disease Control and Prevention; CLIA - Clinical Laboratory Improvement Amendments (CLIA); COVID-19 – coronavirus disease 2019; NAAT – nucleic acid amplification test; POC – point-of-care; RT-PCR – reverse transcription polymerase chain reaction; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; WHO – World Health Organization.

Figure 3.1 Flowchart demonstrating the potential use of rapid antigen testing in settings of widespread community transmission and where there is no nucleic acid amplification test capacity



* WHO recommended duration of isolation; may vary by country

Key: NPV – negative predictive value; PPV – positive predictive value; SARS-CoV-2 – severe acute respiratory syndrome coronavirus disease 2

Source: Adapted from the [World Health Organization: Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays](#). This work is available under the [CC BY-NC-SA 3.0 IGO licence](#)

3.4 Discussion

Although a wide range of alternatives to rRT-PCR are available internationally, the laboratory-based molecular test broadly remains the recommended primary test for the detection of current infection with SARS-CoV-2 in the included countries in this review, as well as by the international public health bodies (that is, the ECDC and WHO). In a small number of countries, following satisfactory completion of validation studies to ensure that tests have met specified performance criteria, RADTs are being used in near-patient settings to diagnose current infection with SARS-CoV-2 in selected symptomatic individuals, with use typically limited to individuals who have recently developed symptoms, as well as asymptomatic individuals in certain clinical contexts and settings. For instance, in Germany, RADTs may be used to screen contacts of confirmed cases, but only in exceptional circumstances, such as when rRT-PCR capacity is limited.⁽¹²⁰⁾ In Canada, RADTs can be used for the purposes of serial testing in at risk-settings such as long-term care facilities or food processing plants.⁽¹⁴⁴⁾ RADTs may also be used for screening purposes in Australia, as determined by individual public health authorities.⁽¹⁵⁶⁾

The review of international practice identified inconsistencies in the wording around rapid and or near-patient testing. From the review, it would appear that a number of the included countries have yet to recommend the use of rapid or near-patient tests for the detection of SARS-CoV-2. However, it is unclear if this relates to all rapid tests, irrespective of where they are used, or specifically to the use of these tests in near-patient (non-laboratory) settings. As noted, it is also possible to deploy rapid molecular tests (including simplified RT-PCR-based rapid tests) in, for example, hospital laboratories to support timely diagnosis in hospital settings. Where deployed in hospital laboratories, it is assumed that they are operated by trained laboratory staff under usual laboratory governance and quality assurance procedures. Therefore from a governance and process perspective, this use is likely distinct from tests deployed in near-patient (point-of-care) settings, including for example clinical settings, or community or 'pop-up' testing centres and operated by non-laboratory staff.

On 11 September 2020, the WHO published interim guidance on the potential use of RADTs for the detection of current infection with SARS-CoV-2.⁽⁹³⁾ The public health body outlined a number of scenarios in which RADTs could be appropriately used. These include, for example, responding to suspected outbreaks in remote settings, institutions, and semi-closed communities; supporting outbreak investigations; monitoring disease incidence in communities, particularly among essential workers and health workers during outbreaks or in regions of widespread community transmission, among other scenarios. The WHO also detailed a number of situations

in which RADTs should not be used, based on currently available information; this includes screening at airports, as well as other border points of entry due to the highly uncertain prevalence of disease and increased likelihood of obtaining false positives.⁽⁹³⁾ In this review, there was no evidence that near-patient tests were being deployed at airports in any of the included countries as a national policy. However, there is anecdotal evidence that testing at airports is being undertaken in a number of countries in Europe, for example. Individual airlines and airports, are offering RT-PCR tests to travellers, often for a fee (including an increased fee for a rapid turnaround time). This is the only evidence from the rapid review that out-of-pocket expenses are being used for testing, including rapid or near-patient tests. There is no evidence that rapid antigen or near-patient tests are being used in airports, to date.^(182, 183)

While rapid or near-patient tests may not be advised at airports or other border points of entry due to the unknown prevalence of infection, alternatives to in-vitro diagnostic techniques are being explored. Researchers at the London School of Hygiene and Tropical Medicine are investigating the use of dogs as biosensors for COVID-19.⁽¹⁸⁴⁾ The team previously demonstrated that trained dogs could detect the presence of malaria in human odour (arising from changes in volatile organic compounds, which can be produced by the body during disease) with an effectiveness greater than 90%, even in asymptomatic individuals and in the early stages of infection.⁽¹⁸⁵⁾ The bio-detection dogs are currently being evaluated in airports. If effective, the trained dogs could be deployed within 8-10 weeks of training and could be used to screen 250 people per hour.⁽¹⁸⁴⁾

Although a comprehensive search of international resources was undertaken, it is possible that the sources identified in this review are not current or do not accurately capture all testing that is being undertaken. For example, in some countries, tests other than NAAT may be used in place of RT-PCR (such as where testing might be outsourced). Pilot testing programmes may be underway internationally, but may not be detailed in the international resources that were searched, suggesting the review might have missed information on potentially relevant alternatives to RT-PCR that may play a role in future testing strategies. Similarly, strategies such as the pooling of samples may be commonly used in reference laboratories, but may not be adequately captured in the included guidance documents, particularly if such an approach has only been used for limited time periods where availability of reagents or test kits were particularly constrained.

The information summarised in this rapid review was correct on 19 October 2020, but is subject to change.

4 Diagnostic accuracy of alternatives to laboratory-based rRT-PCR

Key points

- Diagnostic test accuracy describes the comparison between the estimate of a disease state ('target condition' for example, infection with SARS-CoV-2) by a test of interest (the 'index test') and the current best estimate of the true disease state ('the reference standard' test).
- Key metrics of diagnostic test accuracy include analytic sensitivity and specificity, and clinical sensitivity and specificity.
- Analytic sensitivity indicates the likelihood that a test will be positive for material containing any virus strains, and the minimum concentration the test can detect (limit of detection). Analytic specificity indicates the likelihood that the test will be negative for material containing pathogens other than the target virus.
- Clinical sensitivity represents the proportion of positive index tests in individuals who in fact have the disease in question. Clinical specificity describes the proportion of those without the disease who are correctly classed as negative. Sensitivities and specificities reported in this chapter refer to the clinical, rather than analytical, metrics.
- Where an individual has a negative test result, the probability that they are truly infected with SARS-CoV-2 is a function of the sensitivity of the test and of the pre-test probability of being infected. Pre-test probability depends on factors such as local COVID-19 prevalence, SARS-CoV-2 exposure history, symptoms, and potential additional risk factors for infection.
- In practice, sensitivity of RT-PCR for the diagnosis of infection with SARS-CoV-2 has been estimated as ranging between 71% and 98%; sensitivity depends on various factors, including the timing and type of specimen obtained, the sampling technique, and the quality of particular test kits used to perform RT-PCR. Specificity of RT-PCR tests is generally very high (typically greater than 99.5%).
- In the context of population testing with the aim of controlling infection spread, there are drawbacks both from insufficient sensitivity (for example, leading to missing infected individuals who might otherwise be isolated) and insufficient specificity (for example, imposing isolation measures on

individuals who are not true positives, and imposing restriction of movements on their contacts).

- The WHO published desirable and minimally acceptable product profiles for four types of test situations to support the response to the COVID-19 pandemic. Targets are provided for the estimated sensitivity and specificity of a suitable test; it is stipulated that the lower bound of the estimated confidence interval should equal or exceed the target.
- Two of the WHO test product profiles relate specifically to detection of current infection, and are particularly relevant to the present rapid HTA.
 - The first profile relates to point-of-care testing for suspected COVID-19 cases and their close contacts where rRT-PCR testing is unavailable or where turnaround times obviate clinical utility. Acceptable and desirable levels of sensitivity for this situation are set at $\geq 80\%$ and $\geq 90\%$, respectively, and the corresponding levels of specificity are set at $\geq 97\%$ and $> 99\%$.
 - The second profile relates to testing for the diagnosis or confirmation of acute or sub-acute SARS-CoV-2, for low or high-volume testing needs. Acceptable and desirable levels of sensitivity are set at $\geq 95\%$ and $\geq 98\%$, and the corresponding levels of specificity are set at $\geq 99\%$ and $\geq 99\%$.
- This rapid HTA performed a scoping review to identify systematic reviews, rapid reviews or evidence summaries which have been undertaken to evaluate the diagnostic accuracy of alternatives to laboratory-based rRT-PCR. Reviews published as of 28 September 2020 were included; these reviews captured data published in or before June 2020. The rapid HTA was supplemented with independent evaluations performed by the Foundation for Innovative Diagnostics (FIND) as of 28 September 2020.
- The diagnostic accuracy of commercial rRT-PCR platforms, for example, high-throughput assays such as the Roche Cobas 6800 and Panther Fusion, and automated assays such as GenMark ePlex®, Diasorin Simplexa™ and Cepheid's Xpert® Xpress, was found to be high; in one meta-analysis, the average sensitivity pooled across multiple studies for these tests was found to be $\geq 99\%$ for all but the ePlex platform (pooled sensitivity of 94%). Specificity was $\geq 96\%$ in all cases. Another study identified low sensitivity (68%) for the Mesa Biotech Inc. Accula test.
- Results for the diagnostic accuracy of isothermal amplification based method and test platforms included the study of RT-LAMP, CRISPR, the ID NOW platform, the SAMBA II platform, and several other isothermal amplification methods (iAMP, RT-iiPCR, RT-RPA, RCA, RT-RAA).

- In one systematic review, the diagnostic accuracy of RT-LAMP-based molecular methods appeared to depend on whether crude or purified samples were analysed. Where crude samples were analysed (for example, nasopharyngeal or saliva samples), this resulted in the lowest sensitivity among isothermal methods studied; sensitivity values ranged from 40% to 88%. However, analysis of purified samples led to sensitivity values in excess of 90% in the majority of included studies. Alternative methods using CRISPR or the SAMBA II platform similarly showed sensitivity values above 90% in the majority of cases. The ID NOW™ platform had the lowest sensitivity among isothermal amplification methods. Overall, specificity was high among isothermal amplification methods.
- For rapid antigen tests, only one systematic review of diagnostic accuracy was identified. Sensitivity of such tests was found to vary significantly across test brands; estimates ranged from 0% to 94%, with an average sensitivity of 56.2% (95% CI 29.5% to 79.8%). Average specificity was 99.5% (95% CI 98.1% to 99.9%). Recent clinical evaluations of antigen tests, as conducted by the FIND collaboration, were not included in the aforementioned review; for example, one such recent evaluation for a particular antigen test (SD Biosensor Inc. Standard Q) estimated sensitivity as 77% and 89% in two sites.
- Current estimates of diagnostic accuracy for alternative tests to rRT-PCR are limited by significant flaws in the design, execution and reporting of primary diagnostic accuracy studies; it is not possible to ascertain whether these limitations have led to overestimation or underestimation of test accuracy estimates. In regard to the secondary research literature, as the research landscape for tests involved in the detection of SARS-CoV-2 is rapidly-evolving, recent results will not have been captured in published reviews of diagnostic accuracy. Furthermore, there is a lack of research on the diagnostic accuracy of emerging technologies. Notably, a rapid collaborative review on the diagnostic accuracy of molecular methods is currently being undertaken by EUnetHTA, and is expected to publish in November 2020.
- With respect to transferability of diagnostic accuracy results to certain settings, there is a lack of information on important patient subpopulations and settings. This includes asymptomatic individuals, specific at-risk populations such as healthcare workers, and the validation of diagnostic performance of tests beyond their use in the hospital setting or in self-administered tests. Ideally, clinical sensitivity and specificity of tests should be measured in various clinically-relevant real-life situations, including various sources of specimens, timing of specimens, and degrees of illness severity.

- In addition to considering the limitations of the existing literature, when considering diagnostic accuracy, published metrics should be considered as interrelated with contextual factors which may have implications for accuracy in practice.
- Contextual factors which may affect accuracy in practice include the pre-test probability of infection, which represents the likelihood that a person with a negative test result is a true negative or a false negative, and is dependent on prevalence. Also, the processes involved in testing, for example, sampling, or use of particular reagents, may impact on the performance of a test.
- Diagnostic test accuracy metrics should not be considered as an isolated measure of a test's performance; metrics should be situated within consideration of the overall aim of testing and associated organisational factors which may impact on the value of a particular test.

4.1 Background

4.1.1 The need for alternatives

Testing capacity for SARS-CoV-2 detection has rapidly expanded since the initial phase of the pandemic; the rollout of PCR-based diagnostics from central public health laboratories to regional and local diagnostic laboratories, and the use of high-throughput automated molecular testing platforms, are important developments. However, additional capacity for larger scale testing, or more tailored or efficient testing approaches, are likely to become increasingly necessary. This may include the use of rapid commercial tests, which may present a highly important role, should they demonstrate adequate performance for infection detection.⁽¹⁸⁶⁾

4.1.2 Contextual factors relating to test performance

In judging the utility of a particular test, it is crucial to consider what information different tests can provide; such information relates to the aim of a particular test, the context of providing the test, and the performance of that test, the latter being reflected in diagnostic accuracy (see Section 4.2). Conversely, in deciding policy approaches regarding testing, it is important to contextualise test approaches with respect to the intended purpose of testing, for example, particular diagnosis or screening settings. This includes consideration of the specific target population (such as health professionals, general public), the phase of disease to which a test is applicable (such as asymptomatic or healthy individuals versus confirmed cases at various stages of illness or recovery) and any decision(s) which may be taken based on a test's results.⁽¹⁸⁷⁾ For example, in the context of hospital-based testing, insufficient diagnostic accuracy may have particularly severe consequences in the

immediate-term; the correct classification of patients' infection status is important in minimising nosocomial transmission and the transmission of infection to staff, but also for protecting patients who may unknowingly be infected with the virus and who are due to undergo treatment that would likely undermine their recovery from COVID-19.⁽¹⁸⁸⁾

The European Centre for Disease Prevention and Control has recently published guidance on testing strategies and objectives with respect to SARS-CoV-2 and COVID-19, and outlines five main objectives of testing as follows:⁽¹⁸⁹⁾

1. Control transmission
 - Necessitates the testing of symptomatic individuals and contacts of COVID-19 cases.
2. Reliably monitor SARS-CoV-2 transmission rates and severity
 - Ideally would involve testing for both SARS-CoV-2 and differential diagnoses, for example, seasonal influenza, in symptomatic patients.
 - Suggests the adoption of multiplex molecular testing systems, if available.
3. Mitigate the impact of COVID-19 in healthcare and social care settings
 - For example, adopt periodic screening to prevent nosocomial transmission.
4. Detect clusters or outbreaks in specific settings
 - For example, rapid detection in settings such as workplaces and educational facilities.
5. Maintain sustained control of COVID-19
 - Consider targeted testing and follow up of individuals entering from areas (for example, other countries) considered not to have sustained control.

As such, testing to meet particular objectives may require certain test attributes; for example, multiplex testing to distinguish between different circulating pathogens may be required for certain circumstances, or rapid turnaround of test results may be required in others.

With respect to descriptions of tests within the literature, tests for the detection of SARS-CoV-2 may be grouped according to different, but related, definitions:⁽¹⁸⁷⁾

- scientific rationale, for example, the direct detection of SARS-CoV-2 versus detection of immune response to the virus;
- type of technology — this may include commercial tests, such as tests automated for use on analyser machines or rapid tests, as opposed to in-house assays, or may refer to the technological method underpinning a test's functionality;

- intended user, for example, tests administered by a health professional as opposed to self-testing;
- location of testing, for example, laboratory-based testing or point-of-care or near-patient testing.

Understanding of the interplay of these related definitions is essential for understanding the appropriate use of individual tests.

When considering diagnostic accuracy, specific metrics should not be considered as an isolated measure of a test's value, but should be considered as interrelated with the above factors and considerations. As discussed below and at other points within this review, results pertaining to diagnostic accuracy will also be conditional on factors related to the testing process, such as type of specimen, timing of sampling, sampling technique and the quality of the test kit.

Furthermore, with respect to designing a strategy for the containment of SARS-CoV-2 at a population level, it has been argued that more focus should be placed on the sensitivity of a testing regimen, as opposed to simply focusing on the sensitivity of an individual test.⁽¹⁹⁰⁾ Measuring the sensitivity of a testing regimen requires the consideration of a test in context, with respect to how often it is used, to whom it is applied, when in the course of an infection it works, and whether its results are returned in time to prevent spread.⁽¹⁹⁰⁾ For example, consideration of a testing regimen would include consideration of combining tests to achieve a particular aim; it has been suggested that an effective screening strategy might include frequent, cheap, and rapid tests at scale to mitigate outbreaks, with positive results confirmed using a second rapid test targeting a different protein, or using laboratory-based rRT-PCR.⁽¹⁹⁰⁾

4.2 Assessing the accuracy of diagnostic tests for the detection of SARS-CoV-2

4.2.1 Determination of diagnostic test accuracy

Diagnostic test accuracy is a measure of diagnostic test performance. It describes the comparison between the estimate of a disease state ('target condition', for example infection with SARS-CoV-2) by a test of interest (the 'index test') and the current best estimate of the true disease state ('the reference standard' test). Key metrics of diagnostic test accuracy include analytic sensitivity and specificity, and clinical sensitivity and specificity.⁽¹⁸⁷⁾

Analytic sensitivity indicates the likelihood that a test will be positive for material containing any virus strains, and the minimum concentration the test can detect (limit of detection).⁽¹⁹¹⁾ Analytic specificity indicates the likelihood that the test will

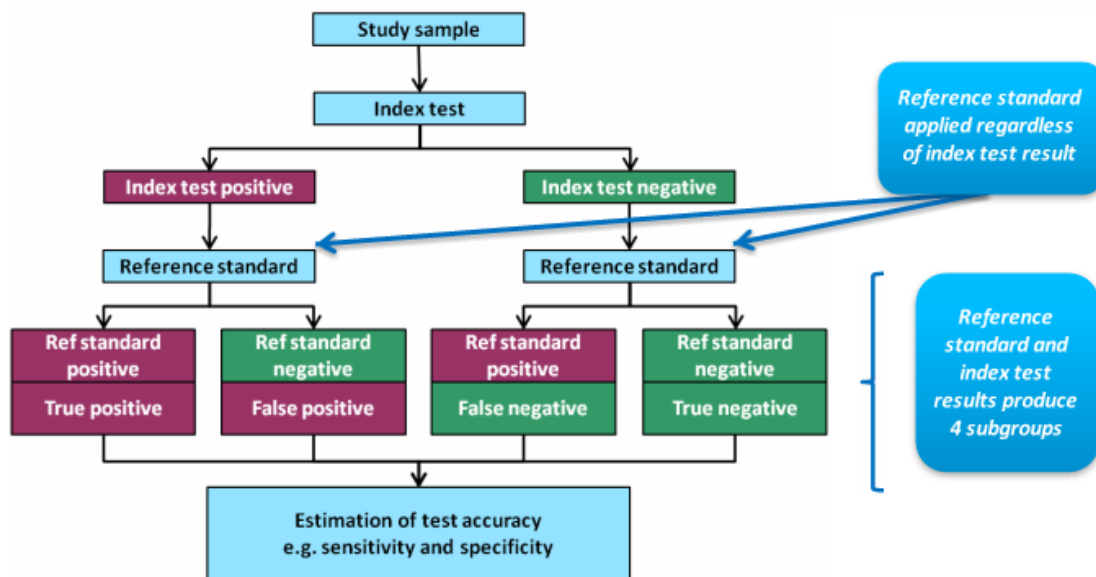
be negative for material containing pathogens other than the target virus. Clinical sensitivity and specificity, in contrast, are determined from studies using specimens from individuals known to have the condition in question. Clinical sensitivity represents the proportion of positive index tests in individuals who in fact have the condition in question. Clinical specificity describes the proportion of those without the condition who are correctly classed as negative. Importantly, high analytical sensitivity does not necessitate acceptable diagnostic (clinical) sensitivity. However, while a large number of commercial detection assays for SARS-CoV-2 RNA or antigen have been recently developed, information on their clinical performance is limited.⁽¹⁸⁶⁾

In the case of assessments of the diagnostic accuracy of alternatives to laboratory-based rRT-PCR testing, the target condition is infection with SARS-CoV-2. For a person with COVID-19, the reference standard test is likely to be a clinical diagnosis, ideally established by an independent adjudication panel whose members are unaware of the results of the index test results.^(191, 192) However, such an approach would exclude individuals who are suspected to be infected with SARS-CoV-2, but do not show symptoms. Also, it is unclear whether the sensitivity of existing commercial tests have been assessed in this way.⁽¹⁹¹⁾ It has been noted that the Food and Drug Administration (FDA), the body responsible for the granting of marketing authorisation for diagnostic tests in the USA, currently permits companies to demonstrate SARS-CoV-2 diagnostic test performance by establishing the index test's agreement with an authorised rRT-PCR test in known positive material from either symptomatic people or contrived specimens. However, use of either known positive or contrived samples may lead to overestimates of test sensitivity due to the potential for swabs to miss infected material in practice (that is, the clinical setting).⁽¹⁹¹⁾ Ideally, clinical sensitivity and specificity of tests should be measured in various clinically-relevant real-life situations, including various sources of specimens, timing of specimens, and degrees of illness severity.⁽¹⁹¹⁾

4.2.2 Calculation of diagnostic test accuracy metrics

In a study aimed at detecting the diagnostic accuracy of an index test, all individuals undergo testing with both the index test and the reference standard, regardless of the results of the index test; Figure 4.1. This process results in four possible subgroups of results, as depicted in Figure 4.2, based on whether or not the target condition has been detected by each test.

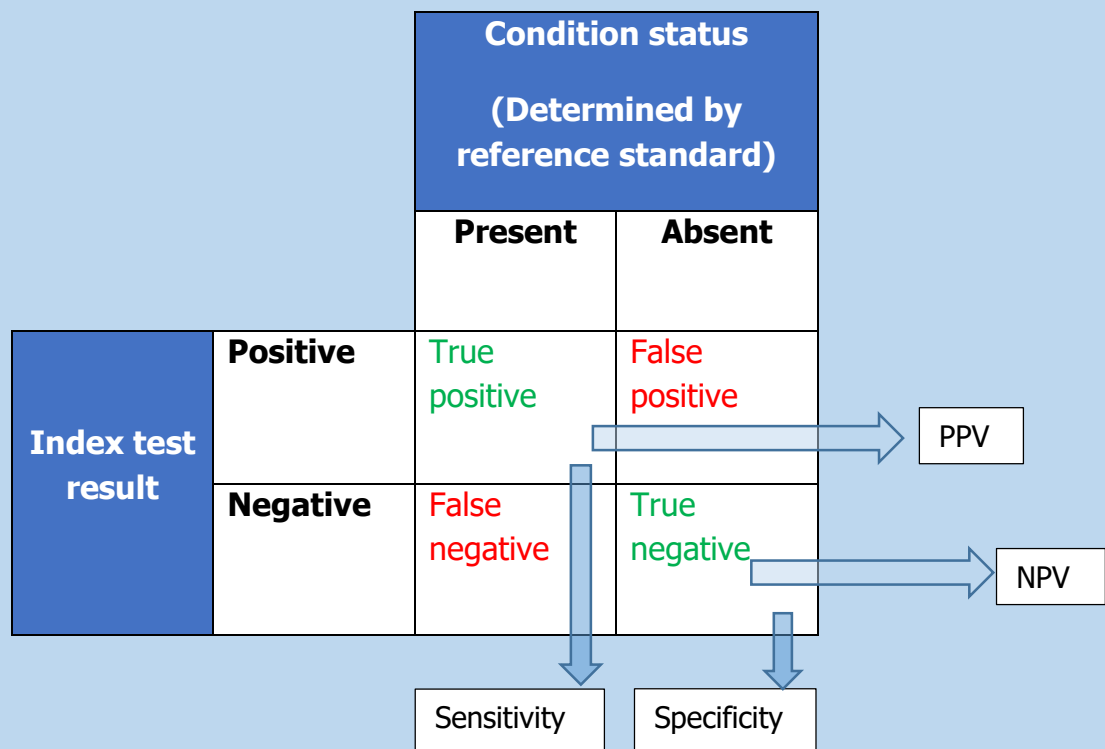
Figure 4.1. Study design for determining test sensitivity and specificity; diagram presented in Cochrane training module on Diagnostic Test Accuracy⁽¹⁹³⁾



- If both the reference standard test is positive (that is, the condition is detected) and the index test is positive, the index test is said to yield a 'true positive'.
- If the reference standard test is negative and the index tests is positive, the index test is said to yield a 'false positive'.
- If the reference standard test is positive and the index text is negative, the index test is said to yield a 'false negative'.
- If both the reference standard test and the index test are negative, the index test is said to yield a 'true negative'.

The above four possible outcomes are represented in the diagnostic 2x2 contingency table, which allows calculation of diagnostic accuracy measures including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) (see Figure 4.2). Sensitivity and specificity are conditional on disease status, as determined by the reference standard. In contrast, PPV and NPV are conditional on the index test results.

Figure 4.2. Relationship between index test result and condition status, and calculation of diagnostic test accuracy metrics



- **Sensitivity** describes the proportion of those with the condition that the index test is capable of detecting:
 - Sensitivity = True positives / (True positives + False negatives)
- **Specificity** describes the proportion of those without the condition who are correctly classed as negative:
 - Specificity = True negatives / (False positives + True negatives)
- The **positive predictive value** describes the proportion of those with a positive index test result who truly have the condition:
 - PPV = True positives / (True positives + False positives)
- The **negative predictive value** describes the proportion of those with a negative index test result who truly do not have the condition:
 - NPV = True negatives / (False negatives + True negatives)

As sensitivity increases, the NPV increases, and false negative test errors decrease. As specificity increases, the PPV increases, and false positive test errors decrease. As such, high sensitivity is associated with the goal of detecting the maximum number of positive individuals, while high specificity is associated with the goal of distinguishing between true and false positives.

4.2.3 Considerations for assessing accuracy of diagnostic tests for the detection of SARS-CoV-2; implications of false negatives, false positives, and context

In April 2020, the European Commission (EC) published a working document on COVID-19 in vitro diagnostic tests and their performance.⁽¹⁸⁷⁾ This proposed a tentative definition of COVID-19 diagnostic test performance criteria. These criteria include analytical sensitivity and specificity, and clinical (or diagnostic) sensitivity and specificity.

As noted by the EC, there can be trade-offs between diagnostic sensitivity and specificity. A test that is very sensitive at detecting the target of interest is more likely to also detect related but distinct targets that are not of interest, that is to say, it may be less specific. Similarly, a low limit of detection may result in a lower reproducibility of the test result. As such, various choices of parameter combinations can be justified in the context of different purposes, for example, rapid screening versus diagnosis or confirmation of results.

In the April 2020 guidance, the EC suggested that when a person is examined for the first time, this should be performed with a test that is highly sensitive and very precise, that is, results in a very low level of false negatives and a low level of false positives. The number of false negative results is a function of the sensitivity of the test, which is generally high for PCR-based tests. If the person tests positive and is examined a few days later, the test may have some tolerance for false positives (since the individual is most likely still positive), but not for false negatives (as this would lead to wrong conclusions). Also, if a test is easy to use and cheap, but has a relatively low specificity, this may be overcome by repeating the test.⁽¹⁸⁷⁾

In the context of population testing with the aim of controlling infection spread, there are drawbacks both from insufficient sensitivity (for example, leading to missing infected individuals who might otherwise be isolated) and insufficient specificity (for example, imposing isolation measures on individuals who are not true positives, and imposing restriction of movements on their contacts). This needs to be taken into account along with the level of transmission in a particular population. For example, where large-scale testing of asymptomatic people is being performed,⁽¹⁹⁴⁾ the total number of people with a false positive result will increase. This number is a function of the specificity of the test, the number of non-infected individuals being tested, and the prevalence of the infection in a population at the time of the test. For PCR-based tests, specificity is generally very high (in the order of >99.5%).⁽¹⁹⁴⁾

In September 2020, the WHO published desirable and minimally acceptable product profiles for four types of tests to support the response to the COVID-19

pandemic.⁽¹⁹⁵⁾ These product profiles provide a framework to weigh the importance of one feature of a test versus another for a specific use case and context. The proposed 'desirable' requirements reflect the specifications that would allow for the broadest uptake and in turn the broadest public health impact. The four profiles are focused on priority scenarios to address the greatest current need:

1. Point-of-care test for suspected COVID-19 cases and their close contacts to diagnose acute SARS-CoV-2 infection in areas where reference assay testing is unavailable, or turnaround times obviate clinical utility
2. Test for diagnosis or confirmation of acute or subacute SARS-CoV-2 infection, suitable for low or high-volume needs
3. Point-of-care test for prior infection with SARS-CoV-2
4. Test for prior infection with SARS-CoV-2 for moderate to high volume needs.

As the scope of the present review focuses mainly on the direct detection of current infection with SARS-CoV-2, the first two profiles are of primary interest. These have been reproduced in Appendix B. The profiles show how acceptable and desirable levels of sensitivity and specificity may vary for different situations. For example, for the first profile (point-of-care testing in areas where reference assay testing is unavailable, or turnaround times obviate clinical utility), acceptable and desirable levels of sensitivity are set at $\geq 80\%$ and $\geq 90\%$, respectively, and the corresponding levels of specificity are set at $\geq 97\%$ and $> 99\%$. In contrast, for the second profile (test for diagnosis or confirmation of acute or sub-acute SARS-CoV-2, for low or high-volume needs), acceptable and desirable levels of sensitivity are set at $\geq 95\%$ and $\geq 98\%$, and the corresponding levels of specificity are set at $\geq 99\%$ and $\geq 99\%$. In both cases, the targets are for the estimated true sensitivity and specificity; therefore, the lower bound of confidence intervals should ideally equal or exceed the target. Additionally, for the first profile, the WHO notes that for both the acceptable and desirable levels, the PPV falls below 50% at a low prevalence of disease and such a test would therefore require a second test for confirmation under these circumstances. When the prevalence increases to 10-20%, the 'acceptable' criterion leads to the PPV increasing to $> 78-89\%$.⁽¹⁹⁵⁾ As such, the applicability of such a test in isolation is dependent on the pre-test probability of infection.

4.3 Note on factors affecting the diagnostic accuracy of rRT-PCR

Tests based on rRT-PCR typically involve the qualitative detection of SARS-CoV-2 RNA from upper respiratory tract samples. In practice, sensitivity of RT-PCR for the

diagnosis of infection with SARS-CoV-2 has been estimated as ranging between 71% and 98%.⁽¹⁹⁶⁾ Sensitivity depends on various factors, including the timing and type of specimen obtained, the sampling technique, and the quality of particular test kits used to perform rRT-PCR (see section 4.5).⁽¹⁹⁷⁾

With respect to the timing of the test, Kucirka et al.⁽¹⁹⁸⁾ analysed the probability of obtaining a false negative rRT-PCR result among COVID-19 patients (symptomatic, inpatients and outpatients, upper respiratory tract samples) with respect to the number of days since exposure. 'Day 5' was used as an estimate for the onset of symptoms following exposure. The probability of a false negative result decreased from 100% (95% CI 100-100) on day one to 67% (95% CI 27-94) on day four. On the day of symptom onset the probability of a false negative test was 38% (95% CI 18-65). This decreased to 20% (95% CI 12-30) on day eight (three days after estimated symptom onset) then began to increase again, from 21% (95% CI 13-31) on day nine to 66% (95% CI, 54-77%) on day 21. As such, the false negative probability was minimised eight days after exposure, or, three days after the average day of onset of symptoms. The authors concluded that this may be the optimal time for testing if the goal is to minimise false negative results.

With respect to sample type, sputum samples have shown the highest rate of positivity in both severe and mild cases of COVID-19, followed by nasopharyngeal swabs and throat swabs.⁽¹⁹⁷⁾ From days 8 to 14 of onset of symptoms, the positivity rate of sputum has been found to remain higher than that of nasopharyngeal swabs, and positive rates of pharyngeal samples have been found to drop to 50% in severe and 29.6% in mild COVID-19 cases. These results are considered to be related to the higher viral load in the upper respiratory tract in the day before and the days immediately following onset of symptoms.⁽¹⁹⁷⁾

4.4 Diagnostic accuracy of specific alternative testing approaches to laboratory-based rRT-PCR; evidence sources identified

4.4.1 Methods

In order to identify literature sources of evidence on the diagnostic accuracy of alternative approaches to laboratory-based rRT-PCR, a literature review was undertaken considering evidence published as of 28 September 2020, in line with HIQA's protocol for this rapid HTA (detailed in Appendix A for reference). Briefly, a scoping review was performed to identify systematic reviews, rapid reviews or evidence summaries which have been undertaken to evaluate the diagnostic accuracy of alternatives to laboratory-based rRT-PCR. In the first instance, a search of the literature was undertaken using the PubMed Clinical Queries Tool and of

Google and Google Scholar to identify recently published manuscripts which may have yet to be indexed within academic search databases. The search was supplemented by targeted searches of the websites of international HTA agencies and public health bodies. PROSPERO was also searched to identify ongoing systematic reviews. Systematic reviews identified as potentially relevant are listed in Appendix C, though not all provided information specifically on test alternatives to laboratory-based rRT-PCR.

Information was first extracted on the scope covered by individual reviews identified, with particular attention to the index test(s) examined. Information on measures of diagnostic accuracy, including average sensitivity, average specificity, and the positive predictive value and negative predictive value, were subsequently extracted.

Relevant reviews identified are described below under the headings of 'Cochrane review evidence', 'Evidence reviews from national and international HTA agencies' and 'Other systematic review evidence'.

In addition to evidence from reviews, independent evaluations performed by the Foundation for Innovative New Diagnostics (FIND) were also included. This data source is described further in section 4.4.5.

4.4.2 Cochrane review evidence

A protocol was published in April 2020⁽¹⁹⁹⁾ for the conduct of five Cochrane 'living systematic reviews' which aim to summarise evidence on the diagnostic test accuracy of different tests and diagnostic features associated with SARS-CoV-2 and COVID-19. These reviews include the following titles:

1. Laboratory-based molecular tests for diagnosis of SARS-CoV-2 infection.
2. Rapid point-of-care tests for diagnosis of SARS-CoV-2 infection.
3. Antibody tests for identification of current and past infection with SARS-CoV-2.
4. Signs and symptoms to determine if a patient presenting in general practice or at the emergency department has COVID-19, COVID-19 pneumonia, or severe COVID-19 pneumonia/acute respiratory distress syndrome (ARDS) requiring intensive care unit (ICU) admission.
5. Routine laboratory testing to determine if a patient has COVID-19 pneumonia or SARS-CoV-2 infection.

To date, two reviews emerging from this protocol have been published. These titles include:

- Antibody tests for identification of current and past infection with SARS-CoV-2⁽¹⁷⁾

- Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection.⁽²⁰⁰⁾

The former review, focusing on the use of antibody tests, concluded that the sensitivity of antibody tests is too low in the first week from symptom onset to have a primary role in the diagnosis of COVID-19, though they may have a role in complementing other testing in individuals presenting later, when RT-PCR tests are negative, or are not done.⁽²⁰⁰⁾ Considering the detection of previous infection, the tests may have a useful role in detecting previous SARS-CoV-2 infection if used 15 or more days after the onset of symptoms.⁽²⁰⁰⁾ As these tests do not have a role in the direct detection of SARS-CoV-2 and do not have a primary role in the diagnosis of COVID-19, they remain outside the scope of the present rapid HTA. As such, this review will not be discussed further.

The second review, on the topic of rapid point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2, is of high relevance to the present rapid HTA and results will be presented in section 4.5, under the heading 'point-of-care testing approaches'.⁽²⁰⁰⁾ This review was published in August 2020 and included evidence published as of 25 May 2020. The review included 22 publications reporting on a total of 18 study cohorts and including 3,198 unique samples, of which 1,775 had confirmed SARS-CoV-2 infection. Data were identified for eight commercial tests, including four antigen tests and four molecular tests, and one in-house antigen test. All of the included studies were considered to be at high or moderate risk of bias.⁽²⁰⁰⁾ It is noteworthy that the Cochrane COVID-19 Diagnostic Test Accuracy Group are in the process of performing an additional evaluation of alternative laboratory-based molecular technologies in their series of Cochrane COVID-19 diagnostic test accuracy reviews; however, the expected publication date is not known.

4.4.3 Evidence reviews from national and international HTA agencies

The European Network for Health Technology Assessment (EUnetHTA) is currently in the process of performing a rapid collaborative review on the diagnostic accuracy of molecular methods (assays based on nucleic acid amplification tests, such as RT-PCR or isothermal RNA amplification) that detect the presence of SARS-CoV-2 virus in people with suspected COVID-19.⁽²⁰¹⁾ This review is being performed by Health Technology Wales (primary author), Healthcare Improvement Scotland (co-author) and Austrian Social Insurance (co-author) and is expected to be published in November 2020.

Authors from Health Technology Wales previously published an evidence appraisal, comprising a rapid systematic review, of the effectiveness of tests to detect the presence of SARS-CoV-2.^(202, 203) This review included consideration of the clinical

effectiveness of tests that detect the presence of SARS-CoV-2 and tests that detect the presence of antibodies to SARS-CoV-2. Information from the review of tests that detect the presence of SARS-CoV-2 (which included evidence available up to 4 May 2020 and included data on molecular tests only) will be discussed further below.

4.4.4 Other systematic review or meta-analysis evidence

Additional reviews identified in this literature search, and which included information relevant to the scope of the present review, included meta-analyses by Subsoontorn et al.⁽²⁰⁴⁾ and Yang et al.⁽²⁰⁵⁾ and a scoping review by Axell-House et al.⁽²⁰⁶⁾

Subsoontorn et al. aimed to consider the diagnostic accuracy of nucleic acid point-of-care tests for human coronaviruses included SARS-CoV, SARS-CoV-2 and MERS-CoV.⁽²⁰⁴⁾ This study included results of analyses for a total of 5,204 clinical samples from 43 individual studies published as of 16 June 2020. Most of these (n=38) considered the diagnosis of infection with SARS-CoV-2 and were published as preprint manuscripts at the time of the review. The majority of the studies (n=24) considered RT-LAMP techniques, followed by CRISPR (n=7), the Abbott ID NOW™ test (NEAR technology) (n=5), and SAMBA II (n=2). Remaining studies examined iAMP, RT-iiPCR, RT-RPA, RT-RAA and RCA techniques (n=1 study for each). Over a third (n=18) of the studies aimed to detect the coronavirus in crude patient samples, for example, nasopharyngeal swabs, sputum or saliva, while the remainder used purified RNA from patient samples. The review by Subsoontorn et al. was in turn considered within the above Cochrane review by Dinnes et al.,⁽²⁰⁰⁾ who concluded that the majority of the 31 RT-LAMP or CRISPR assays included in Subsoontorn et al. would not be suitable for the point-of-care setting.⁽²⁰⁰⁾ No discussion was provided within Subsoontorn et al. with respect to this consideration.

Yang et al. aimed to evaluate the clinical performance of commercial assays and RT-LAMP, as opposed to in-house laboratory rRT-PCR assays, and included studies published as of 25 March 2020.⁽²⁰⁵⁾ Eighteen articles were included in total, which examined five commercial assays and RT-LAMP. A meta-analysis was performed for each of the assays and for RT-LAMP overall, but no commentary was provided on the quality of individual contributory studies.

Axell-House et al. conducted a systematic search of articles published as of 19 June 2020 and included studies which performed tests on at least 10 patients.⁽²⁰⁶⁾ A narrative review of results was provided in place of a meta-analysis, including a detailed description of the quality of reporting within included studies.

4.4.5 Independent evaluations by the Foundation for Innovative New Diagnostics (FIND)

In addition to review evidence, this search identified independent evaluations performed by FIND.^(207, 208) FIND and the Global Fund⁽²⁰⁹⁾ are co-conveners of the Access to COVID-19 Tools (ACT) Accelerator Diagnostics Pillar, which is a global collaboration to accelerate the development, production, and equitable access to COVID-19 tests, treatments, and vaccines. This collaboration was set up in response to a call from G20 leaders in March and launched by the WHO, the European Commission, France and the Bill & Melinda Gates Foundation in April 2020. As part of this action, FIND has conducted independent evaluations of molecular tests and immunoassays, in collaboration with WHO, the University Hospitals of Geneva (HUG) and other groups, to assist in-country decision-making. Evaluations of SARS-CoV-2 assays have been made publicly available on the finddx.org website.

4.5 Diagnostic accuracy findings

4.5.1 Diagnostic accuracy of molecular tests overall

An evidence summary from Health Technology Wales⁽²⁰²⁾, systematic reviews by Subsoontorn et al., Yang et al. and a scoping review by Axell-House et al.⁽²⁰⁴⁻²⁰⁶⁾, and the results of independent evaluations of molecular tests by FIND^(207, 208) were identified as sources of information on the diagnostic accuracy of molecular tests overall (results specific to point-of-care testing are presented in the following section).

Health Technology Wales identified, as of 4 May 2020, one systematic review and 39 sources reporting primary data on molecular test methods.⁽²⁰²⁾ The majority of tests considered were laboratory-based rRT-PCR tests, conducted using standard in-house or commercially available PCR reagents and equipment. The RT-PCR primer used and the method and type of sampling varied between studies, however. Five studies were identified which reported the diagnostic performance of RT-LAMP based methods, which have the potential to be used at point of care. However, none of the studies included in the review reported data from the point-of-care or near-patient setting.

Pooled analysis of 19 studies (1,502 patients) estimated the sensitivity of an initial rRT-PCR test result to be 89% (95% CI 81% to 94%), using results of repeated rRT-PCR as the reference standard.⁽²⁰²⁾ The five studies which reported the diagnostic accuracy of isothermal amplification assays included 972 patients or samples in total. These involved the diagnosis of 130 patients with suspected COVID-19 using equivalent test results from rRT-PCR as a reference standard. The use of a single rRT-PCR test as a reference standard may not be representative of true disease outcomes; as such, Health Technology Wales considered it inappropriate to use the

results of these studies to derive a single pooled estimate of sensitivity and specificity. Reported sensitivity and specificity estimates ranged from 74.7% to 100% and 87.7% to 100%, respectively (see Table 4.1).⁽²⁰²⁾

Table 4.1. Reported values of sensitivity and specificity for isothermal amplification methods as reported by Health Technology Wales⁽²⁰²⁾

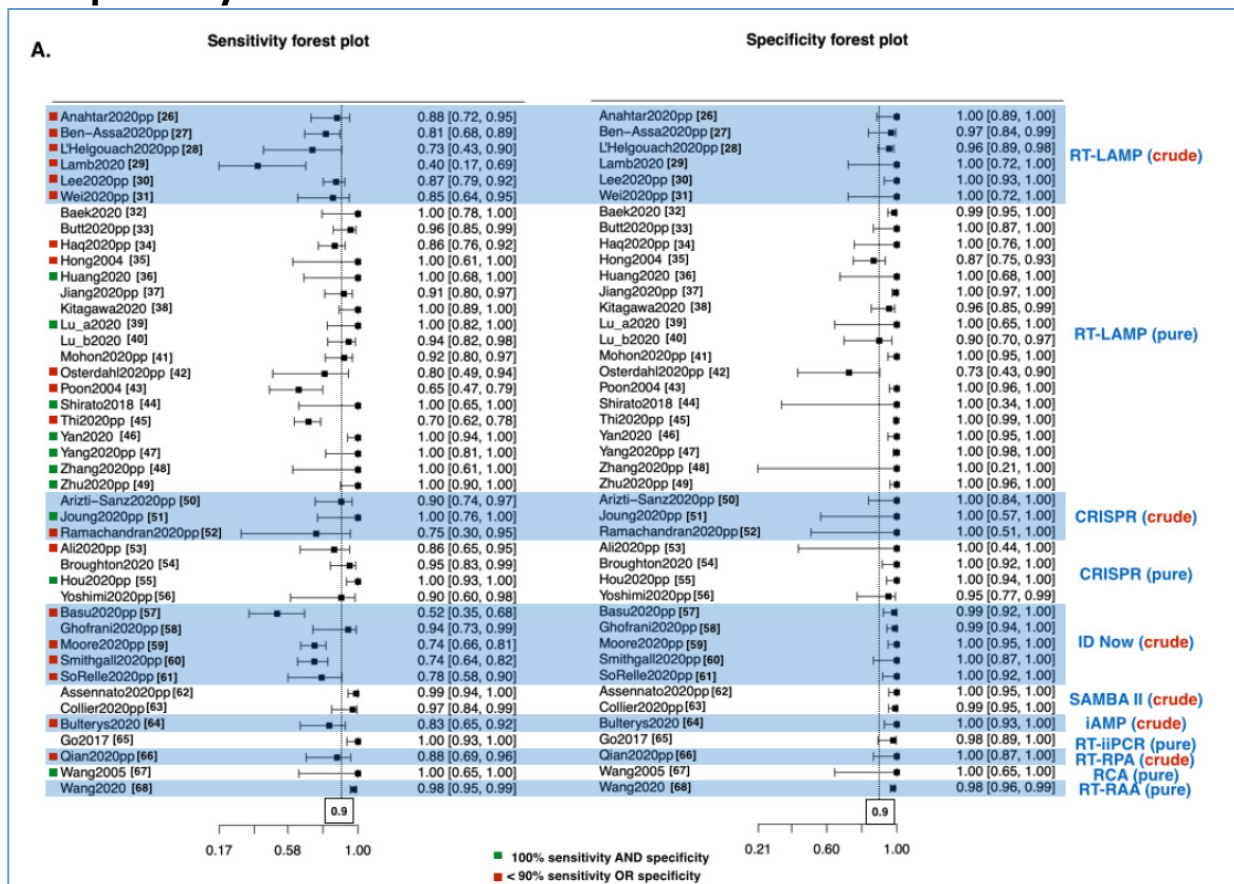
Isothermal amplification method: company, brand	Author of study	Sensitivity, % (95% CI)	Specificity, % (95% CI)
Abbott, ID NOW™ ('NEAR' isothermal amplification)	Harrington et al. 2020	74.7 (67.8 - 80.8)	99.4 (97.8 - 99.9)
Abbott, ID NOW™ ('NEAR' isothermal amplification)	Zhen et al. 2020	87.7 (76.3 - 94.9)	100 (93 - 100)
RT-LAMP* (authors' non-commercialised* RT-LAMP based assay; single-tube, colorimetric detection of N gene)	Baek et al. 2020	100	98.7
RT-LAMP (authors' non-commercialised* RT-LAMP based assay, detection of N gene)	Lu et al. 2020	94.4 (81.3 - 99.3)	90.0 (68.3 - 98.8)
RT-LAMP (extraction using QIAmp Viral RNA Mini Kit, optimisation of RT-LAMP assay for detection of orf1ab and S genes)	Yan et al. 2020	100 (92.3 - 100)	100 (93.7 - 100)

*at time of publication.

Subsoontorn et al. presented results for the individual and pooled sensitivity and specificity of various forms of isothermal amplification methods; these are reproduced in Figure 4.3.⁽²⁰⁴⁾ The diagnostic accuracy of RT-LAMP-based molecular methods appeared to depend on whether crude or purified samples were analysed. Where crude samples were analysed (for example, nasopharyngeal or saliva samples), this resulted in the lowest sensitivity among isothermal methods studied; sensitivity values ranged from 40% to 88%. However, analysis of purified samples led to sensitivity values in excess of 90% in the majority (14 out of 18) of RT-LAMP studies. Subsoontorn et al. classified three contributing studies among these 18 as being of higher quality (based on sample size, and lack of notable concern regarding risk of bias or issues regarding applicability) and noted that the sensitivity estimates

among these ranged from 70%⁽²¹⁰⁾ to 100%.^(211, 212) Subsoontorn et al. considered that this difference in sensitivity might be accounted for by varying concentrations of viral RNA in the samples tested; over one third of the positive samples in the study with lower sensitivity had a cycle threshold (Ct) value between 30 and 40, indicating a low viral load in associated samples, and the sensitivity of RT-LAMP has previously been shown to decrease with decreasing RNA concentration.⁽²⁰⁴⁾ Similar to RT-LAMP in purified RNA samples, alternative methods using CRISPR or the SAMBA II platform showed sensitivity values above 90% in the majority of cases.

Figure 4.3 Figure from Subsoontorn et al;⁽²⁰⁴⁾ Forest plots of sensitivity and specificity for molecular test methods.



Note: Listed studies included some where the target condition included SARS-CoV or MERS-CoV, as opposed to SARS-CoV-2. 'pp' indicates that the study results were from a preprint manuscript (not peer-reviewed). Vertical dotted lines indicate 90% sensitivity or specificity.

Subsoontorn et al. also calculated log diagnostic odds ratios (lnDOR) (see blue box for explanation) to enable comparison across individual tests and subgroups;⁽²⁰⁴⁾ Results for individual studies are reproduced in Figure 4.4; higher ln(DOR) values indicate better overall test performance, in terms of combined sensitivity and specificity. In pooled estimates, the authors found that RT-LAMP using crude

samples (as opposed to pure samples) had the lowest performance ($\ln(\text{DOR}) = 4.46$). This was followed by CRISPR using crude samples ($\ln(\text{DOR}) = 4.85$) and the Abbott ID NOW™ test ($\ln(\text{DOR}) = 5.16$). The SAMBA-II test was found to show the highest performance ($\ln(\text{DOR}) = 8.00$), followed by RT-LAMP and CRISPR diagnosis using purified samples ($\ln(\text{DOR})$ of 6.06 and 5.94, respectively).

Diagnostic odds ratios (DORs) are calculated as the cross product of the 2x2 diagnostic contingency table (Figure 4.2).⁽²¹³⁾ As such, they represent the ratio of the odds of a test being positive where the individual has the condition to the odds of the test being positive where the subject does not have the condition.

$$\text{DOR} = \frac{(\text{True positives} \times \text{True negatives})}{(\text{False positives} \times \text{False negatives})}$$

This results in a single numerical value for describing test performance.

Interpretation of DORs

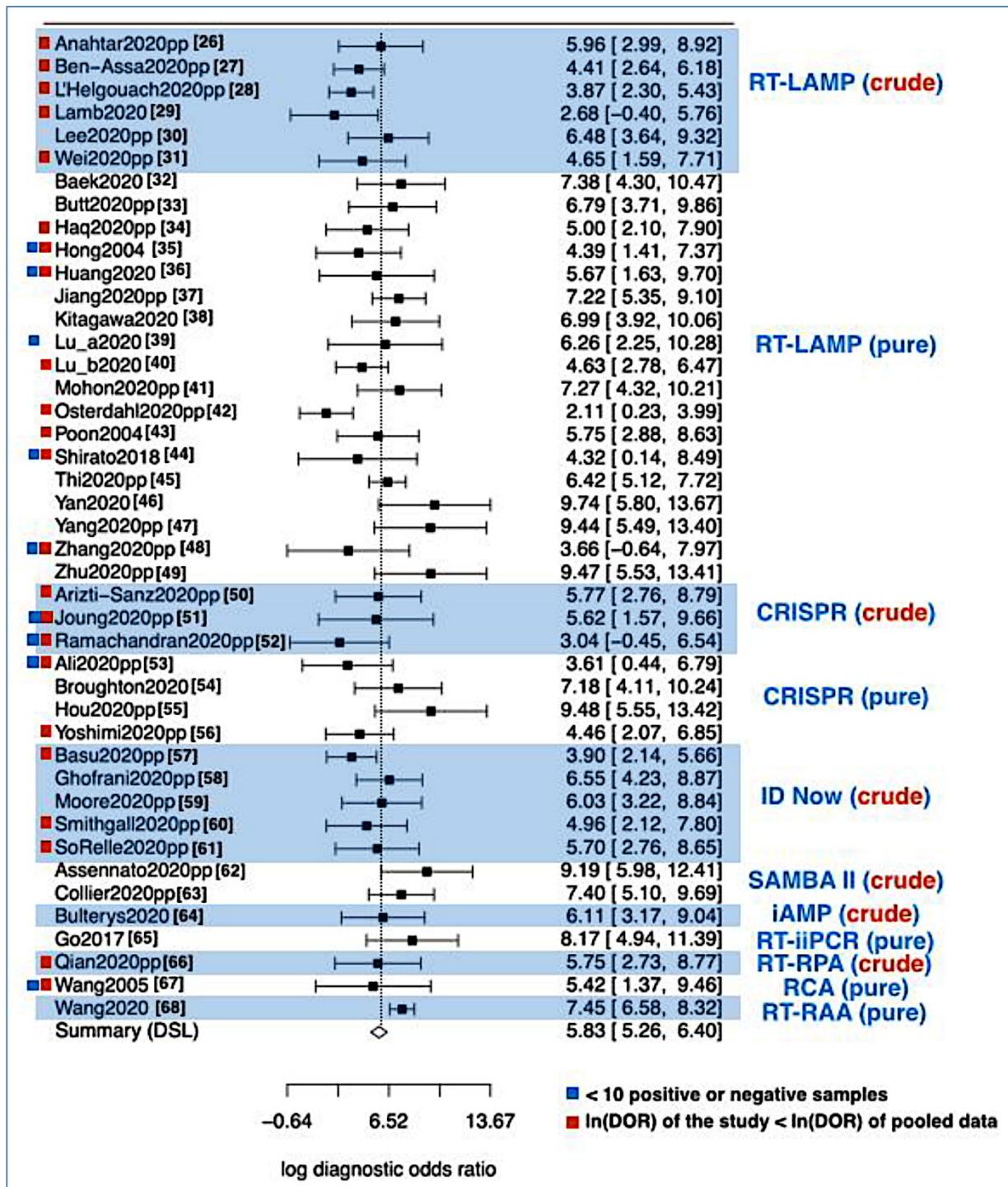
A DOR of 1 represents an uninformative test and increasing DOR values represent increasing discriminatory power of a test.

It is important to note in the interpretation of DOR values that the same DOR may be achieved for different combinations of sensitivity and specificity. For example, a DOR of 36 could be achieved by a test with specificity of 90% and sensitivity of 80%, or by a sensitivity of 90% and a specificity of 80%.

Usage of DORs

DORs may be applied in meta-analyses when making comparisons between tests or between subgroups, and may be expressed using the natural log, that is to say $\ln(\text{DOR})$. As with DOR values, higher $\ln(\text{DOR})$ values indicate better overall test performance, in terms of combined sensitivity and specificity.

Figure 4.4. Figure reproduced from Subsoontorn et al;⁽²⁰⁴⁾ Forest plots of ln(DOR) for molecular test methods. Higher ln(DOR) values indicate better overall test performance, in terms of combined sensitivity and specificity



Yang et al.⁽²⁰⁵⁾ evaluated the sensitivity and specificity of several commercial RT-PCR platforms (automated or multiplex commercial systems) and RT-LAMP. Commercial

assays evaluated included high-throughput platforms (Roche’s Cobas® and Hologic Inc.’s Panther Fusion®) and automated assays (GenMark’s ePlex®, Diasorin’s Simplexa™ and Cepheid’s Xpert® Xpress). Summary pooled results are presented in Table 4.2.

Table 4.2: Yang et al. pooled results for sensitivity and specificity for several commercial RT-PCR assays or platforms (as opposed to in-house rRT-PCR assays) and RT-LAMP based tests⁽²⁰⁵⁾

Test Company, brand	N articles	Pooled Sensitivity % (95% CI)	Pooled Specificity % (95% CI)
GenMark, ePlex®	3	94 (89 - 98)	100 (97 - 100)
Hologic, Panther Fusion®	4	99 (96 - 100)	98 (96 - 100)
Diasorin, Simplexa™	3	100 (98 - 100)	97 (94 - 99)
Roche, Cobas®	4	99 (99 - 1.00)	96 (94 - 97)
Cepheid, Xpert® Xpress	5	99 (98 - 100)	97 (95 - 98)
RT-LAMP	6	98 (94 - 99)	99 (97 - 100)

Axell-House et al. identified 49 articles for review of test performance and provided a comprehensive review of findings.⁽²⁰⁶⁾ Comparisons of interest in their review included:

- agreement of rRT-PCR or automated rRT-PCR platforms or instruments with a ‘composite reference standard’
- performance of other NAAT test methods (for example, isothermal amplification methods such as RT-LAMP) relative to standard rRT-PCR
- performance of NAAT platforms (for example, the ID NOW™ platform, versus rRT-PCR
- agreement between different NAAT platforms.

The authors identified 14 studies which compared alternative NAAT methods to rRT-PCR. These included five studies which evaluated RT-LAMP assays; four reported sensitivity of 100% and specificity of 95.6% to 100% while one reported ‘accuracy’ of 92.9%. Additional studies considered reverse transcription recombinase-aided amplification (RT-RAA), triplex rRT-PCR, an automatic integrated gene detection system and digital droplet PCR (ddPCR).

For performance of NAAT platforms versus rRT-PCR, results are presented in Table 4.3 and broadly corresponded to those presented by Yang et al.⁽²⁰⁵⁾

Table 4.3. NAAT platform test performance characteristics relative to rRT-PCR, as reported by Axell-House et al.⁽²⁰⁶⁾

Test	Description	N articles	Sensitivity (reported as 'percentage positive agreement' or 'sensitivity')	Specificity (reported as 'negative percentage agreement' or 'specificity')
Company, brand			Lowest reported to highest reported	Lowest reported to highest reported
Abbott, ID NOW™	Automated Isothermal NAAT	3	71.7% to 94%	100% in all
Abbott, RealTime	Automated Multiplex RT-PCR	2	93% to 100%	92.4% to 100%
Cepheid, Xpert® Xpress	Automated Multiplex RT-PCR	3	96.1% to 100%	74.3% to 100%
Roche, Cobas® 6800	Automated Multiplex RT-PCR	2	94.2% to 100%	99.5% to 99.6%
Diasorin, Simplexa™	Multiplex RT-PCR	2	96% to 100%	100% to 100%
Aus Diagnostics	Multiplex RT-PCR	1	100%	92.16%
Luminex, NxTAG® CoV	Multiplex RT-PCR	1	97.8%	100%
Mesa BioTech Inc., Accula	Automated PCR with Lateral Flow Assay	1	68%	100%
Hologic, Panther Fusion®	Multiplex RT-PCR	1	98.7%	98.1%
Qiagen, QIAstat-Dx	Automated Multiplex RT-PCR	1	100%	93%

In assessing agreement between different NAAT platforms, the ID NOW™ platform was the most frequently studied test. Summary estimates (and 95% CIs) of ID NOW™ sensitivity, expressed by the individual study authors as 'positive percent agreement' were 75.0% (67.6%-80.6%) and 75.2% when compared with Abbott RealTime, 54.8% when compared with the Xpert® Xpress test, 80.4-87.7% when compared to Panther Fusion®, and 73.9% when compared with the Roche Cobas® 6800. Two studies evaluated the Xpert® Xpress compared to the Cobas® 6800; one found a sensitivity of 98.9% and specificity of 92% (reported by the authors as positive percent agreement and negative percent agreement) and the other reported an overall agreement of 99%.

Overall, Axell-House et al. concluded that several alternative NAAT methods, many of which may involve less complexity and or may be faster to perform, may be comparable to standard rRT-PCR. Also, proprietary multiplex, automated, and or point-of-care methods are comparable in accuracy to rRT-PCR and to each other, although the Abbott ID NOW™ SARS-CoV-2 test was found to have lower comparative agreement with other platforms. However, the authors noted substantial heterogeneity among studies in terms of test types, reference standards, metrics reported, and details of study design and methodology. Furthermore, most of the included studies were at risk of bias and few of the tests were found to have been assessed appropriately; issues noted by the authors included lack of reporting of how samples were selected for evaluation, as well as lack of reporting of patient symptom status, patient demographics, or when the reference standard was conducted on patient samples compared to the index test. Several studies were found to have calculated test performance characteristics based on number of samples instead of number of patients, and many did not report on the management of indeterminate or invalid results. As such, the reported accuracy should be interpreted with caution.⁽²⁰⁶⁾

Finally, the Foundation for Innovative New Diagnostics (FIND) performed independent evaluations of manual molecular test kits as of August 2020.⁽²⁰⁷⁾ These evaluations aimed to verify the limit of detection (LOD) – as reported by the manufacturers – and the clinical performance, of 22 manual molecular test kits in comparison to an in-house rRT-PCR protocol (optimized based on the Tib Molbiol assay).⁽²⁰⁷⁾ The LOD analysis was performed using cultured viral stocks from a clinical isolate from Switzerland; this was quantified using an E gene standard. The clinical performance analysis was conducted on extracted samples from individuals suspected to have COVID-19, 50 of which were reference rRT-PCR positive and 100 of which were reference rRT-PCR negative. Results of these evaluations are presented in Appendix C. While the vast majority of these test results were of rRT-PCR-based technologies, at least one evaluation was of an isothermal amplification

method, the Atila iAMP test, which uses a proprietary 'OMEGA' amplification technique. Across all of the test kits, the lowest clinical sensitivity reported was 90% while the lowest clinical specificity reported was 95%. FIND also evaluated two near-patient automated molecular tests; the results for these evaluations are described in the following section.

4.5.2 Point-of-care and near-patient testing approaches

Molecular tests

The Cochrane review of point-of-care molecular tests identified 13 studies which reported 15 evaluations of four different commercially available rapid molecular tests:⁽²⁰⁰⁾ six evaluated the ID NOW™ test, seven evaluated the Xpert® Xpress, and one evaluation each was performed for the Accula and SAMBA II tests. With respect to technology, ID NOW™ and SAMBA-II use isothermal techniques, Xpert® Xpress is based on rRT-PCR, and Accula is described as a PCR plus LFA (lateral flow assay) test. The review found that no study was at low risk of bias and the authors had concerns about the applicability of results in all studies.⁽²⁰⁰⁾ With respect to tests identified within the Cochrane review overall (both molecular and antigen tests), the authors concluded that the limitations within the design and execution of currently published studies limits the strength of conclusions that can be drawn. The authors noted that it is unclear whether the limitations in the primary studies will lead to overestimates or underestimates of test accuracy, and therefore all results should be interpreted with a high degree of caution.

Table 4.4. Results of Cochrane review of point-of-care molecular-based tests⁽²⁰⁰⁾

Test	Evaluations (studies)	Samples	Cases	Average** sensitivity, % (95% CI)	Average** specificity, % (95% CI)
All tests*	13 (11)	2,194	1,113	95.2 (86.7 - 98.3) Range: 68 - 100	98.9 (97.3 - 99.5) Range: 92 - 100
Individual tests: Company, brand					
Abbott ID NOW™	5	1,003	496	76.8 (72.9 - 80.3)	99.6 (98.4 - 99.9)
Cepheid Xpert® Xpress	6	919	479	99.4 (98.0 - 99.8)	96.8 (90.6 - 99.0)
Mesa Biotech Inc. Accula	1	100	50	68.0 (53.3 - 80.5)	100 (92.9 - 100)
DRW Ltd SAMBA II	1	172	88	98.9 (93.8 - 100)	96.4 (89.9 - 99.3)

DRW: 'Diagnostics for the Real World'

*where 2x2 data were available to enable pooled estimates

** Pooled estimates calculated by Dinnes et al. using a bivariate hierarchical model

FIND performed a limited clinical performance evaluation of the Cepheid Xpert® Xpress SARS-CoV-2 assay at University Hospitals of Geneva.⁽²⁰⁷⁾ A second collaborating site, the Translational Health Science and Technology Institute (THSTI) conducted a similar limited clinical performance evaluation of the Molbio TrueNat™ SARS-CoV-2 assay.⁽²⁰⁷⁾ Both studies were performed using frozen, stored respiratory samples from COVID-19 suspects and the reference standard in each case was a high-throughput laboratory-based rRT-PCR assay. Results are reproduced in Table 4.5.

Table 4.5. Results of FIND independent evaluation of two near-patient automated molecular tests⁽²⁰⁷⁾ (reproduced from finddx.org)

Company, Product name	Gene target	Clinical sensitivity (50 positives), % (95% CI)	Clinical specificity* (100 negatives), % (95% CI)	Reference test
Cepheid, Xpert® Xpress	N2	100 (92 - 100)	99 (95 - 100)*	Roche Cobas ® SARS-CoV-2 N = 44 positive N = 100 negative
	E	97.7 (88 - 100)	100 (96 - 100)	
Molbio Diagnostics TrueNat	E+RdRP	98 (90 - 98)	96 (90 - 98) *	Altona Diagnostics (n=86) /LabGun™ (n=64) and/or Seegene, Inc. (n=12) N = 51 positive N = 111 negative

** Clinical specificity: Further investigation is needed to determine if apparent false positives are truly false positives or whether they are due to a false negative reference standard result*

Subsoontorn et al.⁽²⁰⁴⁾ and Yang et al.,⁽²⁰⁵⁾ as reported above, also included results for tests such as the ID NOW assay and the Xpert Xpress test, which are intended for near-patient use. While the review scope within Subsoontorn et al. was to assess the accuracy of point-of-care tests, it is unclear how point-of-care tests were defined for inclusion within the review. The authors of the Cochrane review of point-of-care molecular tests noted that the majority of the RT-LAMP or CRISPR assay evaluations included in Subsoontorn would not be appropriate for the point-of-care setting.⁽²⁰⁰⁾ Similarly, it is not clear that all tests presented in Yang et al. are suitable for the near-patient setting. As such, overall results from these reviews have been included only within section 4.5.1 of the present report.

Rapid Antigen Detection Tests (RADTs)

The Cochrane review of point-of-care antigen tests identified five studies which reported eight evaluations of antigen tests (four colloidal gold immunochromatographic assays (CGIA), and four fluorescent immunoassays, (FIA)). Seven of these evaluations considered one of five commercially produced tests and one evaluated an in-house CGIA method.⁽²⁰⁰⁾ Based on the full eight evaluations, which included 943 samples, the sensitivity of antigen tests varied greatly, ranging from 0% to 94% and with an average sensitivity of 56.2% (95% CI 29.5 to 79.8%). Average specificity was 99.5% (95% CI 98.1% to 99.9%). There was a limited availability of data for individual antigen tests; no more than two studies were available for any one test. Results are reproduced in Table 4.6. However, as noted above, the authors cautioned that the limitations of the primary studies mean that all results presented should be interpreted with a high degree of caution.

FIND is conducting prospective diagnostic evaluation studies in collaboration with multiple, independent sites to determine the accuracy of RADTs.⁽²⁰⁸⁾ Participants in these evaluation studies include individuals presumed to have COVID-19 due to the presence of symptoms or with close contact with a confirmed case, but may not capture the full spectrum of disease; therefore, sensitivity and specificity estimates may not be indicative of the real-world performance of these tests in all intended use settings. Interim analyses are performed at 25% and 50% enrolment, and the evaluation is stopped if tests do not meet 97% specificity. To date, evaluations have been completed for three assays, as per Appendix D, and summary information was available for two of these (Table 4.7). One of these, the SD Biosensor Inc. Standard Q test, was evaluated in Germany and Brazil in adults suspected of SARS-CoV-2 infection and undergoing community-based testing. Evaluation was based on nasopharyngeal and oropharyngeal swab samples and compared to PCR methods using commercial assays (Germany), such as the Cobas[®] SARS-CoV-2 assay, the Abbott RealTime assay, and an in-house assay (Brazil) based on the US CDC protocol. The Standard Q test was found to have relatively high clinical sensitivity (values of 76.6% and 88.7%) and specificity (97.6% and 99.3%) (see Table 4.7). In contrast, the second test for which evaluation data were available was found to have low specificity in preliminary analysis, and the evaluation was halted early due to sensitivity results being underpowered.

Table 4.6 Results of Cochrane evaluation: Summary of analyses of RADT test accuracy (adapted from Dinnes et al.⁽²⁰⁰⁾)

Test	Evaluations (studies)	Samples	Cases	Average** sensitivity, % (95% CI)	Average** specificity, % (95% CI)
All tests	8 (5)	1180	762	56.2 (29.5 - 79.8)	99.5 (98.1 - 99.9)
				Range: 0 - 94	Range: 90 - 100
Viral load subgroup					
High viral load	7 (5)	400	400	93.2 (63.6 - 99.1)	N/A
Low viral load	7 (5)	341	341	32.6 (17.5 - 52.6)	N/A
Individual tests					
Beijing Savant FIA	1	109	78	16.7 (9.2 - 26.8)	100 (88.8 - 100)
Coris Bioconcept CGIA*	2	466	226	54.4 (47.9 - 60.8)	99.6 (97.7 - 99.9)
Liming CGIA	1	19	9	0 (0 - 33.6)	90.0 (55.5 - 99.7)
RapiGEN CGIA	1	109	79	62.0 (50.4 - 72.7)	100 (88.4 - 100)
Shenzhen Bioeasy FIA*	2	238	162	89.5 (83.8 - 93.3)	100 (95.2 - 100)
In-house FIA	1	239	208	67.8 (61.0 - 74.1)	100 (88.8 - 100)

CGIA: colloidal gold immunoassay; FIA: fluorescent immunoassay

*2x2 tables combined prior to calculating estimates.

** Pooled estimates calculated by Dinnes et al. using a bivariate hierarchical model

Table 4.7 Results of FIND evaluation of RADTs⁽²⁰⁸⁾; SD Biosensor Inc. STANDARD Q COVID-19 test and Coris BioConcept COVID-19 Ag Respi-Strip test

Country of evaluation	SD Biosensor, Inc. STANDARD Q COVID-19 Ag Test		Coris BioConcept COVID-19 Ag Respi-Strip
	Germany	Brazil	Germany and UK
Total N (valid PCR results)	1,259	400	425
Age; mean, (range), N	35, (18-80.4), 1,242	37 (2-94); 397	43, (18-89); 424
Gender (% female)	48.9%, (616/1,222)	57.3%, (229/398)	61% (255/418)
Symptoms present (% yes)	84.7%, (1039/1,227)	98.7%, (392/397)	69.2% (290/419)
Days from symptom onset [median (Q1-Q3); N]	3 (2-4); 1002	5 (4-6); 397	3 (1-5); 282
Days < 0-3 (n, %)	628 (62.7%)	85 (21.4%)	172 (61.0%)
Days 4-7 (n, %)	310 (30.9%)	273 (68.8%)	75 (26.6%)
Days 8+ (n, %)	64 (6.4%)	39 (9.8%)	35 (12.4%)
Positivity [%; (n/N)]	3.7%, (47/1259)	26.5%, (106/400)	1.9% (8/425)
Clinical Sensitivity, % (95% CI); N	76.6 (62.8 - 86.4); 47	88.7 (81.3 - 93.4); 106	50 (21.5 - 78.5); 8 **
Sensitivity days ≤7, N	80 (64.1 - 90.1); 35	90.7 (83.3 - 95.0); 97	42.9 (15.8 - 75.0); 7 **
Sensitivity Ct ≤33, N	87.8 (74.5 - 94.7); 41	91.9 (84.9 - 95.9); 99	
Sensitivity Ct ≤ 25, N	100 (84.5 - 100); 21	95.9 (86.3 - 98.9); 49	
Clinical Specificity, % (95% CI), N	99.3 (98.6 - 99.6); 1212	97.6 (95.2 - 98.8); 294	95.9 (93.5 - 97.4); 412 **
Invalid rate (%; n/N)	0%, (0/1259)	0%, (0/400)	1.2% (5/425)
Analytical Sensitivity (pfu/ml)	5.0 x 10 ³ pfu/ml ~ 7.14 x 10 ³ TCID ₅₀ /ml The claimed LOD by the supplier was 3.06 x 10 ^{2.2} TCID ₅₀ /ml, which is the equivalent of approximately 3.9 x 10 ² pfu/ml. Therefore, the LOD was verified to be 10-fold higher than that found by the supplier, using a different viral strain.		1 x 10 ⁴ pfu/ml ~ 1.43 x 10 ⁴ TCID ₅₀ /ml The supplier reported LOD was 7.14 x 10 ³ TCID ₅₀ /ml, which is the equivalent of about 5 x 10 ³ pfu/ml. Therefore, the LOD was verified to be 2-fold higher than that found by the supplier, using a different viral strain.

**Note: Evaluation stopped after preliminary analysis indicated specificity below 97%; therefore the sensitivity estimate was insufficiently powered.

4.6 Additional factors related to test performance

While this chapter has focused primarily on measures of diagnostic accuracy, other factors may be important in determining test performance, for example performance of alternative sampling sites.

4.6.1 Use of saliva sampling

Saliva-based tests may offer benefits over the current testing procedure, which is primarily based on nasopharyngeal sampling, in terms of ease of use, transmission risk, acceptability to individuals undergoing sampling, and resource use. However, across the literature there is uncertainty surrounding the performance of tests using saliva samples. Some studies have reported that there is an overall higher rate of detection of SARS-CoV-2 associated with reference samples than with salivary samples,⁽²¹⁴⁻²¹⁶⁾ while other studies have found no significant difference in sensitivity between rRT-PCR on saliva samples and nasopharyngeal sampling.^(197, 217-220)

The diagnostic accuracy of saliva-based samples in rRT-PCR testing has previously been reviewed by HIQA,⁽²²¹⁾ and has been assessed in a recent meta-analysis by Czumbel et al.⁽²²²⁾ HIQA found that the detection of SARS-CoV-2 by nasopharyngeal and or oropharyngeal swabs ranged from 79.3% to 100% relative to all known positive samples, while the corresponding detection of SARS-CoV-2 by saliva ranged from 64.7% to 100%. Czumbel et al. calculated summary estimates for the sensitivity of saliva and nasopharyngeal-based tests as 91% (CI 80-99%) and 98% (CI 89-100%), respectively.⁽²²²⁾ Individual studies contributing to the meta-analysis found that the sensitivity of saliva-based tests among known SARS-CoV-2 infected patients ranged from 78% to 100%.

Importantly, methods used to collect saliva samples vary across studies; such collection methods have implications for the specificity of the samples collected, ease of collection, equipment required and required experience level of the clinician. Other factors, including transport medium, temperature during transportation, time passed between specimen collection and RNA extraction, and timing of sampling and study population included (that is, asymptomatic or symptomatic patients) have the potential to affect the outcome of the tests.^(222, 223)

Due to limited timelines within the present review, it was not possible to comprehensively evaluate the suitability of saliva-based sampling for alternative tests to current laboratory-based rRT-PCR, or the diagnostic accuracy associated with saliva-based sampling in conjunction with such tests. However, among the systematic reviews and meta-analyses of tests which were included in this report and which detailed the sample types reported in contributing studies, only one study within one review was noted to have reported on saliva-based testing — Subsoontorn et al. included a study by L’Helgouach et al., which reported results of

'EasyCoV', a direct, saliva-based RT-LAMP detection assay.⁽²²⁴⁾ In the meta-analysis performed by Subsoontorn et al., this assay was associated with the second lowest sensitivity (73%, 95% CI 43-90), and the second widest confidence interval, of six studies which assessed RT-LAMP in crude samples.⁽²⁰⁴⁾

4.7 Limitations of the existing literature

Reviews which considered alternative molecular and antigen tests, and which were included within the present report, consistently reported significant limitations of the primary diagnostic test accuracy literature. In particular, the Cochrane review of point-of-care molecular and antigen tests⁽²⁰⁰⁾ and the review of NAATs by Axell-House et al.⁽²⁰⁶⁾ described important flaws in the majority of the primary research.

The authors of the Cochrane review firstly noted that the vast majority of antigen and molecular assays that were suitable for use at the point of care at the time of the review did not have any published or preprint reports of accuracy. The authors secondly noted that, where accuracy data were reported, the limitations of the primary studies' design and execution limit the strength of the conclusions that can be drawn from a review, and that it was unclear whether these limitations would lead to overestimation or underestimation of test accuracy.⁽²⁰⁰⁾ Axell-House et al. similarly concluded that current studies estimating test performance characteristics have imperfect study design and statistical methods, employ heterogeneous methods, and overall have a high risk of bias.⁽²⁰⁶⁾

For example, as rRT-PCR was the only reference standard for diagnosing the presence of SARS-CoV-2, it is not possible to comment on the accuracy of alternative tests in those who are rRT-PCR negative but meet case definition criteria for SARS-CoV-2 infection.⁽²⁰⁰⁾ Also, the use of a second rRT-PCR assay to determine the disease status of samples with discordant results following rapid molecular testing is considered likely to introduce additional bias.⁽²⁰⁰⁾

Review authors also referred to the lack of information on whether tests perform in the same way or differently depending on whether or not those tested have symptoms of COVID-19, and whether, among symptomatic individuals, the duration of symptoms affects results.⁽²⁰⁰⁾ Dinnes et al. further noted that studies included in the Cochrane review appeared to be based on remnant or residual samples for testing and many selectively included high percentages of samples which were confirmed rRT-PCR positive for SARS-CoV-2 RNA. As such, the generalisability of these results to settings where disease prevalence is low is uncertain; the review underpins this concern by citing evidence⁽²²⁵⁾ that sensitivity and specificity for tests, generally, may be variable under different disease prevalence.

Further to the limitations of the primary research underpinning included reviews, the research landscape for tests involved in the detection of SARS-CoV-2 is rapidly-evolving and recent results of diagnostic accuracy or validation studies are not captured in the secondary research literature (systematic reviews and meta-analyses) described within this report. The most recent literature search performed in a review included in this chapter was carried out on 19 June 2020.⁽²⁰⁶⁾ As such, results included in even the most recent systematic reviews do not account for diagnostic accuracy results from the most recent three months of the pandemic. With respect to the present report, the extremely limited timeline within which this report was conducted precluded the examination of primary literature beyond the evaluations reported by FIND. Also, the short timeline and extensive scope of this rapid HTA, such that all alternatives to laboratory-based rRT-PCR were for consideration, meant that rigorous quality assessment of included secondary research could not be performed, though efforts have been made to highlight strengths and limitations of reviews.

Finally, with respect to transferability of diagnostic accuracy results to certain settings, there remains a lack of information on certain patient subpopulations or settings. This includes asymptomatic individuals, specific at-risk populations such as healthcare workers, and the validation of diagnostic performance of tests beyond their use in the hospital setting or in self-administered tests.⁽²⁰⁰⁾ Also, there is a lack of information on the diagnostic accuracy of emerging technologies.

4.8 Conclusions

Inaccurate diagnostic tests undermine efforts of containment of the SARS-CoV-2 pandemic.⁽¹⁹¹⁾ False positive test results lead to inappropriate labelling of a person as infected; consequences include unnecessary isolation of the person and restriction of movements of their close contacts. Similarly, with imperfect tests, negative results mean only that a person is less likely to be infected with SARS-CoV-2 rather than that they are not infected. The probability of infection with SARS-CoV-2, in the presence of a negative result, is a function of the sensitivity of the test and of the pre-test probability of being infected, which depends on factors such as local COVID-19 prevalence, SARS-CoV-2 exposure history, symptoms, and potential additional risk factors for infection. Such false negative results may be highly consequential due to the potential for missing infected persons who may be pre-symptomatic or asymptomatic and may go on to infect others;⁽¹⁹¹⁾ this may have particularly severe consequences in the hospital setting for both the infected patient — especially where they are to undergo invasive procedures which may interfere with their COVID-19 recovery — and surrounding patients and staff. As such, ensuring optimal diagnostic accuracy is a crucial component of any testing programme.

Due to the need to tailor testing to separate objectives, settings and requirements, diagnostic accuracy is just one of many interrelated components for assessment when evaluating the utility of a particular test. As noted in section 4.2, the WHO has published desirable and minimally acceptable product profiles for tests for four specific purposes considered as high priority in supporting the response to the COVID-19 pandemic.⁽¹⁹⁵⁾ Approximately 30 features are characterised for each type of testing purpose, indicating the complexity involved in an evaluation. Due to the limitations of the timescale for completing this review, all features considered important in the evaluation of a test cannot be considered within this review. Similarly, due to the large volume of technologies and individual commercial tests which are in the process of being brought to market, the vast majority of individual tests cannot be evaluated within the present report; as of end September 2020, 377 molecular tests and 69 antigen tests were listed within the finddx.org COVID-19 diagnostic pipeline database as either in development or commercialised within particular jurisdictions. Instead, this chapter has aimed to provide an overview of considerations in the evaluation of the performance, specifically the diagnostic accuracy, of a particular test for the detection of SARS-CoV-2, and has aimed to summarise existing published reviews of diagnostic accuracy.

Current research on diagnostic accuracy is limited by significant issues with the design and execution of primary diagnostic accuracy studies, and suggests highly variable sensitivity and specificity of both molecular and antigen-based test types for different individual tests and under different circumstances. As such, summary estimates of sensitivity and specificity must be approached with caution.

For alternative molecular tests methods, it is difficult to make conclusions on diagnostic accuracy given the broad range and types of test methods and test platforms considered within the literature, and the limitations of the literature as described in section 4.7. As observed by Axell-House et al., several alternative NAAT methods, including RT-LAMP, may be comparable in accuracy to laboratory-based rRT-PCR. Furthermore, proprietary multiplex, automated and near-patient tests may be comparable in accuracy to laboratory-based rRT-PCR as well as to each other. Nonetheless, much is unknown; for example, the diagnostic accuracy of tests using RT-LAMP as a nucleic acid amplification technique has not been reviewed in the literature with distinction between tests in the laboratory setting and tests in the near-patient setting. A comprehensive review by EUnetHTA of diagnostic accuracy of molecular methods for the detection of SARS-CoV-2 is currently in progress; the publication of the report is currently planned for 17 November 2020.⁽²⁰¹⁾ This review will include evidence published as of 14 August 2020 and may provide valuable insights into the performance and diagnostic accuracy of molecular tests and methods based on NAAT. Furthermore, the Cochrane COVID-19 Diagnostic Test

Accuracy Group is in the process of performing a review of alternative laboratory-based molecular methods for the detection of SARS-CoV-2.^(199, 200)

With respect to rapid antigen tests, specificity broadly appears to be consistently high, but sensitivity appears to show significant differences across test brands.⁽²⁰⁰⁾ Recent clinical evaluations of antigen tests as conducted by the FIND collaboration have not been included within systematic reviews. For at least one rapid antigen test, the SD Biosensor Inc. Standard Q test, the lower sensitivity value reported from among two FIND collaborating countries was 77% (see Table 4.6); this value exceeds the sensitivities reported for all but one test reviewed in the Cochrane review of rapid antigen tests.⁽²⁰⁰⁾ It is important to consider that iterative development of diagnostic tests over time may result in improved sensitivity and specificity among technologies.

As discussed in chapter 3 of this report, various jurisdictions have started to adopt the large-scale use of rapid antigen tests. The Haute Autorité de Santé (HAS), the national health technology assessment agency of France, recently issued formal opinion on the diagnostic accuracy of antigen tests for the detection of SARS-CoV-2 based on nasopharyngeal swab samples.⁽²²⁶⁾ The HAS considered that there was a lack of available data on the use of such tests in asymptomatic individuals (for example, for the screening of case contacts or more general population screening) and therefore based their consideration on the use of antigen tests in symptomatic patients. It was concluded that antigen tests are associated with lower sensitivity than that reported with RT-PCR, but that such results are highly variable among different tests. Also, while the high specificity associated with antigen tests was noted, the importance of optimal specificity was stressed due to the potential for false positive results in the context of circulating seasonal (winter) viruses. As such, HAS concluded that minimum thresholds for sensitivity and specificity must be reached in order for an antigen test to be recommended for use; a test must have a clinical sensitivity $\geq 80\%$ and a clinical specificity $\geq 99\%$. These values must be established by a manufacturer on the basis of a prospective comparative clinical study involving a series of individuals of unknown virus status recruited consecutively or randomly. Furthermore, the lower limit of the 95% CI for sensitivity must be no lower than 10 points below the threshold value and the test must be CE marked if it is to be used.

With respect to the adoption of specific alternative molecular or antigen-based tests in Ireland, findings on diagnostic accuracy should be considered with respect to the limitations of the studies generating such results. Furthermore, findings of diagnostic accuracy should be considered alongside contextual factors which may have implications for accuracy in practice, in addition to consideration of organisational factors important to decisions on the value of a particular test.

5 Operational considerations

Key points

- COVID-19 diagnostic testing is currently carried out in the National Virus Reference Laboratory (NVRL) and at least 42 hospital laboratories in Ireland using real-time reverse transcription polymerase chain reaction (rRT-PCR). Current median test turnaround (from sample collection to reporting of results) for swabs taken in the community is 29 hours and 16 hours for swabs taken in hospitals.
- Effective testing strategies rely on a portfolio of tests based on different technologies that can be used in different settings and situations. Real-time RT-PCR relies on laboratory infrastructure and highly skilled staff. Rapid molecular tests can be used to expedite clinical decision-making in both laboratory and non-laboratory settings. At present, for the purposes of diagnosis, international guidelines suggest that the clinical utility of rapid antigen detection tests (RADTs) is limited to settings with limited or no access to laboratory testing. RADTs may be used for screening purposes, with consideration to the disease prevalence.
- The benefits of rapid testing are dependent on the accuracy of the test and how the results of the test will affect patient treatment or the initiation of public health interventions. Near-patient testing (NPT) can be deployed to non-laboratory settings where more rapid turnaround times are likely to result in improved clinical and public health outcomes.
- Many rapid molecular tests are only suitable for use in laboratory settings due to requirements for RNA purification prior to the reverse transcription and amplification reactions. RNA purification is a time-consuming laboratory procedure requiring specialised kits and reagents, which are subject to supply shortages. Tests that effectively integrate all steps necessary for molecular analysis are highly desirable.
- Not all reagents (for example, protein buffer) used in RADTs are compatible with downstream amplification reactions. The use of rapid tests that are compatible with swabs and reagents in current use or devices with broad compatibility (to circumvent shortages due to supply chain issues) enables laboratory-based confirmatory testing using rRT-PCR to be carried out on the same specimen.
- Consideration should be given to the criteria recommended in national guidelines for deployment of near-patient testing. In the context of near-patient testing for COVID-19, these would ideally include:
 - For initial introduction of rapid tests into clinical practice, settings where rRT-PCR testing is currently available should be selected so that staff can gain experience in the use of the test, confirm

performance of the selected test, and troubleshoot any operational or implementation issues.

- NPT settings should establish a close link with local hospital laboratories to ensure NPT is provided in a safe and effective manner. Prior to the establishment of near-patient testing, the capacity of local laboratories to provide testing support should be considered.
 - Rapid testing devices and associated consumables could be accommodated within existing near-patient settings without the need for significant alteration of or investment in premises. Changes to work processes will require consideration at a site-specific level.
 - Operators of near-patient testing devices should receive training and demonstrate competence in all aspects of near-patient testing. Patient results should be reviewed and interpreted by appropriately qualified persons.
 - Appropriate supervision and governance must be provided. Quality assurance programmes should be implemented and monitored to ensure the results obtained in near-patient settings are accurate and reliable.
- Test turnaround times for near-patient testing will be reduced compared with laboratory based rRT-PCR owing to reduced requirements for sample handling and the eliminated need for sample transportation to centralised laboratories for processing. However, manufacturer reported turnaround times or turnaround times when tests are conducted in laboratory settings by highly skilled technical staff may not reflect the expected turnaround time for tests carried out by users with less experience and training in near-patient settings or in settings with busy workflows.
 - The results of diagnostic and screening tests carried out in near-patient and laboratory settings should be recorded in the patient's health record and reported to the Medical Officer of Health (MOH) and or Director of Public Health and notified onwards to the Health Protection Surveillance Centre (HPSC) to enable monitoring of infection trends and tracing and isolation of close contacts.

5.1 Background

The current testing strategy in Ireland involves laboratory-based testing using nucleic acid amplification technology (NAAT) methods.⁽²²⁷⁾ As described in Chapter 1, this involves the use of real-time reverse transcription polymerase chain reaction (rRT-PCR) techniques to amplify and detect viral RNA in clinical samples. Testing for SARS-CoV-2, as part of the national strategy, is currently conducted in the National Virus Reference Laboratory (NVRL) and in hospital diagnostic laboratories with additional surge capacity provided by other designated laboratories.⁽²²⁷⁾

Improvements to the current testing pathway or the establishment of decentralised testing capacity under the supervision of the NVRL could help to ensure adequacy of the national testing strategy during times of increased demand for testing (for example, rising prevalence or co-circulation of other respiratory pathogens with common symptoms). This assessment will focus on changes to the current testing pathway, in particular, the potential for testing in near-patient settings.

Near-patient (or point-of-care) tests can be defined as 'any device that is not intended for self-testing, but is intended to perform testing outside a laboratory environment, generally near to, or at the side of, the patient by a health professional'.⁽²²⁸⁾ The establishment of COVID-19 near-patient testing (NPT), particularly for settings not already undertaking other NPT services, will require changes to workflow and some work processes. Faster access to results may increase clinical effectiveness, contribute to improved outcomes for patients and facilitate timely implementation of infection prevention and control measures. It is imperative that the result provided by the device is accurate, reliable and visible in the data captured through the Track and Trace programme and in the individual patient's health records, where applicable.⁽²²⁹⁾ However, accurate and reliable results can only be obtained if near-patient testing is performed as part of a well-structured and properly governed service.⁽²³⁰⁾

Due to the unique circumstances of the COVID-19 pandemic, updated Irish National Near-Patient Testing Guidelines have been released in the interests of having an updated authoritative guideline available and to provide an opportunity for early feedback.⁽²²⁹⁾ The final version is expected to be published in 2021.

The purpose of this chapter is to outline the potential organisational considerations and resource implications associated with changes to the current laboratory-based testing process for the detection of SARS-CoV-2.

5.2 Current testing pathway

For suspected cases of COVID-19 in the community, the current COVID-19 testing pathway in Ireland comprises:

- GP or GP out-of-hours service telephone based assessment (severity of symptoms and indication for testing) and referral for testing (as necessary)
- presentation for the test appointment at a designated test centre or testing at home for those that are unable to present for testing for medical or socioeconomic reasons
- sample collection and transportation to a laboratory for processing
- laboratory-based sample processing using rRT-PCR

- delivery of results and recording of positive cases by the HPSC.

Individuals identified as close contacts through contact tracing are referred directly to designated test centres.

In the hospital setting, there are a number of testing pathways for distinct patient subgroups, including pre-admission diagnostic testing of individuals for planned surgeries, procedures or treatments using rRT-PCR, testing of unplanned admissions to inform patient allocation and testing of symptomatic in-patients. For symptomatic in-patients in hospital or acute care settings, the current pathway comprises:

- assessment by the attending physician and ordering of the test
- determine the urgency of results to inform the selection of the appropriate test (that is, rRT-PCR for non-critical cases, rapid molecular testing for critical cases)
- sample collection and delivery to laboratory reception
- laboratory-based sample processing using rRT-PCR or rapid molecular testing (if indicated)
- delivery of results and recording of positive cases by the HPSC.

There are a number of centres nationwide carrying out sample collection. The precise number of test centres active at any time is dependent on the local or regional demand for testing. Mobile testing units have also been deployed for sample collection.

Forty-three laboratories in Ireland are carrying out or equipped to carry out COVID-19 testing using rRT-PCR, including the NVRL, and 42 public or private hospital laboratories. Over 70% of sample processing takes place in the NVRL, using samples collected from testing centres nationwide. Diagnostic testing capacity has been expanded to provide capacity for over 100,000 tests per week operating on a seven-day per week basis.^(231, 232) For a swab taken in the community, the median test turnaround time (that is, from sample collection to delivery of test result) is 29 hours. For swabs taken in hospitals, the median test turnaround time is 16 hours, due to the elimination of sample transportation requirements.

5.2.1 Current indications for testing

At present, testing for the general population is largely limited to patients with symptoms suggestive of COVID-19 or close contacts of a confirmed case of COVID-19.⁽²³³⁾

Serial testing (that is, repeated testing at different points in time) may be more likely to detect acute infection among individuals with repeat exposures or potential

exposures than testing performed at a single point in time.⁽²³⁴⁾ Serial testing is offered as a precaution to people in certain settings where, despite implementation of infection prevention and control measures, infection is more likely to occur due to the essential working arrangements (for example, certain workplaces (such as food factories) or facilities (such as residential care facilities, direct provision centres)).⁽²³³⁾ Testing of some at-risk groups such as those working in food processing factories or the homeless population is also currently being provided. Testing of any suspected cases amongst people experiencing homelessness in Dublin is currently provided through Safetynet Primary Care – a registered charity funded by the HSE. The programme is intended to make testing more accessible. Safetynet is currently carrying out COVID-19 testing for homeless patients on the basis of GP referrals and also in hostels where they are prioritising the most vulnerable.⁽²³⁵⁾

Guidance on the management of planned hospital admission for non-COVID-19 care applicable to those undergoing planned surgery or medical interventions that will impact the patient's immune system (for example, surgery and immunosuppressive treatments such as chemotherapy or radiotherapy) recommend testing for COVID-19 within 48 hours of the scheduled admission, in addition to minimising the risk of exposure to others who may be infectious with SARS-CoV-2 for 14 days prior to admission.⁽²³⁶⁾ It is recommended that patients discharged to another healthcare or long stay residential centre (LSRC) are tested for COVID-19 within 24 hours of discharge.⁽²³⁶⁾

Currently, sample collection is located in COVID-19 testing centres, with drive-through capacity in many locations to reduce the risk of transmission. Individuals with symptoms suggestive of COVID-19 are advised not to attend GP surgeries, pharmacies or hospitals, and instead to contact the GP by phone for referral to a COVID-19 Assessment hub if appropriate.⁽²³³⁾ The expansion of testing to non-laboratory settings is intended to be in addition to the existing recommendations, which remain unchanged based on the current evidence. The expansion of testing aims to prioritise those who cannot easily access testing through the existing systems.

5.3 Expansion of test capacity

In addition to the gold standard rRT-PCR testing conducted in laboratory settings, and contingent on the availability of accurate and reliable assays whose performance has been validated in the setting in which they are intended to be used, additional testing may be carried out in selected near-patient settings to improve access to testing, and potentially the quality of national-level surveillance data. The expansion of test capacity will be considered in accordance with the framework outlined in Chapter 2:

- identification of the goal of testing in a given setting
- selection of the most appropriate test technology
- taking into account the necessary resources to establish testing in this setting
- and investigation of available CE-marked devices, with consideration to key device characteristics.

5.4 Testing strategies

As described in Chapter 1, testing for SARS-CoV-2 may be used for diagnostic, screening or surveillance purposes. When considering the appropriate technology for testing (that is, rapid antigen detection tests (RADTs) or automated molecular-based testing) consideration should be given to the aim of testing and the setting in which testing is to be implemented. For example, RADTs typically require less technical skill and are potentially suitable for near-patient testing; however, they may not be suitable for large scale testing due to their single-use design. Automated molecular testing methods designed for use in near-patient settings may not be suitable for some non-laboratory-based applications due to the requirement for some additional instrumentation and longer turnaround times; however, increased accuracy and throughput is achievable using molecular methods.

5.4.1 Diagnosis or screening

Diagnostic testing for SARS-CoV-2 is intended to identify current infection in symptomatic individuals and in asymptomatic individuals with a recent known or potential exposure to SARS-CoV-2.⁽²³⁷⁾ The aim of diagnostic testing is to inform clinical management of the patient and the initiation of infection and prevention and control measures such as isolation and contact tracing. Screening for SARS-CoV-2 is the use of testing to identify asymptomatic individuals, without known or potential exposure to SARS-CoV-2. Screening facilitates the identification of individuals who may be infectious, so that measures can be taken to prevent further transmission.⁽²³⁷⁾ In general, screening of asymptomatic individuals without a known or potential exposure, who are not at increased risk of poor outcomes from COVID-19, is not undertaken to facilitate optimum use of testing resources.⁽²³⁸⁾ However, in certain settings, screening of asymptomatic people may be carried out in response to public health need to potentially prevent onward transmission of SARS-CoV-2 or to ensure maintenance of critical activities.

The application of a test for diagnosis or screening depends on numerous factors including site-specific characteristics, the urgency of the result, the intended population for testing (for example, hard-to-reach populations) and the accuracy and reliability of the test result.

Real-time RT-PCR is recommended for the acute diagnosis of COVID-19.⁽²³⁹⁾ Rapid molecular testing may be carried out for diagnostic purposes in health or social care settings (such as, accident and emergency departments, residential care facilities) with limited or no access to laboratory testing, or where delayed turnaround times preclude clinical utility. Rapid molecular methods designed for use in near-patient settings may also be used for screening for SARS-CoV-2 in settings in which a short delay in the reporting of results is acceptable.

At present, the WHO advises that RADTs should not be used as a standalone test for the diagnosis of COVID-19 in settings where there is access to laboratory testing.⁽²³⁸⁾ The clinical performance of RADTs largely depends on the circumstances in which they are used. Optimal performance is achieved when an individual is tested early in the course of infection when viral load is generally highest.^(237, 240) The accuracy of alternatives to laboratory-based rRT-PCR is discussed in detail in Chapter 3. RADTs are considerably less sensitive compared with rRT-PCR and may not detect all current infections when the viral load is low.⁽²³⁸⁾ However, they are said to be highly specific in a person who has COVID-19 symptoms, although there is potential for cross-reactivity with seasonal coronaviruses in widespread circulation.⁽²³⁷⁾ Therefore, these tests may be suitable for screening of symptomatic patients to inform rapid clinical decision-making. When used for the purpose of screening, the results of RADTs can be considered unconfirmed or presumptive, and may require confirmatory testing, depending on the prevalence of COVID-19 and the clinical context.⁽²³⁷⁾ Negative results from a RADT do not preclude infection with SARS-CoV-2 and may need to be confirmed with a rRT-PCR test prior to making treatment decisions or to prevent onward transmission due to false negative results, especially if the result of the RADT is inconsistent with the clinical context (the patient is symptomatic), the pre-test probability is high or the person has a known exposure to a confirmed case of COVID-19.^(237, 241) There are limited data to guide the use of RADTs as screening tests on asymptomatic persons to detect or exclude COVID-19, or to determine whether a previously confirmed case is still infectious.⁽²³⁷⁾ Due to the single-use, low through-put design of RADTs, they are not suitable for large-scale screening in settings where alternative high-throughput options are available.

The WHO has agreed to a global partnership with some manufacturers of RADTs to provide affordable high-quality COVID-19 RADTs to low and middle-income countries that do not have access to extensive laboratory facilities or trained health workers to implement laboratory-based rRT-PCR testing.⁽²⁴²⁾ In the absence of reliable transportation services and laboratory infrastructure, a near-patient test may be the only feasible option to increase the pace of testing, contact tracing and treating people with COVID-19 in areas with under-resourced health systems.^(242, 243)

The following include a list of potential settings or situations in which different tests could be deployed. All are contingent on the availability of accurate and reliable assays whose performance has been validated in the setting in which they are intended to be used.

Diagnosis or screening during outbreak situations

Mobile NAAT platforms, such as the Abbott ID NOW™, are small and portable, and are potentially suitable for deployment to non-laboratory, outbreak and crisis situations. It may not be feasible to test, for example, a facility with a high volume of staff or residents within a short period of time with a mobile testing platform.⁽²⁴¹⁾ In such a situation, near-patient testing could be used to test the highest priority (symptomatic) individuals, while test orders for asymptomatic individuals could be sent out for processing at an off-site laboratory using high-throughput platforms.⁽²⁴¹⁾

RADT may also be used to respond to suspected outbreaks of COVID-19 in situations where molecular methods are not available.⁽²³⁸⁾ Positive antigen results from multiple suspects is highly suggestive of a COVID-19 outbreak and could allow for early implementation of infection control measures. In COVID-19 outbreaks that have been confirmed using molecular methods, RADTs could be used to screen at-risk individuals, and prioritise sample collection from negative RADT results for laboratory-based testing.⁽²³⁸⁾ Where possible, all (or at least a subset) of samples should be transported to laboratories for confirmatory testing.⁽²³⁸⁾

Facility-based diagnosis of high-priority specimens

Larger facility-based NAAT platforms, such as the Cepheid GeneXpert® Xpress, have been used in hospitals and medical centres. They have higher throughput than mobile platforms, but still have reduced throughput compared with rRT-PCR. Rapid, facility-based near-patient platforms can be used to test healthcare providers and symptomatic patients, facilitating maintenance of an adequate workforce and rapid diagnosis of critically ill patients.⁽²⁴¹⁾ Molecular-based methods with reduced turnaround times may be used to test high-priority specimens that require a rapid results to inform clinical management.⁽¹⁶³⁾

Screening in congregate settings

Settings for which molecular tests or RADTs may be considered appropriate for the purpose of screening to identify those who require immediate medical or public health intervention include closed or semi-closed population networks (that is, the population within the network is closed or semi-closed). Certain settings can experience rapid spread of SARS-CoV-2, in particular, congregated settings (that is, environments in which a number of people live or meet in close proximity for either a limited or extended period of time such as homeless shelters, prisons, schools and workplaces). Approaches for early identification of infected individuals in

congregated settings include, initial testing of everyone in the setting, periodic (for example, weekly) testing of everyone in the setting, testing of new or returning entrants into the setting or a risk-based approach to testing based on potential exposure and adherence to infection prevention and control measures to quickly identify those with SARS-CoV-2 infection to inform infection prevention and control measures.^(237, 244-246) In settings where a rapid turnaround is required, there may be value in providing immediate results with RADT despite reduced sensitivity when compared with rRT-PCR tests.⁽²³⁷⁾

Serial testing in at-risk populations

Healthcare workers or workers in high-density settings such as food processing facilities in which workers are present for long time periods (for example, 8-12 hours per shift), and are in close contact with co-workers (within two metres for 15 minutes or more) may be at increased risk of exposure to SARS-CoV-2.⁽²³⁴⁾ A serial testing strategy can detect infected workers earlier and exclude them from the workplace, thus potentially preventing or reducing disease transmission and subsequent outbreaks.⁽²³⁴⁾

In such a scenario, testing practices should aim for rapid turnaround times in order to facilitate effective action. Strategies involving serial testing (for example weekly or every three to four days; facility-wide or limited to a subgroup) are dependent on rapid test turnaround times in order to effectively reduce transmission. In circumstances where turnaround times for laboratory-based rRT-PCR prevent timely exclusion of potentially infectious individuals from the workplace, the use of rapid molecular methods or RADTs may be considered. It has been suggested that the results of RADTs may not require confirmatory rRT-PCR testing in those who will receive RADTs on a recurring basis.⁽²³⁷⁾ However, it is noted that the requirement for confirmatory testing may also depend on the specified criteria for consideration as a case. Currently, the case definition is based on laboratory-based rRT-PCR using an assay for which validated data in that setting are available.

Other applications

Screening in open settings (that is, environments that are open to the public) is more difficult to implement due to challenges with follow up, estimating testing volumes, the implementation of safety and quality assurance processes, and variable prevalence, particularly at airports or points of entry. However, screening may be feasible in some open settings, such as airports, if adequate measures are taken to address the above challenges.⁽²³⁸⁾ Currently, the WHO has noted that the use of RADT in settings where confirmatory testing is not readily available is not recommended due to the reduced sensitivity of RADT and the resulting increased potential for false negative results which may lead to a false sense of security.⁽²³⁸⁾

A potential alternative strategy could involve the use of antigen testing to indicate infection with viable, replicating virus, suggesting potential to transmit the virus to others. Contingent on the availability of a highly sensitive test, antigen tests may be clinically useful to inform whether a patient with confirmed COVID-19 is still infectious later in their disease course.

Challenges associated with serial testing for COVID-19

Molecular testing of asymptomatic people at increased risk of exposure to SARS-CoV-2 (for example, healthcare workers) can result in the detection of low levels of viral RNA.⁽²⁴⁷⁾ In general, viral loads peak around the time of symptom onset and are likely to be of clinical and infection prevention and control significance.⁽²⁴⁰⁾ Detection of low levels of SARS-CoV-2 viral RNA in asymptomatic individuals may represent pre-symptomatic infection, sub-clinical or asymptomatic infection or residual levels of detectable RNA following recovery.⁽²⁴⁷⁾ The relationship between viral load and infectivity is not yet fully characterised, although it is thought that those with high viral loads are most likely to transmit the virus to others, making the interpretation of such results and recommendations for subsequent action challenging.^(240, 243)

5.4.2 Surveillance

Epidemiological data on infection trends is essential to inform policy and to monitor the effectiveness of interventions. Epidemiological data can be gathered through public health surveillance — the 'ongoing, systematic collection, analysis, and interpretation of health-related data essential to planning, implementation, and evaluation of public health practice'.⁽²³⁷⁾ Unlike diagnostic or screening, surveillance testing is used to gain information at a population level, rather than an individual level, and the results of surveillance testing are presented in aggregate format.⁽²³⁷⁾

In the context of COVID-19, diagnostic testing and surveillance are linked, whereby the results of all diagnostic tests performed are reported to the HPSC (as COVID-19 is a notifiable disease) to facilitate monitoring of infection rates and trends in order to inform infection prevention and control measures.⁽²⁴⁸⁾ Improvements in access to diagnostic testing will result in more robust surveillance data to monitor for increasing or decreasing prevalence (at the regional and national level), the spatiotemporal spread of the virus, and determining the population effect from community interventions (for example, local lockdowns).⁽²³⁹⁾ This could enable improved disease forecasting and resource allocation.

The application of whole genome sequencing (WGS) for surveillance purposes can provide information on the early emergence and spread of new SARS-CoV-2 strains, to inform policy development on prevention and control.

5.4.3 Sample pooling strategies

In circumstances where the testing capacity is fixed (that is, access to reagents or other laboratory consumables cannot be readily increased), sample pooling strategies may be used to increase testing coverage.

Sample pooling strategies are technically challenging to carry out and require significant automation (for example, robotic systems, software supporting the algorithms to identify positive samples, laboratory information systems) to avoid errors, and therefore are only suitable strategy in high-throughput clinical laboratories. Importantly, pooling strategies are only useful in low prevalence populations due to the increased time requirements associated with preparation of sample pools and the requirement to confirm the results of positive pools.

Ongoing monitoring of disease prevalence is necessary as the most effective pooling strategy will depend on the community prevalence of SARS-CoV-2 (in general, a lower disease prevalence may enable a laboratory to use a larger optimal pool size). The laboratory must validate the performance of the rRT-PCR assay for the pooling strategy to limit the potential for false-negative results. Pool sizes may need to be re-evaluated and adjusted accordingly to ensure the safety and efficiency of the pooling strategy with consideration to the disease prevalence in the intended population.

5.5 Application of alternative approaches for SARS-CoV-2 testing in the Irish setting

Different testing approaches are suited to different circumstances of testing for SARS-CoV-2. As outlined in chapters 2 and 4, testing may be intended to fulfil different aims (for example, diagnosis or surveillance) or objectives (for example, isolation of cases in the community, ruling out of infection in a patient prior to admission for surgery, serial testing for outbreak prevention) and different settings will be associated with different risks. As discussed in chapter 4, in the absence of 100% accurate tests, the pre-test probability (that is, the estimated risk of infection at the time of testing) must be considered alongside the reported accuracy of a test, in order to select an appropriate test for a particular setting.

In the context of SARS-CoV-2 infection, the pre-test probability is based on factors associated with the individual (for example, known exposure status, symptoms of COVID-19, individual risk factors for infection, likelihood of an alternative diagnosis) and factors associated with the individual's environment that may increase or decrease their probability of being infected. Where measures in place include physical distancing, restrictive measures (for example, national or regional

lockdowns), disinfection and hand sanitation and use of face coverings, which collectively reduce viral transmission within the general population, the pre-test probability decreases. Also, where capacity for testing increases such that more asymptomatic individuals are enabled to undergo testing, this will also lower the pre-test probability of infection. Where the pre-test probability of infection is particularly low, and where a positive test result is obtained, this positive test may require confirmation. Conversely, where the pre-test probability of infection is high and a 'not detected' test result is obtained, these 'negative' test results may similarly require confirmation. This need for confirmatory testing is increased where tests are associated with lower diagnostic accuracy, and where the consequences of the test returning an inaccurate result are more severe. As the extent of testing in Ireland increases with expanded laboratory-based rRT-PCR testing capacity, and potentially with the use of alternative tests, careful targeting of tests and use of confirmatory testing as appropriate becomes increasingly important.

Figure 5.1 outlines the potential applications of SARS-Cov-2 testing in the Irish setting, with consideration to the disease prevalence in the intended setting, and essential test characteristics for each setting or scenario. At present diagnostic testing is carried out using laboratory-based rRT-PCR. Also, in settings where rapid turnaround is necessary (such as, unplanned hospital admissions), validated rapid molecular methods are used to inform rapid clinical decision-making. Consideration may be given to the use of RADTs to detect SARS-CoV-2 in symptomatic patients early in the course of infection, or in close contacts; this approach has been adopted in other European countries.

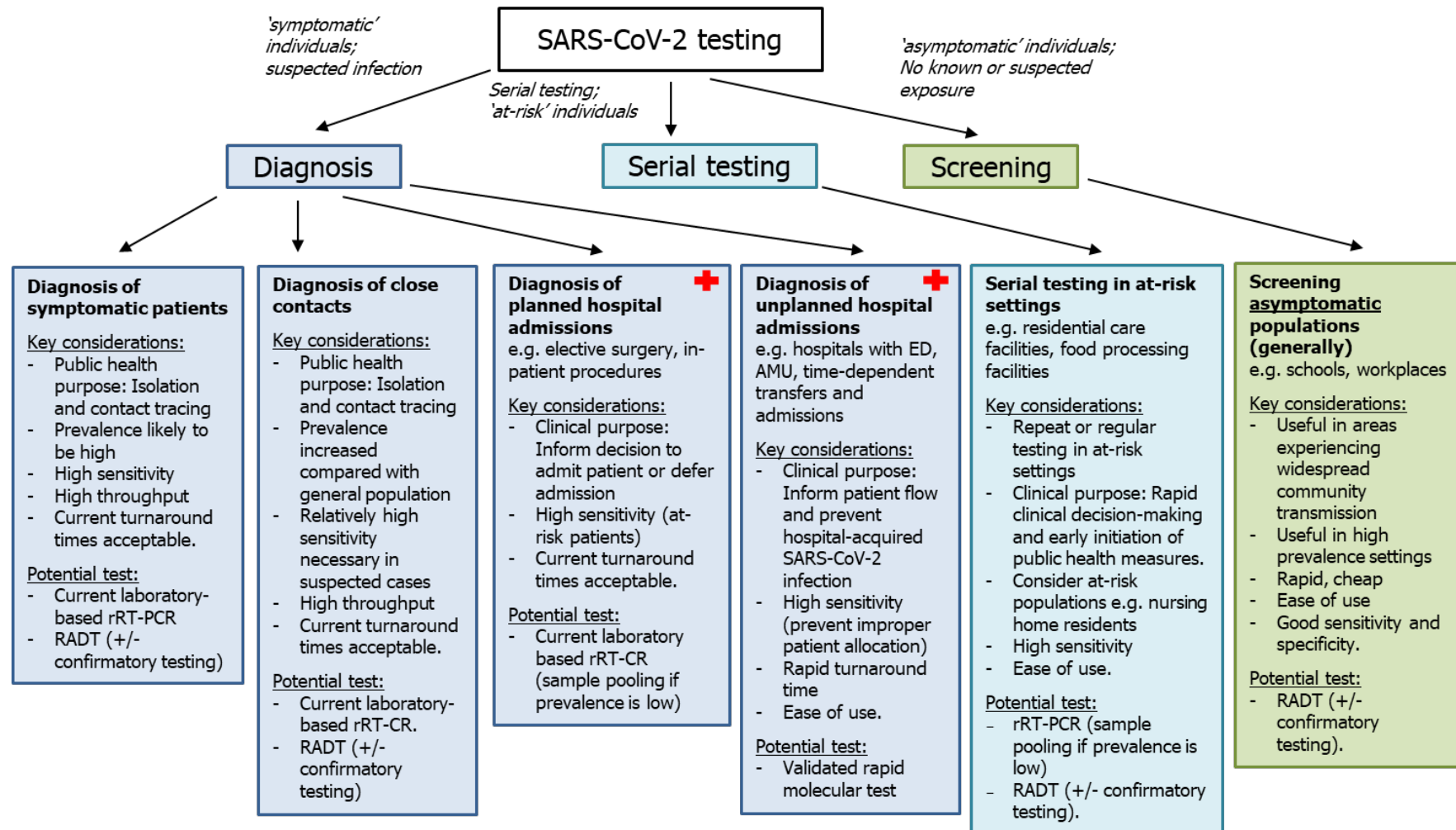
In order to expand access to testing within existing resources, where testing capacity is fixed, a pooled sample strategy may be considered to increase population coverage. A pooling strategy will only increase the efficiency of testing if the prevalence of SARS-CoV-2 is low due to the requirement to confirm positive results of pooled samples. Therefore, ongoing monitoring and regular review of pooling strategies is necessary as the disease prevalence changes.

The WHO have recommended the use of RADTs in settings where there is widespread community transmission, or in circumstances where the health system may be over-burdened and testing of all suspected cases using molecular methods may not be feasible. RADTs may be used to improve access to testing in such a scenario. However, based on the present evidence, confirmatory testing with rRT-PCR would be necessary to verify infection with SARS-CoV-2. The requirement for confirmatory testing is dependent on the pre-test probability. In general, when the pre-test probability is relatively high, the results of negative tests should be confirmed using rRT-PCR, particularly if the patient is symptomatic or has a known exposure to a confirmed case of COVID-19.^(237, 249) While in circumstances where the

pre-test probability is low, positive results should be interpreted with caution and confirmed prior to diagnosis. In practice, the feasibility of confirmatory testing using laboratory-based rRT-PCR is dependent on the availability of laboratory resources. A requirement to confirm all RADT results would overwhelm available laboratory resources and negate any efficiency gains arising from the introduction of RADT. Results that may be prioritised for confirmatory testing include:

- negative test results in symptomatic individuals
- positive results in asymptomatic populations
- positive results in at-risk groups undergoing serial testing (for example, nursing home residents).

Figure 5.1: Potential applications of rapid tests in SARS-CoV-2 diagnosis and screening.



Note: The requirement for confirmatory testing depends on the accuracy of the rapid test, the serial testing strategy (e.g. the time interval between testing), laboratory resources, and the disease prevalence. AMU – acute medical unit; ED – emergency department; RADT – rapid antigen detection test; rRT-PCR – real time reverse transcription polymerase chain reaction

5.6 Non-laboratory settings for diagnostic testing or screening

COVID-19 testing sites can include:⁽³⁾

- laboratories that perform diagnostic testing (for example, regional diagnostic laboratories)
- non-laboratory settings within the health and social care system that perform diagnostic or screening (for example, accident and emergency departments, residential care facilities)
- other facilities or settings offering COVID-19 near-patient diagnostic or screening tests (for example, schools, workplaces).

Suggested priority settings for the introduction of non-laboratory testing in circumstances where access to laboratory testing is limited or its use precludes clinical utility include:^(234, 238, 250)

- settings identified as having a potential or confirmed outbreak or cluster of infection
- settings with individuals at high risk of complications from SARS-CoV-2 infection (for example, nursing homes)
- settings in which workers are carrying out critical activities (such as, healthcare workers)
- high-density settings in which workers are present for long periods of time in prolonged close contact (within two metres for 15 minutes or more)
- settings in which expansion of test capacity is anticipated to significantly reduce the sample processing burden on local or regional laboratories.

Prior to the implementation of testing in a non-laboratory setting the following should be considered to determine the most suitable testing technology and feasibility of testing, specifically the:^(230, 251)

- goal of testing (diagnosis, screening or surveillance)
- intended population for testing within the setting
- clinical or public health need (for example, faster access to treatment, change in patient management, timely initiation of isolation and quarantine, benefits to staff or patients)

- the capacity of the local laboratory to provide support
- estimated daily and weekly volume of tests
- site-specific considerations, including:
 - operational efficiencies or workflow processes that may need to be modified to facilitate testing and subsequent care (if necessary)
 - human resource constraints.

For all settings in which patients are not resident, a robust system for follow up and referral should be put in place to ensure linkage to care, as necessary.

For initial introduction of rapid tests into clinical practice, the WHO has recommended that settings where rRT-PCR testing is currently available should be selected so that staff can gain experience in the use of the test, confirm performance of the selected rapid test, and troubleshoot any operational or implementation issues encountered.⁽²³⁸⁾ The learnings from the initial introductory phase can be used to inform the development of standard operating procedures (SOPs) and quality assurance processes prior to widespread implementation. A number of pilot sites targeting settings known to have a high prevalence, in particular settings with individuals at risk of complications associated with COVID-19, may be considered if there are concerns regarding the affordability of national level rollout.

5.6.1 Readiness for testing

The ability of the intended testing site to carry out accurate and effective testing, with appropriate linkage to care (if necessary), should be considered. A facility readiness assessment should be undertaken to evaluate the response capacity of the facility.⁽²⁴⁵⁾ The ability of laboratory services to support near-patient testing must also be considered.

Readiness of hospital laboratories

Prior to the implementation of near-patient testing (NPT) in a given setting, careful consideration should be given to the capacity of a local or regional hospital laboratory to provide a responsive service with short test turnaround times (Figure 5.2).⁽²²⁹⁾ Provision of rapid molecular testing through local or regional hospital laboratories could provide an acceptable turnaround time which meets the needs of the near-patient setting if a regular reliable sample transport service and or electronic result reporting system is available, potentially negating the need for the establishment of community-based NPT with the associated quality assurance requirements.⁽²²⁹⁾ If community-based NPT is still considered necessary, national guidelines suggest that such services should have access to laboratory support and

include close cooperation and dialogue with clinical laboratories.⁽²²⁹⁾ The ability of a laboratory to handle large volumes of samples is dependent on the site-specific throughput. Laboratories with the highest number of tests processed may not be the laboratories with the greatest resource constraints. An assessment of laboratory capacity should be based on the number of tests performed relative to the maximum capacity of the laboratory for SARS-CoV-2 sample processing, in order to identify bottlenecks within the laboratory-based testing system.

The expansion of testing to near-patient settings may reduce sample processing needs in laboratory settings (in situations where confirmatory testing is not required) and requirements for sample transport. However, the additional requirements for oversight and support of NPT activities by hospital laboratories will necessitate the introduction of new processes, thereby resulting in a repurposing of human resources rather than a reduction in requirements, particularly during the early implementation phase.

Readiness of proposed NPT sites

The testing capacity of the NPT site should be defined at the outset. Consideration must be given to the ability of staff on the intended setting to carry out testing within the available resources. Staffing plans should be developed to redistribute or allocate additional staff for testing, as necessary. The potential impact of fluctuations in transmission levels and co-circulation of other respiratory pathogens with common symptoms on demand for testing should be considered when developing the staffing plan to ensure that adequate staffing levels are maintained throughout the influenza season when the demand for testing is likely to increase.

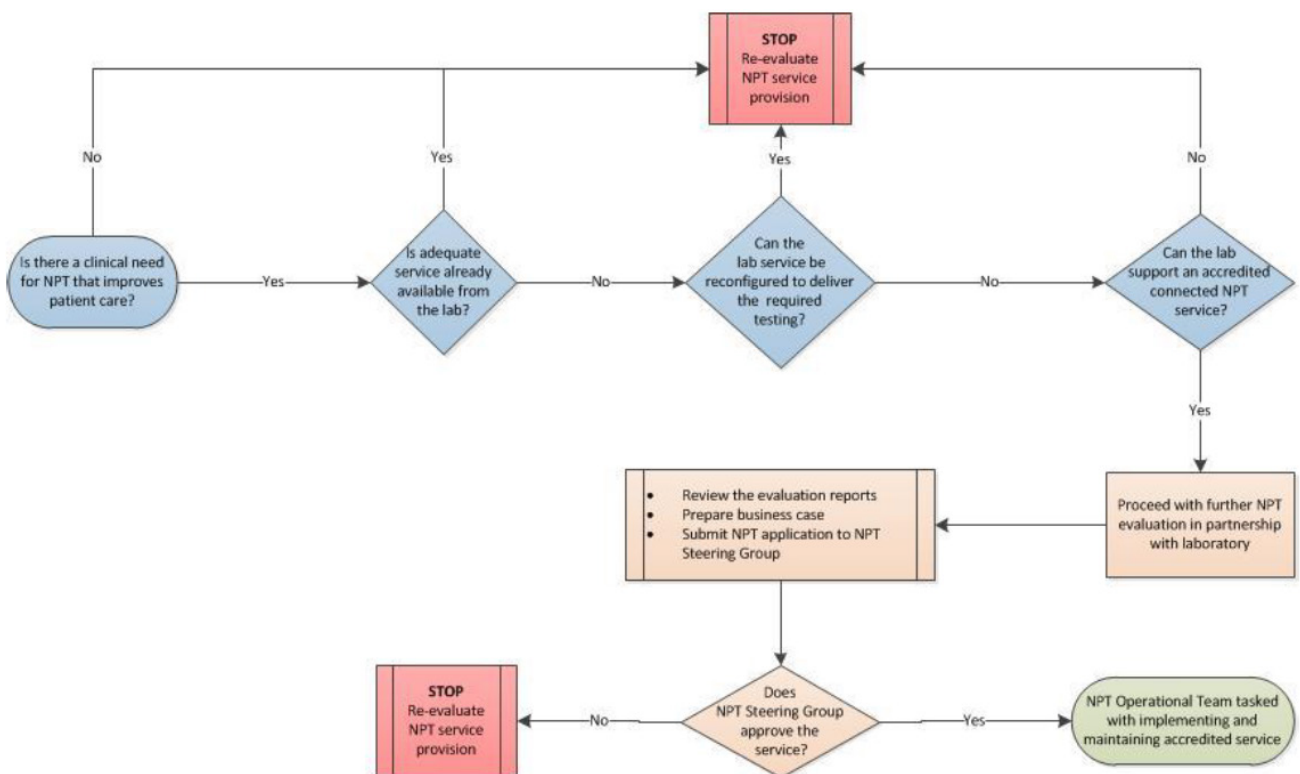
In the event of increased transmission, a plan for surge capacity should be established. A supply procurement and distribution plan for personal protective equipment (PPE) and any ancillary equipment should be developed, including a contingency plan for shortages.⁽²⁴⁵⁾ Efficient lines of communication between laboratories overseeing test processes and NPT settings should be established, which can provide specialist advice and expertise if required.⁽²²⁹⁾

Maintenance of essential activities

It is imperative that the expansion of COVID-19 testing to non-laboratory settings does not adversely impact the ongoing activities in these settings. Context-relevant core activities (for example, access to care in nursing home and residential care facilities, teaching and supervision in school settings) should not be significantly compromised.⁽²⁴⁵⁾

Provision of support to NPT sites by hospital laboratories should not significantly impact the ability of the laboratory to continue to carry out COVID-19 diagnostic testing, or other routine or urgent tests.

Figure 5.2 Process for the assessment of laboratory readiness to support NPT testing



Key: NPT – near-patient testing.

Guidelines for Safe and Effective Near-Patient Testing.⁽²²⁹⁾

5.6.2 Surge capacity

Rapid deployment of NPT to aid in the investigation or control of a newly identified case cluster or outbreak may also be considered, if available resources allow.⁽¹⁶³⁾ Infection prevention and control measures must be observed in the transfer of equipment between outbreak sites.

Outsourcing rRT-PCR testing is another potential means to rapidly increase testing capacity, without compromising the accuracy and reliability of results, when available laboratory testing capacity is overwhelmed. However, the implications for delays in the reporting of results for testing outsourced to other laboratories internationally must be considered.

5.7 Selection of the appropriate technology and device

The most appropriate technology for a given application should first be selected, with consideration to the goal of testing, setting-specific characteristics and the estimated prevalence of SARS-CoV-2 in the setting (Table 5.1). Once the appropriate technology for the intended setting and application has been selected, consideration

can be given to the available CE-marked devices using the underlying technology (Table 5.2). New and emerging diagnostic products will not necessarily meet all the desired criteria for a particular testing scenario, thus it is important to weigh the importance of one device characteristic against another for a specific use (diagnosis or screening) and context (population and setting). Ultimately, a test that does not possess several minimum key characteristics is unlikely to be suitable for testing in the Irish context.⁽²⁴³⁾

Devices should be independently clinically validated prior to implementation, and used only with the specimen type(s) used during validation.

Consideration of disease prevalence

The positive and negative predictive values (the post-test probability) of *in vitro* diagnostic tests vary depending on the prevalence (the pre-test probability) of the infection in the population for whom the test is intended. The expected prevalence of current infection with SARS-CoV-2 will vary across populations being tested, and is therefore an important consideration when selecting a test and interpreting test results for a specific population subgroup. Clinical performance will vary depending on the testing strategy, whether this is screening in asymptomatic populations where the prevalence is anticipated to be low (such as $\leq 2\%$ in the asymptomatic general population), or screening of suspected cases where the likelihood of a positive test result is high (such as 10-30% in symptomatic healthcare workers).⁽²⁵²⁾

Table 5.1 Considerations for selection of the appropriate rapid test technology

	Rapid molecular testing	RADT
Purpose	Diagnosis; Screening.	Diagnosis; Screening.
Intended use	Detect current infection <ul style="list-style-type: none"> ▪ diagnosis of priority specimens ▪ screening 	Detect current infection <ul style="list-style-type: none"> ▪ diagnosis of priority specimens ▪ diagnosis in hard-to-reach populations[‡] ▪ screening
Test accuracy*	High	Moderate
Priority	Accuracy; throughput.	Emergency or rapid clinical decision-making.
Setting	Near-patients settings or hospital laboratories.	Near-patients settings.
Human resource requirements	Higher-throughput testing reduces human resource requirements for sample processing. [†]	Dependent on scale of testing. Increased human resource requirements for large-scale testing due to single-use design.
Equipment and consumables	Bench-top device for nucleic acid amplification.	Variable dependent on test design: <ul style="list-style-type: none"> ▪ Self-contained RADT ▪ RADT with reader
Turnaround time	Variable (hours)	<30 minutes

Cost of test	Moderate	Low
Test complexity	Variable dependent on device. Some technical skill required.	Less technical skill required (typically).
Training	Training required.	Minimal training required (typically).

Key: RADT – rapid antigen detection test.

* Test accuracy is dependent on the technology underpinning an individual device and adherence to quality assurance processes (including sample handling and processing and staff training).

† Some automated molecular methods for use in near-patient settings are designed for testing a number of clinical sample in a small series. At present, batch processing capacity for molecular methods intended for use in near-patients settings is typically reduced compared with rRT-PCR, however this may be offset by reduced turnaround times.

‡ Groups of the population that are difficult to reach or involved in research or public health programmes due to their physical and geographical location (e.g. in mountains, forests or deserts) or their social and economic situation.⁽²⁵³⁾

Table 5.2 Considerations for selection of the appropriate test

Test aspect	Key considerations
Manufacturing quality	<ul style="list-style-type: none"> CE-IVD, WHO EUL, PQ, EU-FDA or other approval. Independent validation data. Manufacture under ISO.
Targets	<ul style="list-style-type: none"> Number and type of SARS-CoV-2 targets. Multiplex capacity: Number of bacterial and/or viral targets in panel (if applicable; increases specificity). Specificity for SARS-CoV-2 or other sarbecoviruses.
Controls	<ul style="list-style-type: none"> For manual NAAT testing, a positive template control (PTC) and at least one negative template control (NTC) should be included. Use of an extraction control and an internal human housekeeping gene specimen adequacy control is also recommended.
Instrumentation	<ul style="list-style-type: none"> Compatibility with available systems in the laboratory. Compatibility with existing or multiple swab materials and viral transport medium. Platforms can be used for other applications (flexibility). Platform is compatible with assays from a number of manufacturers (consider reagent shortages) Ease of use and operational utility. Cost of platform and maintenance. Ability to calibrate remotely or no calibration needed. Ease of access to maintenance provider/troubleshooting. Additional instrumentation needed (e.g. calibration panel before running the test, extraction platforms, heat block, vortex, magnetic stand or centrifuge).
Impact on workflow	<p><u>Laboratory testing:</u></p> <ul style="list-style-type: none"> Can the kit be implemented in the existing workflow of the laboratory, while assuring minimal disruption on other diagnostics? Can diagnostic testing for other diseases be transferred to the new instrument? Is the instrument validated for other purposes? <p><u>Near-patient testing:</u></p> <ul style="list-style-type: none"> Requirements for additional staff. Requirements for task shifting or task sharing, and relevant changes in responsibility.

	<ul style="list-style-type: none"> Time-critical results (requires immediate interpretation)
Facilities	<p><u>Near-patient testing:</u></p> <ul style="list-style-type: none"> Repurposing of existing spaces for testing. Biosafety facility requirements. Access to clinical waste disposal.
Ease-of-use	<ul style="list-style-type: none"> Complexity of assay. Number of steps (consider potential for cross contamination and manual handling errors). Training requirements.
Storage and shipment requirements	<ul style="list-style-type: none"> Requirements for cold chain conditions during shipment and storage. Some kits contain lyophilised enzymes that do not require the kit to be shipped and sometimes stored cold. Shelf life: To be prepared for periods of intense testing, stocks might be needed. A longer shelf life may be needed. <p><u>Near-patient testing:</u></p> <ul style="list-style-type: none"> Repurposing of existing spaces for storage. Requirements for refrigeration of reagents.
Training requirements and access to support	<ul style="list-style-type: none"> Instructions for use available. Training available by manufacturer or others. Response time with the supplier regarding repairs/replacement. Troubleshooting options provided and accessible support (24hour/seven days a week agreement). <p><u>Near-patient testing:</u></p> <ul style="list-style-type: none"> Communication line with local/regional laboratory.
Need for ancillary reagents	<ul style="list-style-type: none"> Complete kit for extraction/amplification or test kit requires additional reagents or tools. Compatibility with existing reagents.
Continuity of supply	<ul style="list-style-type: none"> Long-term supply agreement. Secured routes of delivery if lockdowns occur. Assay and ancillary reagents costs. Contingency plan for supply shortages.

Adapted from the WHO guidance on diagnostic testing for SARS-CoV-2. (239, 243)

Key: CE-IVD - CE Marking for In Vitro Diagnostic (IVD) devices; FDA - Food and Drug Administration; ISO - International Organization for Standardization; PQ – performance qualification; WHO EUL – World Health Organization Emergency Use Listing Procedure.

5.8 Testing processes

5.8.1 Sample collection and handling of specimens

All testing for SARS-CoV-2 is directly impacted by the integrity of the specimen, which depends on specimen collection, storage and transport.⁽²³⁷⁾ Improper specimen collection may result in retrieval of inadequate amounts of viral genetic or antigenic material for detection. Specimen collection should be in accordance with the instructions for use provided with the device.

Deterioration of biomolecules in clinical tissues is an inevitable part of the pre-analytical process. Thus, delays from sample collection to testing can lead to

increased sample deterioration and should be minimised as far as possible to reduce the risk of significant deterioration, in particular for molecular-based testing, which may impact downstream analysis.⁽²³⁷⁾ Quality assurance processes should be developed and implemented for NPT sites to ensure maintenance of sample integrity and the safety of test operators. It is imperative that proper specimen labelling practices are adhered to when batch processing specimens in laboratory or non-laboratory settings to ensure proper patient identification and subsequent interventions.⁽²³⁹⁾

Purification of viral RNA from crude patient samples removes other sample components that can inhibit the amplification reaction and reduce test sensitivity. RNA purification is a time-consuming laboratory procedure requiring specialised kits and reagents, which are subject to supply shortages. Many rapid molecular tests are only considered suitable for use in laboratory settings due to requirements for RNA purification prior to the reverse transcription and amplification reactions. Tests that effectively integrate all steps necessary for molecular analysis are highly desirable.

5.8.2 Sample storage and shipment

For laboratory-based testing, clinical samples should be transferred to the hospital diagnostic laboratory or the laboratory associated with the test centre for processing as soon as possible.⁽²³⁹⁾ Diagnostic laboratories can only control the processes between sample arrival and the test result, it is therefore critical that upstream processes (sample storage and transport) are carried out in a timely and effective manner to ensure rapid turnaround of results and sample integrity.

For on-site testing locations, shipment to a centralised laboratory for testing is not required. Consideration must be given to the manufacturer's instructions regarding maximum time from sample collection to processing, or the availability of stabilising agents that allows for a short testing delay.⁽²⁴³⁾ While it is intended that testing will be performed in near-patient settings, consideration should also be given to the compatibility of the test device with preserved samples.⁽²⁴³⁾

5.8.3 Confirmatory testing

Confirmatory testing using laboratory-based rRT-PCR may be necessary for results obtained using RADTs, in particular if the result of the RADT is inconsistent with the clinical context.⁽²³⁷⁾ The ECDC advises that due to the reduced sensitivity of RADTs, there is a risk that 'not detected' results comprise false negatives, though positive results in this context are very likely to be true positives. Confirmatory testing can increase the accuracy and reliability of the testing strategy.⁽¹⁸⁹⁾ Depending on the scale at which RADTs are deployed, confirmatory testing of all test results may not be feasible.

If confirmatory testing is necessary, consideration must be given to how this will be operationalised. Some RADTs are designed for immediate analysis of the specimen (that is, without placement in viral transport medium [VTM]) as dilution of the sample in VTM may result in decreased test sensitivity.⁽¹⁴⁷⁾ For rRT-PCR, placement in VTM is essential to stabilise the specimen and prevent RNA degradation during sample transport for laboratory-based sample processing – the dilution of RNA in the clinical specimen (if present) prior to rRT-PCR is not clinically relevant due to the subsequent amplification of RNA during sample processing.⁽²⁵⁴⁾

The suitability of a RADT to a given testing strategy will depend on the approach to confirmatory testing. RADTs that recommend against placing swabs in VTM immediately (due to the risk of diluting the sample), cannot be reliably used for confirmatory testing with rRT-PCR. Therefore, if confirmatory testing using the same specimen is to be carried out, careful consideration must be given to selection of a RADT that can support confirmatory testing using the same patient sample. Confirmatory testing using the same patient sample will reduce the number of swabs necessary to carry out testing, therefore conserving limited resources. Factors to consider when deciding the optimal approach to confirmatory testing for a given context are outlined in Table 5.3.

Standard practice in Ireland is a combined nasopharyngeal and oropharyngeal specimen. A single swab is used to first sample the oropharynx and then the nasopharynx. To facilitate confirmatory testing, a variation on this approach is for two sample swabs to be used simultaneously (that is, duplicate swabbing), so that two specimens are obtained as part of a single swabbing experience. Swabs are typically very fine, so this option may be reasonable for the majority of patients. This retains many of the advantages in terms of patient acceptability and reduced loss to follow up. While still vulnerable to inadequate sampling, each specimen can be placed in the appropriate medium or test solution.

The CDC recommends that confirmatory testing with an rRT-PCR test should be carried out on a second clinical specimen, within two days of the initial sample collection for the rapid test.⁽²³⁷⁾ The ECDC advises that a second confirmatory test may be carried out on the same patient sample.⁽¹⁷¹⁾ WHO guidance acknowledges that confirmatory testing using molecular methods is not always feasible. Where possible, a context-specific approach to confirmatory testing is advised, whereby positive samples collected during an outbreak should undergo confirmatory testing on the same specimen. In circumstances where there is widespread community transmission and the NPV is low, a negative RADT result cannot completely exclude an active COVID-19 infection. In such circumstances, a second clinical specimen may be needed, particularly in symptomatic patients.⁽²³⁸⁾

Table 5.3 Considerations for clinical specimen collection strategy for confirmatory testing.

Confirmatory testing using the same clinical sample	Confirmatory testing using a second clinical sample	Confirmatory testing using a 'duplicate swabbing' technique*
Advantages		
<ul style="list-style-type: none"> ▪ Conservation of laboratory consumables (e.g. sample swabs) ▪ Increases patient acceptability ▪ Reduced risk of loss to follow-up ▪ Avoids potential risk of exposure or viral clearance during the interval between tests. 	<ul style="list-style-type: none"> ▪ False negative RADT results due to pre-analytical vulnerabilities (e.g. inadequate sampling) may be identified. ▪ No requirement for storage of initial patient sample after sample processing. ▪ Each swab can be stored as per the manufacturer's instructions. 	<ul style="list-style-type: none"> ▪ Increases patient acceptability ▪ Reduced risk of loss to follow-up ▪ Avoids potential risk of exposure or viral clearance during the interval between tests ▪ Each swab can be stored as per the manufacturer's instructions.
Disadvantages		
<ul style="list-style-type: none"> ▪ Instructions for use for some RADT may not be compatible with rRT-PCR confirmatory testing. ▪ Clinical samples requiring confirmatory testing must be decided in advance of sample collection to facilitate appropriate sample storage for subsequent confirmatory testing ▪ Testing is directly impacted by the integrity of the specimen. Testing clinical samples that were subject to pre-analytical vulnerabilities (e.g. inadequate sampling) will give inaccurate results ▪ All testing is subject to pre-analytical and analytical vulnerabilities. 	<ul style="list-style-type: none"> ▪ Increased resource use (e.g. laboratory consumables, staff time) ▪ Potential loss to follow-up for confirmatory testing ▪ Potential exposure to SARS-CoV-2 or viral clearance during the interval between tests. ▪ Confirmatory testing is time-dependent. Sample collection must occur within 2 days of the initial RADT. ▪ All testing is subject to pre-analytical and analytical vulnerabilities. Repeat testing can confirm reproducibility of results. 	<ul style="list-style-type: none"> ▪ Two swabs are required for the 'duplicate swabbing' technique. ▪ Clinical samples requiring confirmatory testing must be decided in advance of sample collection to ensure two individual swabs are collected and stored appropriately. ▪ Testing is directly impacted by the integrity of the specimen. Testing clinical samples that were subject to pre-analytical vulnerabilities (e.g. inadequate sampling) will give inaccurate results. ▪ All testing is subject to pre-analytical and analytical vulnerabilities.

Key: RADT – rapid antigen detection test.

*'Duplicate swabbing' is the use of two sample collection swabs simultaneously during a single sample collection to facilitate parallel testing.

5.8.4 Referral

Appropriate referral criteria should be in place to ensure access to confirmatory testing and further medical attention, as necessary.^(229, 245) The capacity of the referral system must also be taken into consideration. Transfer vehicles and ambulances for severe suspected or confirmed COVID-19 cases based on the results of near-patient testing should be available.

5.8.5 Turnaround times

Rapid test turnaround times are important to inform clinical management, particularly in critically ill patients or those at risk of complications from COVID-19. Confusion often arises given the distinction between reported test run times or processing times and the total time taken to complete the testing process. Furthermore, when a large volume of testing is required, the stated advantages of a rapid sample processing time may be negated by the requirement to process samples individually or in small batches compared with conventional laboratory-based testing in which large batches are processed concurrently, albeit over a longer period.

Laboratory turnaround time can be defined as the time interval between receipt of the specimens at the testing site to the time of dispatch of the results.⁽²⁵⁵⁾ The therapeutic or overall turnaround time includes:

- ordering of the test
- sample collection
- sample transportation
- sample processing
- reporting of results
- analysis of results and subsequent clinical action (as necessary).⁽²⁵⁵⁾

At present, for swabs taken in the community, the median test turnaround time for laboratory-based COVID-19 testing in Ireland is over 24 hours.⁽²³¹⁾ Therapeutic test turnaround times can be subject to delays along the entire testing pathway, and are therefore more variable than laboratory test turnaround.⁽²⁵⁵⁾ Near-patient or point-of-care testing occurs at the time and place of patient care (for example, patient's bedside or physician's office) thus reducing the potential for delays in the testing pathway.⁽²⁴¹⁾

Test turnaround time varies according to the test device and associated characteristics, including the extent to which pre-analytical handling is required, performance time and requirements for an additional reader. Turnaround times may be reduced for devices that do not depend on a reader for analysis of results,

however the subjectivity of interpretation may result in reduced reproducibility and reliability.⁽²⁵⁶⁾ The time for sample processing reported by manufacturers of nucleic acid amplification instrumentation may not include any additional steps necessary for sample processing such as cell lysis, isolation and purification of RNA from samples. Manufacturer reported turnaround times or turnaround times for tests conducted in laboratory settings by highly skilled staff may not reflect the expected turnaround time for tests carried out by staff with minimal training in near-patient settings.

A realistic test turnaround time relevant to near-patient settings should be established to ensure confidence in the system. Potential delays in the reporting of results due to task-shifting in near-patient settings without dedicated testing staff should be minimised. Regular review of test turnaround times is important to identify potential problems in the testing pathway.

The choice of device for a particular setting should take into consideration the workflow. For some devices, the accuracy of the result may decline after the optimal read time, which may be a challenge for busy settings with regular task-shifting.⁽²³⁸⁾ In such settings, a long-lasting and stable result is desirable.

5.8.6 Disruptions to near-patient testing

Links to laboratory settings that can provide support should be set out prior to the establishment of near-patient testing in a given setting. If testing in near-patient settings cannot be carried out (for example, reagents shortages, excessive demand, lack of skilled operators, device malfunction) the associated laboratory should be alerted at the earliest possible time to enable redirection of sample processing to an alternative site.

5.9 Resource requirements

5.9.1 Diagnostic testing or screening

National near-patient testing (NPT) guidelines highlight that a designated area for the provision of NPT should include suitable facilities for sample collection, test performance, instrument storage, safe disposal of clinical waste and storage of consumables in accordance with the appropriate conditions as defined by the manufacturer and applicable legislation.⁽²²⁹⁾ Bench-top molecular devices could be accommodated within existing near-patient settings without the need for significant alteration of or investment in premises. The adoption of a compact portable device could potentially facilitate its use across a number of sites, eliminating the need for capital investment in multiple devices. Additional equipment required to support NPT for COVID-19 (such as sample collection tools) may already be available in some, but not all, near-patient settings.

The implementation of NPT may require changes to workflow arrangements. Individual settings may need to consider their own staffing, infrastructure and culture when establishing the workflow for COVID-19 NPT. Patient flows need examination at a site-specific level.⁽²⁵⁷⁾ Queuing or seating systems should be appropriately spaced to facilitate physical distancing. Convening groups for mass sample collection should be avoided to reduce the risk of disease transmission. Depending on the scope of testing and existing capacity, staff requirements will vary.⁽²²⁹⁾

5.9.2 Whole genome sequencing

Whole genome sequencing (WGS) requires specialised instrumentation, large data storage capacity and highly skilled staff for sample processing. The resultant data are very complex, and their interpretation requires expert bioinformatics assistance.⁽²⁵⁸⁾ A large number of mutations may be identified from clinical samples, however only clinically significant mutations are relevant, which can be challenging to identify. Thus, WGS can only be conducted in specialised laboratories certified to perform high-complexity testing.

This WGS workflow requires substantial initial and sustained investments in laboratory equipment, computing infrastructure and training.⁽²⁵⁹⁾ The necessary tools and resources include basic laboratory facilities (for example, culturing, DNA extraction), high-throughput sequencing instruments, advanced laboratory and IT infrastructure, bioinformatics tools and resources, and teams of highly skilled personnel who can efficiently handle and process samples from various sites.⁽²⁵⁹⁾ WGS also requires substantial data storage capacity, data security measures and data-sharing policies and procedures.⁽²⁵⁹⁾ Standardised software for analysis and workflow management should be used to facilitate data-sharing (if appropriate) with other members of a surveillance network.⁽²⁵⁹⁾

The cost of establishing and maintaining one or more WGS laboratories depends on existing laboratory facilities and the intended sequencing capacity.⁽²⁵⁹⁾ WGS is more cost-effective in higher throughput laboratories.⁽²⁵⁹⁾ Centralised sequencing can significantly reduce the cost of sequencing if the challenges associated with sample transportation can be overcome and the central laboratory has sufficient capacity to serve all submitting laboratories with an acceptable wait period.⁽²⁵⁹⁾

5.10 Training requirements

National NPT guidelines highlight the benefits of a designated operational team to provide oversight and monitoring of training and certification by thereby ensuring consistency across all NPT sites.⁽²²⁹⁾ Options include that training is provided by the NPT operational team directly, by dedicated staff members from specialist

departments under guidance of the NPT operational team and or the device manufacturer under guidance of the NPT operational team.⁽²²⁹⁾

The WHO emphasises that training must cover all stages of the testing pathway. It notes that, given the challenges associated with adequate sample collection and the potential pre-analytical and analytical vulnerabilities associated with testing, sample collection and processing should only be performed by trained personnel.⁽²³⁹⁾

Additional training requirements include the use of test devices and readers (if applicable), systems for recording results (patient results and quality assurance), internal and external quality assurance systems, troubleshooting methods and an understanding of health and safety legislation.⁽²⁵⁷⁾ While training requirements for the operation of some low-complexity tests are said to be minimal, training in all aspects of NPT must be provided to ensure that testing is carried out in line with best practice procedures, ensuring that results are accurate and reliable.

User experience and familiarity with the device are an important consideration for achieving good performance. To ensure provision of a safe and effective NPT service, a range of control measures have been suggested to limit the performance and interpretation of near-patient tests to staff that have been trained in the use of the device and for whom competency has been documented. These include verification of user competency before operating the device,⁽²⁶⁰⁾ restricting access to devices to certified users (for example, requirements for barcodes or passwords),⁽²⁶⁰⁾ and maintaining a record of the staff trained in the conduct and interpretation of tests.⁽²²⁹⁾ Refresher training courses should be available for operators in near-patient settings, particularly for operators or settings (for example, settings with access to an on-demand mobile testing device) with a break period in the conduct of testing or in facilities with low test volumes to ensure competency standards are maintained. Web-based training has been identified as a valuable aid to delivering training modules to facilitate these requirements, particularly in the current context.⁽²⁶⁰⁾ Essential components of a NPT training programme are outlined in the Guidelines for Safe and Effective Near-Patient Testing.⁽²²⁹⁾

5.11 Safety protocols

A site-specific and activity-specific (that is, sample collection, sample processing) risk assessment to identify safety risks and determine if additional biosafety precautions are warranted based on situational needs.⁽¹⁶³⁾ Irish-specific health and safety legislation for handling chemical reagents in accordance with the Safety, Health and Welfare at Work Act 2005 should be followed.⁽²⁶¹⁾

5.11.1 Safety procedures during specimen collection

Those collecting clinical specimens from suspect cases must adhere rigorously to infection prevention and control guidelines by wearing appropriate personal protective equipment (PPE) and following standard precautions when handling clinical specimens, including hand hygiene.^(163, 239) Staff should be appropriately trained in specimen collection, packaging, shipment and storage including proper use of the sampling instrument and ways to minimise the risk of exposure.^(163, 239)

At present, the WHO does not recommend the use of saliva as the sole sample type for routine clinical diagnosis. However, it is recognised that collection of nasopharyngeal and oropharyngeal swabs can be particularly problematic for some groups in settings where non-laboratory may be considered feasible, such as screening or serial testing in schools or long-term care facilities, respectively. In these scenarios, less invasive collection methods may be considered acceptable if there is lower risk of exposure of staff to SARS-CoV-2 during sample collection, as compared with the collection of upper respiratory tract (URT) specimens.⁽²³⁹⁾ Prior to widespread implementation of alternative sampling methods (such as respiratory or oral fluid sampling methods) the proposed sampling method should be validated in the intended patient group(s).⁽²³⁹⁾

5.11.2 Safety procedures during sample processing

Sample handling for molecular testing using standard rRT-PCR requires biosafety level (BSL) 2 or equivalent facilities with the use of a biosafety cabinet (BSC) or a primary containment device. A risk assessment should be undertaken and adequate risk-mitigation measures put in place prior to the establishment of NPT may be performed outside a biosafety cabinet.⁽²³⁹⁾

Staff must be trained in the proper use of the instrument and ways to minimise the risk of exposures. The use of automated instruments and analysers reduces the risk of transmission to test operators.⁽¹⁶³⁾ The instrument and workstation must be decontaminated after each test run to reduce the risk of transmission and contamination between samples, which may lead to false positive results.

5.11.3 Clinical waste disposal

The handling and disposal of clinical waste should be conducted in accordance with the appropriate health and safety and or infection control legislation.⁽²²⁹⁾

5.12 Quality assurance processes

Current Irish guidelines for near-patient testing (NPT) highlight the importance that testing performed outside of a central laboratory is assured of the same quality and

standards and does not represent a patient safety risk.⁽²²⁹⁾ The guidelines note that NPT should be conducted with appropriate supervision, governance and accreditation to ensure that the service is fit for purpose. However, the ultimate responsibility for the quality control of the NPT lies with the service provider. Considerations for the organisation and delivery of near-patient testing are outlined in table 5.3 using the STEP-UP framework. Of note, the public or patient perspective is not captured by this framework. Factors influencing patient acceptability (for example, sample type, test turnaround time, access to testing) are likely to influence uptake of testing.

Consultation with the local laboratory

In the event that NPT sites are established as part of the national testing strategy, consideration should be given to establishing a link with their local hospital pathology service to ensure that testing is provided in a safe and effective manner and to be ultimately accreditable to the required ISO 15189/22870 standards (standards for accreditation of medical testing in medical laboratories and point-of-care testing, respectively) as implemented by the Irish National Accreditation Board (INAB) in Ireland.⁽²²⁹⁾ The clinical laboratory is a source of expertise and has an essential role in the leadership and co-ordination of NPT.⁽²²⁹⁾ During the introductory phase of implementation, and contingent on resources being available, an option is that hospital laboratory teams could provide support to facilitate consistent provision of a quality-assured service across laboratory and non-laboratory testing sites. This may involve participation in the networking of NPT sites, management of external quality assurance (EQA) schemes and or provision of advice on request.^(229, 239)

Governance

The Guidelines for Safe and Effective Near-Patient Testing set out that a NPT steering group should be established for the delivery of all NPT services in Ireland.⁽²²⁹⁾ These recommend that laboratory consultants from participating disciplines provide direction for their particular NPT service. The guidelines recommend that NPT services (both new proposals and or services already in use elsewhere within the health service) should only be introduced in consultation with the NPT steering group and have the approval of the laboratory consultant from the appropriate discipline.⁽²²⁹⁾

If NPT for COVID-19 were to be deployed as part of the national testing strategy, and in line with the Guidelines for Safe and Effective Near-Patient Testing, consideration should be given to the establishment of an NPT operational team to oversee the day-to-day operation of COVID-19 NPT testing including the development of standard operating procedures (SOPs), training requirements, and quality assurance programmes.⁽²²⁹⁾

Standard operating procedures

If COVID-19 NPT sites are established, protocols should be developed and implemented for all sites. These should comprise SOPs which are developed and implemented for all aspects of testing, including sample collection, test performance, interpretation of results, record keeping, patient referral criteria, expert laboratory guidance, quality assurance, patient and staff health and safety.⁽²²⁹⁾

As with all testing that is undertaken, laboratory and non-laboratory-based testing personnel should follow the manufacturer's instructions for use. Deviation from the standard protocol will result in suboptimal test performance and increase the risk in inaccurate results.

Quality assurance programme

Participation in an external quality assurance (EQA) scheme, performance of regular internal quality control (IQC) testing, and adequacy of test volumes to maintain operator competency can provide assurance of the accuracy and reliability of NPT. IQC provides assurance that the device is performing as expected and must be performed in accordance with the manufacturer's instructions.⁽²²⁹⁾ Again, if NPT for COVID-19 is deployed as part of the national testing strategy, consideration should also be given to the development of an EQA scheme with oversight and management by the local or regional clinical laboratory to provide assurance that results are reliable and comparable irrespective of where testing is performed.⁽²²⁹⁾ Consistent with criteria established by the National Pathology Accreditation Advisory Council, actions to be taken when quality control results are unacceptable must be documented.⁽²⁶²⁾

Ongoing monitoring and review

In line with national guidelines for NPT, any NPT sites for COVID-19 established as part of a national testing strategy, would be required to establish a system to support full traceability of COVID-19 rapid tests, including reagents and consumables, in line with the guidelines for safe and effective NPT.⁽²²⁹⁾ Consistent with these guidelines, difficulties with the operation of tests used in decentralised or near-patient settings or discordance between the results of screening and confirmatory tests should be reported as part of quality improvement processes. All adverse incidents that occur with near-patient rapid test must be reported to the manufacturer, the hospital overseeing NPT activities and the Health Products Regulatory Authority (HPRA).⁽²²⁹⁾

Table 5.3 Potential logistical challenges outlined using the STEP-UP framework.⁽²⁵⁷⁾

Logistical challenge		Considerations
S	Skills	<ul style="list-style-type: none"> testing will be carried out by operators who have not received specialist laboratory training. minimum competencies for test performance and interpretation must be documented. systems for verification of user competency.
T	Training	<ul style="list-style-type: none"> training by device manufacturers or specialist laboratory staff. training protocols as part of the SOPs. training for all aspects of the testing pathway (use of test devices and readers (if applicable), recording of results (patient results and quality assurance), IQC and EQA systems, troubleshooting, health and safety). access to refresher training. availability of e-Learning materials.
E	Equipment	<ul style="list-style-type: none"> COVID-19 rapid test and reader (if applicable). maintenance and repairs of equipment. additional consumables and reagents (e.g. sample collection tools). refrigerator for storage of reagents (if necessary). clinical waste disposal bins. password protection of test devices.
P	Premises	<ul style="list-style-type: none"> accommodation of devices and additional storage capacity within existing spaces. suitable facilities for sample collection that ensure dignity and respect for the patient. requirements for network points/Wi-Fi and power sockets.
U	User perspective	<ul style="list-style-type: none"> accuracy and reliability of the test. ease-of-use. impact of existing workflows and buy-in. test turnaround times.
P	Primary-secondary interface	<ul style="list-style-type: none"> ICT infrastructure. integration of NPT testing with existing reporting systems.

Key: EQA – external quality assurance; ICQ – internal quality control; ICT – information and communications technology; SOP – standard operating procedure.

5.13 Connectivity and reporting

The public health response to COVID-19 depends on comprehensive testing data which contribute to understanding the impact of COVID-19, positivity trends and testing coverage. All test results (positive and negative) and quality control results should be recorded appropriately in accordance with defined procedures and the General Data Protection Regulation (GDPR).^(172, 229) As COVID-19 is classified as a notifiable disease, all medical practitioners, including clinical directors of diagnostic laboratories, are required to notify the Medical Officer of Health (MOH) and or Director of Public Health of a confirmed case of COVID-19. The MOH reports the notification onwards to the Health Protection Surveillance Centre (HPSC).⁽²⁴⁸⁾ If

testing is implemented in any settings not currently carrying out COVID-19 testing, clear guidance should be provided regarding who is responsible for reporting of positive cases.

In general, according to Ireland's national near-patient testing guidance,⁽²²⁹⁾ results of diagnostic and screening carried out in near-patient and laboratory-based settings should be recorded in the individuals testing record or patient health record, as appropriate. In the context of COVID-19, where individuals may undergo testing as part of contact tracing efforts, serial testing on a regular basis or large scale testing in outbreak settings, recording of results in a patient's medical record may not be feasible or necessary. However, test results should be reported to the HPSC to ensure results obtained in non-laboratory settings are documented in the national level statistics to facilitate accurate monitoring of infection rates and trends, and to provide accurate data on the extent and setting of testing. It is important that the results of diagnostic and screening tests are distinguishable in the reporting system according to the type of test used (for example rRT-PCR, rapid molecular test, RADT).⁽²³⁷⁾ These data should be reported regularly (for example, within 24 hours of test completion) after all personally identifiable information has been removed in accordance with GDPR.⁽¹⁷²⁾ The recording of the results of both results from screening, using rapid tests, and laboratory confirmed rRT-PCR represents a challenge for duplication of results in the reporting system that are later confirmed. Patient test results can be linked to a unique patient identifier in order to determine the total number of tests carried out, and the number of individuals tested.

Electronic reporting options are favoured to reduce the administrative burden on providers reporting test results and minimise the risk of reporting errors.^(3, 262) Connectivity between disparate computer systems and COVID-19 NPT devices allows NPT devices to be controlled and managed centrally and facilitates exchange of information (for example, patient results and quality parameters) from the remote NPT site to the laboratory or hospital information system.⁽²²⁹⁾ Connectivity can be achieved through deployment of a device management system known as 'NPT middleware'.⁽²²⁹⁾ For devices without reader technology (for example, lateral flow assays), consideration should be given to the possibility to transform test results into a digital format using third party readers to circumvent manual reporting errors.^(243, 256)

It is anticipated that the new national medical laboratory information system (MedLIS) will be available in 2020. MedLIS aims to deliver a complete national pathology record for all patients in Ireland including integration of NPT results.⁽²²⁹⁾ In order to reduce reporting burdens for testing facilities and ensure that COVID-19-related test data is accurate and consistent, standardised terminology should be

used (for example, SNOMED-CT) to improve the accuracy of reporting tests for the SARS-CoV-2 virus.^(3, 263)

5.14 Implications for contact tracing efforts

At present, only the results of laboratory-confirmed rRT-PCR tests (standard or rapid), that have been obtained using tests for which satisfactory validation data are available, are recorded by the HSE and used to inform contact tracing efforts. Positive RADT results that have not been appropriately validated for use in near-patient settings are not currently recognised as a diagnosis of COVID-19 by the HSE, and require further confirmatory testing using validated laboratory-based rRT-PCR prior to being recorded in the national surveillance estimates.

However, national regulations in respect of infectious diseases specify a system of dual notification, that is, by the clinician who may make the diagnosis on clinical grounds and separately, where supported, by the laboratory. If the referral for testing is made by a clinician, consistent with management of other notifiable infectious diseases, there would be a requirement for suspected cases to be reported to local public health authorities. In the context of an ongoing pandemic, where public health authorities are working at capacity, ad hoc manual reporting of such cases will likely pose challenges, particularly when the accuracy of the diagnosis is uncertain due to the use of tests that do not meet the requirements for a diagnosis of confirmed COVID-19.

In the context of any testing undertaken outside the national publicly-funded COVID-19 testing strategies, individuals with a positive RADT result who do not present for follow-up testing, at present, would not be captured in the national surveillance data, and would therefore not be included as part of the resultant contact tracing efforts. However, to minimise the potential for onward transmission of SARS-COV-2 by individuals who are potentially infectious, consideration may need to be given as to how positive RADT results are managed and how they might be used to inform contact tracing efforts, particularly if there is a risk of loss to follow up for confirmatory testing. Only accurate and reliable tests that have been appropriately validated for use in the intended setting should be used to inform contact tracing efforts to circumvent the close contacts of individuals with a false positive result being contacted during contact tracing efforts and the unnecessary harms associated with this (such as, emotional distress, absence from work and reduced productivity).

5.15 Testing conducted outside the national testing strategy

The purpose of the HTA is to inform the national publicly funded testing strategy. At present, private hospitals carrying out COVID-19 diagnostic testing using rRT-PCR

report positive cases to the MOH for inclusion in the national level surveillance data. Several commercial tests for COVID-19 are available in Ireland, which are not coordinated under the HSE and are not in line with the national testing strategy and associated quality assurance processes.⁽²⁶⁴⁾

COVID-19 testing conducted under the national testing strategy requires sample collection to be carried out by trained healthcare professionals.⁽²⁶⁴⁾ Results are reported centrally for monitoring, surveillance and contact tracing purposes.⁽²⁶⁴⁾ Near-patient tests provided by commercial entities may not meet the definition of confirmed SARS-CoV-2 infection set out by the HSE (laboratory-confirmed rRT-PCR).⁽²⁶⁴⁾ Only results that have been obtained using tests for which satisfactory quality-assurance and validation data are available are recorded by the HSE. In addition, while testing carried out within the national testing strategy is provided free of charge, individuals availing of commercially available testing will incur out-of-pocket costs.⁽²⁶⁴⁾

It is recommended that individuals eligible for testing, access testing through the national testing strategy to ensure the results are quality assured and can be identified for use in contact tracing efforts.

5.16 Cost of introducing a near-patient testing service

Consideration of the cost-effectiveness and estimated budget impact of introducing NPT for COVID-19 were outside the scope of this rapid HTA. It is noted however that the provision of rapid testing in near-patient settings is intended to supplement the current national testing strategy, and as such, will likely require additional investment in the immediate term.

In general, the cost per test of NPT exceeds that of equivalent tests undertaken in the clinical laboratory, taking the cost of devices and other consumables, training, establishment and maintenance of quality assurance programmes and device servicing and repairs into account.⁽²²⁹⁾ Taking all requirements into consideration, the cost per test of laboratory-based batch RT-PCR tests is typically less than the cost of laboratory-based rapid molecular tests which are less costly than rapid molecular tests provided in near-patient settings. While the cost of RADT devices are considerably cheaper than molecular tests, consideration must also be given to where in the testing strategy these tests are most appropriately deployed as this may influence the requirement for confirmatory testing. The strategy adopted for laboratory-based confirmation of RADT results (for example, confirmation of positive (or negative) RADT test results or in selected population subgroups; confirmation using the same clinical specimen or a second specimen) has the potential to significantly impact the overall cost of adopting RADTs as the primary test.

The cost of ancillary consumables, disposal, training and quality assurance requirements must also be considered. Thus, inappropriate or excessive NPT can significantly increase health service expenditure. Standard practice is that the provision of NPT should not be considered when the laboratory can provide the result in a timely manner; however, in the context of an ongoing pandemic, consideration must be given to alternative testing methods that can improve access to testing.

5.17 Conclusion

Near-patient testing should be conducted with appropriate supervision, governance and accreditation to ensure that the service is fit for purpose. All tests are subject to pre-analytical and analytical vulnerabilities that can impact the accuracy and reliability of test results, and represent a potential patient safety risk if not properly managed. To mitigate such risks, training and quality assurance procedures are required to ensure that test samples are appropriately identified and reported (right result, right patient), and to ensure adequate procedures for correct specimen collection and sample processing.

6 Summary & Development of Advice

This rapid HTA was undertaken by HIQA to assess the alternatives to laboratory-based rRT-PCR that could be used to rapidly detect current infection with SARS-CoV-2. In particular, the assessment:

- considered a wide range of alternatives, both commercially available and in development
- investigated the extent that alternatives to rRT-PCR are being used or recommended for use internationally
- summarised the diagnostic accuracy of different alternatives to rRT-PCR, where possible, in terms of clinical sensitivity and specificity
- and addressed the potential organisational considerations and resource implications that might arise from the use of alternative tests for direct detection of SARS-CoV-2 infection in Ireland.

Since the assessment was undertaken as a rapid assessment within very restricted timelines and in the context of a global pandemic, the approach differed from a standard HTA in its scope and approaches adopted. For instance, a systematic review of the literature is routinely used in HTA to assess diagnostic test accuracy, with a meta-analysis performed to estimate a common effect and investigate the certainty of the evidence overall. However, given the complexities involved in evaluating the diagnostic test accuracy of individual tests, which are subject to a wide variety of clinical considerations, (such as disease severity, disease prevalence, participant selection, specimen handling, timing and or location of the test, and so on), such an approach was not considered suitable within the time constraints and broad scope of this rapid HTA. A number of systematic reviews that are currently completed or underway have aimed to examine particular subsets of alternatives to rRT-PCR (for example, considering alternative molecular testing approaches, or examining rapid tests suitable for point-of-care use). This rapid HTA is therefore limited to summarising and critiquing, where possible, the available evidence on diagnostic test accuracy from systematic reviews, rapid reviews or evidence summaries, as identified from a scoping review.

A discussion of the findings of the rapid HTA is provided below, separately for each research question.

6.1 Description of the technology

Testing for the detection of SARS-CoV-2 can be considered under three strategic approaches; diagnosis, screening or surveillance testing. Effective testing strategies rely on a portfolio of tests based on different technologies that can be used in different settings and situations. There is no single test that is suited to all contexts. Hence, it is necessary to investigate how different technologies can potentially be applied as part of the national testing strategy for COVID-19. Additional capacity for larger scale testing will most likely be necessary to fully meet urgent clinical and public health needs in the coming months. This may include the use of alternative technologies for detection of SARS-CoV-2, including 'rapid tests' that have been clinically validated for use in near-patient settings. The technology underpinning a given test has a significant impact on the test's performance (including its sensitivity, specificity, and reproducibility), in addition to key device characteristics such as multiplexing capacity and throughput. The performance of the device in clinical practice and device characteristics ultimately determine the ability to safely and effectively use the device in a given setting, for a given purpose.

In Ireland, diagnosis of infection with SARS-CoV-2 is currently accomplished using laboratory-based rRT-PCR. Although rRT-PCR is the gold standard diagnostic test and has high diagnostic test accuracy when used correctly, its use is associated with several limitations. In particular, the current test turnaround time exceeds 24 hours for community-based samples, which poses challenges for rapid clinical decision-making and the early initiation of public health interventions. Testing for the detection of SARS-CoV-2 infection has evolved over the course of the pandemic as more has become known about the structure of the virus and the dynamics of infection. New and emerging technologies, such as rapid molecular tests and rapid antigen detection tests (RADTs), suitable for use in near-patient settings. As such use would eliminate the requirement for transporting samples to centralised laboratories, these technologies have the potential to reduce test turnaround times and thereby improve clinical outcomes and facilitate expedited public health interventions.

Rapid molecular tests using simplified rRT-PCR technology or isothermal amplification technology apply the same basic principles of rRT-PCR; however, the number and complexity of the steps involved in sample processing is reduced, which may facilitate their use outside of the clinical laboratory. However, the integration and automation of all steps involved in molecular analysis typically results in reduced test sensitivity, compared with rRT-PCR. Some of the available tests are limited by the small number of samples which can be processed in a single run, and may

therefore be most suited to testing high-priority specimens in situations where the turnaround times associated with rRT-PCR precludes clinical utility.

RADTs are designed to directly detect SARS-CoV-2 antigens, which indicate the presence of actively replicating virus in biological samples. The vast majority of RADTs intended for use in near-patient settings are based on lateral flow assay technology. In general, RADTs are less accurate than molecular methods; however, the reliability and clinical utility of RADTs can potentially be increased where the technology includes a reader device. Despite potential limitations in performance, the WHO has suggested that if tests with acceptable performance are carried out and interpreted correctly, RADTs could play a significant role in guiding patient management and public health decision-making. Although more evidence is needed on real-world performance and operational aspects, RADTs are most likely to perform well in patients with high viral loads (Ct values ≤ 25) which usually appear in the pre-symptomatic (one-to-three days before symptom onset) and early symptomatic (within the first five-to-seven days after symptom onset) phases of the infection course. There are concerns regarding the potential for low or variable viral loads to go undetected. Thus, RADTs are likely to have the greatest clinical utility in confirming infection in symptomatic patients, who are likely to be most infectious.

Irrespective of the technology or particular device, the ability of any diagnostic test to achieve acceptable clinical performance is contingent on it being performed within the appropriate time frame with due consideration to the principles of good pre-analytical and analytical testing practice. Ease of use is an important consideration for tests performed by operators with minimal training in order to limit the potential for human error. It is important that clinical samples are processed in an environment with adequate quality assurance processes in place, particularly when considering implementation in near-patient settings.

6.2 Review of international testing methods

Both the WHO⁽⁹³⁾ and ECDC⁽⁹⁴⁾ recommend using nucleic acid amplification tests (NAATs), such as rRT-PCR, for the direct detection of current infection with SARS-CoV-2. All 18 countries included in this review similarly recommend using rRT-PCR for the detection of SARS-CoV-2, with some countries allowing other approaches such as RADTs. Nasopharyngeal swabs from the upper respiratory tract are the typically preferred specimen for PCR testing, while bronchoalveolar lavage, sputum, or endotracheal aspirate specimens are preferred from lower respiratory tract, but only if obtainable or a patient is severely ill or hospitalised. An increasing number of countries are also accepting salivary specimens, but in specific circumstances. For example, salivary specimens are acceptable in France in symptomatic patients only,⁽¹¹⁶⁾ while in Ontario, Canada, saliva specimens are acceptable in cases where

other specimen sites may not be possible or tolerated.⁽¹⁴²⁾ Some countries have also moved away from requiring a nasopharyngeal swab from certain groups due to the uncomfortable feeling associated with the swab. For example, in Ireland, a nasal swab is an acceptable specimen type for use in children in the community in Ireland,⁽¹⁶⁵⁾ while in British Columbia, Canada, children may provide a mouth rinse and gargle sample.⁽¹⁴¹⁾

Although antibody or serological testing remains broadly focused on the serosurveillance of defined target cohorts or populations for the purpose of understanding the spread of the disease, for example, a number of countries suggest serological testing can be used to support diagnosis of COVID-19 in a specific clinical scenarios. In Switzerland,⁽¹³⁴⁾ a serology test may be used as an additional diagnostic method in hospitalised patients if the RT-PCR test is negative, but the clinical picture and radiological image indicate an infection. In the US,⁽¹⁴⁵⁾ it may be used to support clinical assessment for individuals who present late in their illness. In Sweden, serological testing may provide utility beyond diagnosis of COVID-19. For example, serological testing may be of value in high-risk groups where the result may provide reassurance to people that they have some protection or immunity against the virus.⁽¹³⁰⁾

None of the included countries appear to be using whole genome sequencing for the purposes of detection of SARS-CoV-2. However, the method is being used in some countries to investigate outbreaks and study routes of transmission, as well as host response and evolution of the virus.^(166, 167, 169) In Australia, public health laboratories are expected to start sequencing the virus genomes of all positive COVID-19 tests to track the spread of the virus across the country.⁽¹⁷⁰⁾

In Australia,⁽¹⁵³⁾ Canada,⁽¹⁴⁴⁾ Germany,⁽¹²⁰⁾ Spain⁽¹²⁸⁾ and the US,⁽³⁾ rapid antigen detection tests may also be used to detect SARS-CoV-2, but in limited or specific clinical scenarios. For example, in Spain, rapid antigen tests may be used in symptomatic patients at the point of care, provided the time since symptom onset has not surpassed five days.⁽¹²⁸⁾ Depending on whether patients require hospitalisation, specific criteria is outlined for interpreting the results of rapid antigen tests. For example, if a patient does not require hospitalisation, a diagnosis is confirmed if the result is positive (detected) or ruled out if it is negative (not detected). In this scenario, it is presumed that the prevalence of infection is between 10% and 30% and the negative predictive value is between 97.2% and 99.3%, while the positive predictive value is between 94.5% and 98.5%. In contrast, if a patient requires hospitalisation, and the test is performed in a health or social centre, such as a nursing home, where the prevalence of COVID-19 may be as high as 50%, there's a higher risk of obtaining false negatives. As such, negative test results should be confirmed by rRT-PCR since the negative predictive value of the

test falls to 93.7%, while the positive predictive value rises to 99.4%.⁽¹²⁸⁾ The same criteria has been specified by the CDC⁽³⁾ in the US and is consistent with the recommendations made by the WHO;⁽⁹³⁾ however, the recommendations likely extend to both scenarios since 10%-30% prevalence of infection is relatively high (that is, confirmatory testing of negative samples should be undertaken, irrespective of whether an individual requires hospitalisation).

The WHO provides guidance on interpreting rapid antigen detection test results, depending on the scenario in which the test is deployed.⁽⁹³⁾ Typically, results should be guided by the pre-test probability (the likelihood that the patient has COVID-19 before their results are known, based on epidemiological and clinical factors) which, in part, determines the negative and predictive values of a rapid antigen test, as illustrated in Chapter 3. For example, when monitoring trends in disease incidence or attempting to quickly detect and isolate cases when there is widespread community transmission, the WHO suggests that the positive and negative predictive value of a rapid antigen test result should be used to enable effective infection control, or pre-test probability. In this scenario, the pre-test probability is high (due to a high prevalence of disease, for example) so a negative test result cannot completely exclude an active COVID-19 infection (low predictive value). As a consequence, repeat rapid antigen testing, or preferably confirmatory testing, is recommended within 48 hours.

In some countries, RADTs may also be used to screen asymptomatic individuals. For instance, in Germany, RADTs may be used to screen contacts of confirmed cases, but only in exceptional circumstances, such as when rRT-PCR capacity is limited. In Canada, RADTs can be used for the purposes of serial testing in at risk-settings such as long-term care facilities or food processing plants. RADTs may also be used for screening purposes in Australia, to be determined by individual public health authorities. Although RADTs have not yet been introduced in France, the Haute Autorité de Santé (HAS) has specified that these tests could be used to screen asymptomatic individuals who are not close contacts (for example, for the purposes of testing target populations, such as those who live, study, or work in confined places, where the risk of infection is greater than in the general population). The HAS specified that rRT-PCR should be used to screen asymptomatic contacts who have been identified via contact tracing since there is limited evidence to date to support the use of RADTs in these individuals.

The HAS additionally specified that RADTs must meet a minimum performance criteria of $\geq 80\%$ sensitivity and $\geq 99\%$ specificity (to ensure that positive cases are indeed cases of COVID-19 and not other seasonal respiratory viruses).⁽¹¹⁷⁾ This is somewhat consistent with the WHO recommendation, which specified a specificity requirement of 97%.⁽⁹³⁾ However, it is unclear where or how rapid antigen tests will

be deployed in France if the recommendations of the HAS are adopted, or how test results should be interpreted.

While a number of countries are supporting the use of RADTs at the point of care for the detection of SARS-CoV-2, it is worth noting that the WHO only recommends using these devices when rRT-PCR is unavailable or where prolonged turnaround times preclude clinical utility.⁽⁹³⁾ The WHO also recommends only using the test in symptomatic individuals (or asymptomatic individuals with known exposure), since the test has only been validated in this group. It further advises against the use of rapid antigen testing at airports or other border points of entry, since the prevalence of disease will be highly variable and predictive values of the test unknown.⁽⁹³⁾ However, as determined in this assessment, there is no evidence, to date, that rapid antigen or other point-of-care testing is being used at airports.

6.3 Diagnostic accuracy

Chapter 4 of this rapid HTA reviewed the clinical sensitivity and specificity of alternative tests to laboratory-based rRT-PCR. A scoping review was performed to identify systematic reviews, rapid reviews or evidence summaries which have been undertaken to evaluate the diagnostic accuracy of alternatives to laboratory-based rRT-PCR. Independent evaluations performed by the Foundation for Innovative Diagnostics (FIND) were also included.

When considering diagnostic accuracy, it is firstly important to understand the consequences of reduced sensitivity and specificity. In the context of population testing with the aim of controlling infection spread, and where the test method in place has suboptimal accuracy, insufficient sensitivity will result in missing infected individuals who might otherwise be isolated, and insufficient specificity may result in the imposition of isolation measures on individuals who are not true positives, and the imposition of restriction of movements on their contacts. In the context of hospital-based testing, insufficient diagnostic accuracy may have particularly severe consequences in the immediate term. The correct classification of patients' infection status is important in minimising nosocomial transmission and the transmission of infection to staff, but also for protecting patients who may unknowingly be infected with the virus and who are due to undergo treatment that would likely undermine their recovery from COVID-19.

In practice, sensitivity of rRT-PCR, the benchmark test for the diagnosis of infection with SARS-CoV-2, has been estimated as ranging between 71% and 98%; the exact sensitivity depends on various factors, including the timing and type of specimen obtained, the sampling technique, and the quality of particular test kits used to perform rRT-PCR. Specificity of rRT-PCR tests is generally very high (typically greater than 99.5%). In considering an alternative test, the relative gains and losses in

sensitivity and specificity, and the context in which the test is to be used, should be carefully considered.

The WHO has specified desirable and minimally acceptable targets for the sensitivity and specificity of tests to be used in various situations of testing to support the response to the COVID-19 pandemic. For the diagnosis or confirmation of acute or sub-acute SARS-CoV-2 in the context of both low and high-volume testing needs, acceptable and desirable levels of sensitivity are set at $\geq 95\%$ and $\geq 98\%$, and the corresponding levels of specificity are set at $\geq 99\%$ and $\geq 99\%$. For the circumstance of point-of-care testing for suspected COVID-19 cases and their close contacts, where rRT-PCR testing is unavailable or where turnaround times obviate clinical utility, acceptable and desirable levels of sensitivity are set at $\geq 80\%$ and $\geq 90\%$, respectively, and the corresponding levels of specificity are set at $\geq 97\%$ and $>99\%$. For each of these sets of specifications, when considering the estimates for the sensitivity or specificity of a particular test, it is stipulated that the lower bound of the estimated confidence interval should equal or exceed the target. As such, the degree to which the estimate is precise, and the degree to which the lower estimate of precision exceeds sensitivity and specificity thresholds, will dictate the acceptability of a particular test.

The present scoping review found that the diagnostic accuracy of commercial rRT-PCR platforms, for example, high-throughput assays such as Roche's Cobas® 6800, and automated assays such as GenMark's ePlex®, Diasorin's Simplexa™ and Cepheid's Xpert®Xpress, was found to be high. In one meta-analysis, the average sensitivity pooled across multiple studies for these tests was found to be $\geq 99\%$ for all but the ePlex platform (pooled sensitivity of 94%). Specificity was $\geq 96\%$ in all cases. Another study identified low sensitivity (68%) for Mesa Biotech Inc.'s Accula test, however.

Results for the diagnostic accuracy of isothermal amplification based assays and test platforms included estimates for the sensitivity and specificity of RT-LAMP, CRISPR, Abbott's ID NOW™ platform, the SAMBA II platform, and several other isothermal amplification methods (iAMP, RT-iiPCR, RT-RPA, RCA, RT-RAA). In one systematic review, the diagnostic accuracy of RT-LAMP-based molecular methods appeared to depend on whether crude or purified samples were analysed. Where crude samples were analysed (for example, nasopharyngeal or saliva samples), this resulted in lower sensitivity, with values ranging from 40% to 88%. However, analysis of purified samples led to sensitivity values in excess of 90% in the majority of RT-LAMP based studies. Alternative methods using CRISPR or the SAMBA II platform similarly showed sensitivity values above 90% in the majority of cases. The ID NOW™ platform had the lowest sensitivity among isothermal amplification methods. Overall, specificity was high among isothermal amplification methods.

For rapid antigen tests, only one systematic review of diagnostic accuracy was identified. The sensitivity of RADTs was found to vary significantly across test brands; estimates ranged from 0% to 94%, with an average sensitivity of 56.2% (95% CI 29.5% to 79.8%). Average specificity was 99.5% (95% CI 98.1% to 99.9%). Recent clinical evaluations of antigen tests, as conducted by the FIND collaboration, were not included in the aforementioned review; for example, one such recent evaluation for a particular antigen test (SD Biosensor Inc. Standard Q) estimated sensitivity as 77% and 89% in two sites. It is important to consider that iterative development of diagnostic tests over time may result in improved sensitivity and specificity among technologies.

Current estimates of diagnostic accuracy for alternative tests to rRT-PCR are limited by significant flaws in the design, execution and reporting of primary diagnostic accuracy studies, and it is not possible to ascertain whether these limitations have led to overestimation or underestimation of test accuracy estimates. With respect to the secondary research literature, as the research landscape for tests involved in the detection of SARS-CoV-2 is rapidly-evolving, recent results will not have been captured in published reviews of diagnostic accuracy. Furthermore, there is a lack of research on the diagnostic accuracy of emerging technologies.

There is also a lack of information on the transferability of diagnostic accuracy results to certain subpopulations or settings. These include asymptomatic individuals, specific at-risk populations such as healthcare workers, and the validation of diagnostic performance of tests beyond their use in the hospital setting or in self-administered tests. Ideally, clinical sensitivity and specificity of tests should be measured in various clinically-relevant real-life situations, including various sources of specimens, timing of specimens, and degrees of illness severity.

In addition to considering the limitations of the existing literature, when considering diagnostic accuracy, published metrics should be considered as interrelated with contextual factors which may have implications for accuracy in practice. These include the pre-test probability of infection, which affects the likelihood that a person with a negative test result is a true negative or a false negative, and the processes involved in testing, for example, sampling, or the use of particular reagents may impact the performance of a test.

Finally, diagnostic test accuracy metrics should not be considered as an isolated measure of a test's performance; metrics should be situated within consideration of the overall aim of testing and associated organisational factors which may impact on the value of a particular test.

6.4 Organisational considerations

Expansion of testing to include alternatives to laboratory-based rRT-PCR has the potential to improve access to timely testing, contributing to improved outcomes for patients and facilitate implementation of infection prevention and control measures. However, reliable and accurate testing in near-patient testing (NPT) settings can only be achieved if accurate and reliable tests are employed that have been validated for the setting in which they are intended to be used, and that NPT is provided as part of a well-structured and properly governed service. Current Irish guidelines for near-patient testing highlight that it is important for testing performed outside of a laboratory to be assured of the same quality and standards and is not a patient safety risk. If NPT for COVID-19 were to be deployed as part of the national testing strategy, then consideration should be given to ensure such a service is in line with the national guidelines for safe and effective near-patient testing. This includes recommendations that a NPT operational team should be established, with appropriate representation from participating disciplines, to oversee the day-to-day operation of COVID-19 NPT. This would include the development of standard operating procedures, training requirements, and quality assurance programmes. Consideration should also be given to establishing a link with the local hospital pathology service to ensure that testing is provided in a safe and effective manner and to be ultimately accreditable to the required ISO 15189/22870 standards. Consideration must be given to the capacity of clinical laboratories to provide the level of support necessary to ensure consistent provision of a quality-assured service across laboratory and non-laboratory testing sites settings on an ongoing basis, but particularly during the early implementation phase.

The expansion of testing is intended to supplement, not replace, the current laboratory-based testing strategy. Testing for the general population with symptoms associated with COVID-19 or those identified during contact tracing efforts will continue to take place in clinical hospital laboratories with established quality assurance processes. However, in some scenarios or settings, such as the management of critically ill patients, at-risk groups, outbreak settings or settings carrying out essential activities, NPT has the potential to improve clinical outcomes and facilitate faster initiation of public health interventions. It is imperative that the expansion of COVID-19 testing to near-patient settings does not adversely impact the conduct of context-specific core activities at these sites where testing is to be carried out within the existing resources. While rapid tests are likely to be easily accommodated within existing near-patient settings without the requirement for substantial alteration of investment in the premises, the impact on workflow arrangements will require consideration at a site-specific level.

It is important that the results of near-patient testing are captured in the national surveillance estimates using an electronic reporting system, where possible, to reduce the administrative burden on providers reporting test results and to minimise the risk of reporting errors. The tests recognised by the HSE within this reporting system have implications for both national surveillance data and contact tracing efforts, which requires careful consideration. At present, only the results of laboratory-confirmed rRT-PCR tests (standard or rapid), that have been obtained using tests for which satisfactory validation data are available, are recorded by the HSE. Positive RADT results that have not been appropriately validated for use in near-patient settings are considered to be indicative of infection with SARS-CoV-2, and require further confirmatory testing using validated laboratory-based rRT-PCR prior to being recorded in the national surveillance estimates. Under the current testing strategy, individuals with a positive RADT result who do not present for follow-up testing would not be captured in the national surveillance data, and would therefore not be included as part of the subsequent contact tracing efforts. Accurate data on the extent of testing that is being undertaken would be helpful to inform national surveillance estimates; however, it is important that these data are based on tests that achieve minimum performance criteria and that have been clinically validated for the purpose for which they are being used.

The reporting system for COVID-19 testing should include the results of both diagnostic and screening testing, which should be distinguishable in the reporting system according to the type of test used (for example rRT-PCR, rapid molecular test, RADT). However, the use of RADTs to inform contact tracing efforts is reliant on the results being accurate and reliable. Only tests that have been appropriately validated for use in the intended setting should be used to inform contact tracing efforts. This prevents the close contacts of individuals with a false positive result being contacted and the unnecessary harms associated with this (such as, emotional distress, absence from work and reduced productivity).

6.5 Development of advice for NPHE

The advice provided to NPHE is informed by research evidence developed by HIQA's COVID-19 Evidence Synthesis Team and with expert input from HIQA's COVID-19 Expert Advisory Group (EAG). Topics for consideration are outlined and prioritised by NPHE. This process helps to ensure rapid access to the best available evidence relevant to the SARS-CoV-2 outbreak to inform decision-making at each stage of the pandemic.

A draft document was developed by the Evidence Synthesis Team to address the four questions that informed the scope of this report. Individual members of the COVID-19 Expert Advisory Group (EAG) with expertise in specific areas (for example,

laboratory testing practices) were contacted for support in respect of specific aspects of the report. A meeting of the COVID-19 EAG was convened for clinical and technical interpretation of the research evidence, and a draft of the report was circulated in advance of the meeting. Feedback was provided on the draft and on the basis of discussions at the meeting. This feedback was incorporated into this document with revisions made to the text, where appropriate.

At the EAG meeting, the evidence was then considered in terms of its application to the policy question and, specifically, to address the alternatives to laboratory-based real-time RT-PCR that could be deployed in Ireland to detect SARS-CoV-2 infection within the coming weeks (that is, quarter 4 of 2020) and in the near future (for example, the first half of 2021). Specific consideration was given to whether these alternatives would differ according either to the purpose of the test or the setting in which it might be deployed. Consideration was also given to the organisational issues that would need to be considered depending on where the test is deployed.

Based on the research evidence presented, the Expert Advisory Group reasoned that the following were options that could potentially be adopted:

- Within high-throughput laboratory settings, sample pooling strategies could be expanded to increase rRT-PCR testing capacity. Given potential supply constraints for rRT-PCR consumables (for example, reagents), pooling would allow additional testing to be provided without a proportional increase in requirements for the related consumables. While there are acknowledged technical and logistical challenges to sample pooling, it was considered feasible that use of this strategy could be expanded within the coming weeks. The following circumstances were identified as potentially suitable for pooling of samples:
 - specimens collected for the purpose of serial testing of asymptomatic individuals in at-risk settings (for example, nursing homes, healthcare workers, food processing facilities, vulnerable communities)
 - specimens collected from patients as part of pre-admission precautions prior to elective procedures.
- Near-patient testing, including the use of rapid antigen detection tests, was identified as a potential option to expand test capacity and to improve test turnaround and access. However, a number of limitations were identified that prevent immediate deployment of these tests. Specifically the:

- substantial variability in reported performance within individual technologies and devices that precludes a class-based endorsement of such tests
 - uncertainty regarding the suitability of certain tests for use in the near-patient setting, such as devices based on RT-LAMP technology and certain RADTs that may require additional handling steps
 - lack of performance data in asymptomatic populations
 - requirement for clinical validation of any test compared with laboratory-based rRT-PCR both for the purpose and the setting in which they are intended to be used.
- The Expert Advisory Group reasoned therefore that initial immediate investment is required to commence a number of clinical validation studies in the Irish setting. Pending satisfactory performance in these clinical validation studies, the relevant tests could be adopted as part of a national testing strategy in 2021. Potential circumstances identified for validation included:
 - supplementing the capacity of the high-throughput laboratories for the diagnosis of symptomatic patients early in the course of infection
 - serial testing for the prevention of outbreaks in at-risk settings (for example, nursing homes, healthcare workers, food processing facilities, vulnerable communities)
 - testing for the investigation and management of outbreaks (for example, in nursing home and university settings).
 - Adoption of an alternative test requires consideration of factors including clinical performance (sensitivity and specificity), turnaround time, and ease of use. Similar to global guidance issued by WHO Exact specifications should be outlined for what constitutes a suitable test for each relevant purpose in the Irish setting.
 - It was noted that hospital-based laboratories have validated and adopted a range of simplified rRT-PCR tests. These tests offer comparable accuracy and facilitate rapid clinical decision-making. These devices typically have limited throughput and can be subject to supply chain shortages. Therefore the use of these simplified rRT-PCR tests should be reserved for high priority clinical circumstances.

- It was emphasised that the adoption of near-patient testing would present substantial organisational and logistical settings. Pending the outcome of the clinical validation studies outlined above, consideration should be given in the interim to how such testing in devolved settings (for example, in a community testing hub) would be resourced. This could require consideration of the quality assurance, governance, training and reporting requirements essential to delivering a safe and effective service. It was highlighted that the introduction of near-patient testing should be within the context of a supporting national quality management system. It was highlighted that adoption of such testing would likely be in addition to existing centralised testing and would therefore require substantial investment.
- It was also emphasised that there is a need to mitigate potential risks associated with testing performed outside of the publicly-funded national Test and Trace programme. This should include multilateral communication with stakeholders, including members of the public and private providers with the goal that all testing should be undertaken in the context of an ongoing quality assurance programme to provide confidence in the test results for both the physician and the patient.

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Appendix A: Protocol for the rapid HTA

A.1 Purpose and Aim

The purpose of this protocol is to outline the process by which the health technology assessment (HTA) team will conduct a rapid HTA of alternatives to laboratory-based real-time RT-PCR to diagnose current infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This assessment is to inform the work of the National Public Health Emergency Team (NPHEM) in their response to the COVID-19 (coronavirus disease 2019) pandemic.

The rapid HTA will be conducted in line with the processes and timelines outlined for Phase 2 of HIQA's COVID-19 response. The report commenced on 7 September 2020 and a final draft will be completed by 2 October 2020 with a view to providing advice and recommendations to NPHEM on 7 October 2020.

Draft outputs from the rapid HTA will be circulated to the COVID-19 Expert Advisory Group (EAG) for review in two phases. The first two research questions (detailed below, in section 2.1) will be completed and circulated to the EAG on 25 September as part of the first phase of the rapid HTA. The final two questions will be completed during the second phase, with a final draft of the report completed and circulated to the EAG on 2 October 2020 and discussed at a meeting of the EAG on the 6 October. The findings from the rapid HTA will be provided as advice to NPHEM on Wednesday, 7 October 2020.

A.2 Process outline

It is important that a standardised approach to the process is developed and documented, to allow for transparency and to mitigate risks which may arise due to changes in staff delivering and or receiving the information.

Four distinct steps in the process have been identified. These are listed below and described in more detail in sections 2.1-2.5:

1. Develop research question(s) and formulate protocol(s)
2. Address research question(s), as defined by the associated protocol(s)
3. Summarise findings
4. Provide advice to NPHEM.

Given the rapidly changing environment, this protocol will be regarded as a live document and amended when required to ensure it reflects any changes made to the outlined processes.

A.3 Research questions

The following research questions (RQs) were developed, in line with the request from NPHE, and following feedback from key stakeholders, and will inform the scope of the assessment:

1. What are the potential tests or testing methods that can detect SARS-CoV-2?
2. What testing methods are currently being used internationally for the detection of SARS-CoV-2?
3. What is the diagnostic accuracy of alternatives to laboratory based real-time RT-PCR testing, for the purpose of diagnosis of current infection with SARS-CoV-2?
4. What are the potential organisational considerations and resource implications that might arise from the use of alternative tests for direct detection of SARS-CoV-2 infection in Ireland?

The protocols for the above questions are detailed below.

A.4 Protocols

A.4.1 RQ 1

RQ 1: What are the potential tests or testing methods that can detect SARS-CoV-2?

One key step is involved in the process of this RQ:

- Describe the range of alternative tests to laboratory-based real-time RT-PCR as informed by a scoping review of the literature, including the:
 - direct detection of viral ribonucleic acid (RNA), such as:
 - real-time reverse transcription polymerase chain reaction (rRT-PCR)
 - digital droplet PCR (ddPCR)
 - Isothermal nucleic acid amplification tests (for example, reverse transcription loop mediated isothermal amplification (RT-LAMP))
 - DNA sequencing
 - DNA microarray
 - direct detection of viral proteins (antigens) , such as:
 - enzyme immunoassay (EIA) – also termed ELISA
 - chemiluminescence immunoassay (CLIA), fluorescent (FIA) immunoassays, lateral flow immunoassays (LFIA) including lateral flow fluorescent immunoassays
 - protein microarrays

A.4.2 RQ 2

RQ 2: What testing methods are currently being used internationally for the detection of SARS-CoV-2?

Three distinct steps in the process have been identified. These are listed below and described in more detail in sections 2.2.2.1-2.1.2.3.

1. Perform a rapid review by searching relevant international resources.
2. Review and extract relevant information on international testing methods for the detection of SARS-CoV-2.
3. Summarise findings.

Perform a rapid review by searching relevant international resources

The international resources included in this rapid review are from a range of ministries of health and public health agencies. These were chosen based on them being in a similar phase of pandemic response, widespread use of the organisation's advice, and or the working constraints of the HTA team. In addition to including information from the World Health Organization (WHO) and European Centre for Disease Control and Prevention (ECDC), guidance from 21 countries will be sought. Where guidance could not be found or is unavailable, this will be detailed in the report.

Guidance from the following national or international public health bodies, ministries of health and associated governmental departments will be sought for this rapid review:

International public health bodies

- WHO
<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance-publications?healthtopics=b6bd35a3-cf4f-4851-8e80-85cb0068335b&publishingoffices=aebab07-3d0c-4a24-b6ef-7c11b7139e43&healthtopics-hidden=true&publishingoffices-hidden=true>
- ECDC
https://www.ecdc.europa.eu/en/search?s=&sort_by=field_ct_publication_date&sort_order=DESC&f%5B0%5D=diseases%3A2942

United Kingdom

- England (Public Health England (PHE))
<https://www.gov.uk/government/collections/coronavirus-covid-19-list-of-guidance>
- Scotland (Health Protection Scotland; Scottish Government)

<https://www.hps.scot.nhs.uk/a-to-z-of-topics/covid-19/>

- Northern Ireland (NI) (Public Health Agency of NI)

<https://www.publichealth.hscni.net/>

- Wales (Welsh Government)

<https://gov.wales/coronavirus>

<https://phw.nhs.wales/>

Other European countries

- Austria

<https://www.sozialministerium.at/public.html>

- Belgium

<https://www.info-coronavirus.be/en/>

- Denmark (The Danish Health Authority)

<https://www.sst.dk/en/english/corona-eng>

- France

<https://solidarites-sante.gouv.fr/>

- Germany (Federal Ministry of Health, Robert Koch-Institut (RKI))

<https://www.zusammengeng corona.de/en/>

https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Vorl_Testung_nCoV.html

- Iceland (Ministry of Health)

<https://www.covid.is/faq>

- Ireland

<https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/>

- Norway (Norwegian Institute of Public Health (NIPH))

<https://www.fhi.no/en/id/infectious-diseases/coronavirus/>

- Spain (Ministry of Health)

<https://www.mscbs.gob.es/profesionales/saludPublica/ccayes/alertasActual/nCov/documentos.htm>

- Sweden (The Public Health Agency of Sweden)

<https://www.folkhalsomyndigheten.se/the-public-health-agency-of-sweden/>

- Switzerland (Federal Office of Public Health (FOPH))

<https://www.bag.admin.ch/bag/en/home/krankheiten/ausbrueche-epidemien-pandemien/aktuelle-ausbrueche-epidemien/novel-cov.html>

- The Netherlands (Ministry of Health)

<https://www.rivm.nl/en/novel-coronavirus-covid-19>

North America

- Canada (Government of Canada)

<https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/guidance-documents.html>

- United States (US) (Centers for Disease Control and Prevention (CDC))
<https://www.cdc.gov/coronavirus/2019-ncov/whats-new-all.html>

Australasia

- Australia (Department of Health)
<https://www.health.gov.au/>
- New Zealand (NZ) (Ministry of Health)
<https://www.health.govt.nz/our-work/diseases-and-conditions/covid-19-novel-coronavirus>

Asia

- Singapore (Ministry of Health)
<https://www.moh.gov.sg/covid-19/advisories-for-various-sectors>

Review and extract relevant information on testing methods for the detection of SARS-CoV-2

All identified testing methods will be extracted by one reviewer. A second reviewer will verify all extracted data and ensure no information is missing.

In the first instance, data on the recommended primary SARS-CoV-2 test by country/organisation will be extracted (including information on the type, sample, and specimen). Information on any other tests that have been recommended for use will also be extracted. Where alternative tests have been recommended or are in use internationally for the detection of current infection, the following information will be extracted:

- Type of test (e.g., rapid antigen test)
- Location/specialty required (e.g., laboratory-based or near patient testing (point-of-care))
- Setting (e.g., nursing homes, airports, schools)
- Criteria for testing (e.g., vulnerable groups)
- Testing strategy (e.g., given a positive/negative test result)
- Quality assurance processes
- Centralised reporting or data linkage
- Out-of-pocket expenses

Where information is extracted, the exact source will be recorded and detailed in the summary findings.

Summarise findings

A summary of the findings will be drafted with all extracted data presented in the report.

A.4.3 RQ 3

RQ3: What is the diagnostic accuracy of alternatives to laboratory based real-time RT-PCR testing, for the purpose of diagnosis of current infection with SARS-CoV-2?

Four distinct steps in the process have been identified. These are listed below.

1. Perform a scoping review to identify systematic reviews, rapid reviews or evidence summaries which have been undertaken to evaluate the diagnostic accuracy of alternatives to laboratory-based rRT-PCR, as currently undertaken in Ireland, in accordance with the research question. Studies will be assessed for inclusion according to the hierarchy of evidence.
 - a. Reviews of diagnostic accuracy which consider the PIRD elements (population, index test, reference standard, diagnosis of interest) in Table A.1 will be considered relevant.
 - b. In line with standard operating procedure for the conduct of scoping reports, a search of the literature will be undertaken using the PubMed Clinical Queries Tool. The results will be limited to English-language studies conducted in humans and published since January 2020. The search will be supplemented by ad hoc Internet searches, in addition to targeted searches of the websites of HTA agencies and public health bodies. PROSPERO will also be searched to identify any ongoing systematic reviews.
 - i. The following search terms will be used: (diagnostic test* OR rapid test* OR near-patient test* OR point-of-care test*) AND (COVID-19 OR coronavirus OR SARS-CoV-2).
2. If a high-quality systematic review(s) is available, review and extract relevant information on the diagnostic accuracy of tests for the direct detection of SARS-CoV-2 as per PIRD. Due to the time limitations associated with this review, updating of existing systematic reviews will not be possible.
3. Summarise findings.

Table A.1 Research question outlined in the PIRD format

Criteria	Definition
Population	Individuals (any age) tested for active SARS-CoV-2 infection: <u>Subgroups of interest:</u> <ul style="list-style-type: none"> - Adults (≥ 18 years) vs children (<18 years) - Symptomatic vs asymptomatic
Index test	Alternative testing approaches to laboratory-based real-time RT-PCR for the detection of SARS-CoV-2 based on nucleic acid or antigen. <u>Subgroups of interest:</u> Where combined with additional testing approaches (e.g. rRT-PCR) and or clinical assessment or imaging.
Reference standard	Laboratory-based real time RT-PCR
Diagnosis of interest	Accurate diagnosis of current SARS-CoV-2 infection, as measured by sensitivity, specificity and predictive values: <u>Measures of diagnostic accuracy to be reported:</u> <ul style="list-style-type: none"> - Average sensitivity of tests (and associated uncertainty), range of values for sensitivity. - Average specificity of tests (and associated uncertainty), range of values for specificity. - Positive predictive value (and associated uncertainty) - Negative predictive value (and associated uncertainty)
Study design	Study designs to be considered within systematic reviews: Retrospective and prospective cohort, case series/case control studies (with a minimum of 10 participants) and cross-sectional studies, which evaluate diagnostic accuracy of near-patient tests for the detection of SARS-CoV-2.

PIRD – population, index test, reference standard, diagnosis of interest

A.4.4 RQ 4

RQ 4: What are the potential organisational considerations and resource implications that might arise from the use of alternative tests for direct detection of SARS-CoV-2 infection in Ireland?

One key step is involved in the process of this research question:

- Describe the potential operational considerations that might arise from the use of alternative testing approaches in Ireland, as informed by findings of the rapid HTA and the current health care landscape in Ireland. Consideration will be given to the various settings in which rapid tests could be deployed.

A.5 Summarise findings

A descriptive overview of the findings of the rapid HTA will be prepared. The potential strengths and limitations of the evidence will be detailed. A draft of the report will be circulated to the EAG for review.

A.6 Provide advice and recommendations to NPHE

Informed by feedback and input from the EAG, the draft rapid HTA will be updated and provided to NPHE as advice, in line with HIQA's processes for Phase 2 of its COVID-19 response.

Appendix B: WHO COVID-19 Target product profiles for priority diagnostics to support response to the COVID-19 pandemic, v.0.1⁽¹⁹⁵⁾

Background rationale and key considerations:

1. Point of care test for suspected COVID-19 cases and their close contacts to diagnose acute SARS-CoV-2 infection in areas where reference assay testing is unavailable, or turnaround times obviate its clinical utility

For many reasons, including shortages of reagents, lack of technical expertise and inadequate laboratory capacity, rRT-PCR/reference assay testing has either not been broadly available or its availability has not translated into timely diagnostic results because the human and lab capacities have been insufficient to meet demand in many countries. Delayed transport of samples and return of results is a critical problem given the crippling social and economic impact of quarantine and lack of facilities to properly isolate patients awaiting test results. Many countries, especially low- and middle-income countries, rely on centralized testing facilities that rarely meet the needs of patients, caregivers, health workers and society as a whole. Therefore, a highly specific, rapid and easy-to-use test that could identify the majority of patients with early, acute SARS-CoV-2 infection, allowing for immediate implementation of isolation and other efforts to arrest transmission of the virus, would reduce the number of people with suspected infection requiring secondary testing. Such a test would be particularly useful during suspected SARS-CoV-2 outbreaks; in areas with confirmed SARS-CoV-2 community-wide transmission; confirmed outbreaks in closed or semi-closed communities; in high-risk groups; among contacts of confirmed cases; and as a tool to monitor disease incidence.

It is considered acceptable to target patients with high viral loads often present in the first week following infection^{1,8,2,3} because they are most likely to transmit the infection to others.

Depending on the known or estimated prevalence of COVID-19 among suspected cases or contacts, a test that meets the profile's acceptable or desirable performance characteristics should be sufficient to diagnose SARS-CoV-2 infection and/or exclude a diagnosis without additional confirmatory testing.

Regarding programmatic suitability, tests that do not require any additional equipment are desirable, and any equipment required must be portable and battery powered. The profile's acceptable test kit stability and shelf-life characteristics will not meet the needs of many tropical countries where distribution is challenging and cool storage (< 30°C) is not consistently feasible. Test developers are strongly encouraged to achieve desirable characteristics to maximize access in remote settings with hot climates.

1. Point of care test (POCT)¹ for suspected COVID-19 cases and their close contacts to diagnose acute SARS-CoV-2 infection in areas where reference assay testing is unavailable, or turnaround times obviate its clinical utility

Notes

Intended Use

In areas with confirmed SARS-CoV-2 community wide transmission or confirmed outbreaks in closed or semi closed

The primary objectives of the COVID-19 global response are to slow and

¹ Wölfel, R., Corman, V.M., Guggemos, W. *et al.* Virological assessment of hospitalized patients with COVID-2019. *Nature* **581**, 465–469 (2020). <https://doi.org/10.1038/s41586-020-2196-x> ⁸ Bullard J, Dust K, Funk D, *et al.* Predicting infectious SARS-CoV-2 from diagnostic samples [published online ahead of print, 2020 May 22]. *Clin Infect Dis.* 2020; ciaa638. doi:10.1093/cid/ciaa638

² Wang-Da Liu, Sui-Yuan Chang, Ming0Jui Tsai *et al.* Prolonged virus shedding even after seroconversion in a patient with COVID-19. *Journal of Infection.* In press.

<https://doi.org/10.1016/j.jinf.2020.03.063> 0163-4453

³ Van Kampen J, van de Vijver D, Fraaij P *et al.* Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants. doi:

<https://doi.org/10.1101/2020.06.08.20125310> medRxiv preprint

	<p>communities and in high risk groups: Early detection of SARS-CoV-2 cases where molecular/reference assays are not available or services are overloaded, leading to turnaround times that are not useful for guiding clinical case management and infection control measures.</p> <p>In suspected SARS-CoV-2 outbreak situations: multiple positive cases highly suggestive of SARS-CoV-2</p> <p>Monitor trends in disease incidence</p>	<p>stop transmission; find, isolate and test every suspect case; and provide timely appropriate care of patients with COVID-19. This test would allow for rapid and early detection of the most infectious SAR-CoV2 cases (highest viral loads). Where SARS-CoV-2 is known to be circulating (prevalence high), positive results would trigger immediate infection control measures and contact tracing. Mild or no symptoms would be referred for self-isolation and self-care, and those with moderate/severe and or risk factors would be hospitalized and isolated. Confirmatory testing for people who test positive is only recommended where enough tests are available, where disease prevalence is low (<5%) and to confirm suspect outbreaks (positive predictive value unknown). Patients with negative test results should be tested and treated or treated empirically for other diseases as per national guidelines, and those with respiratory symptoms should take precautions to reduce onward transmission and should have repeat SARS-CoV-2 POCT during the first 10 days post onset of symptoms if symptoms persist or worsen.</p>	
Key Feature	Acceptable	Desirable	Notes
Target use setting	The tests can be performed outside laboratories including	Same as acceptable but can be self-administered and/or	

	at routine and ad-hoc triage/screening points of health care facilities such as emergency units, mobile units and in the community (contact tracing) by health care workers or laboratory technicians with appropriate training in sample collection, biosafety and in the use of the test.	performed by trained lay workers (volunteer/community health workers).	
Target molecule (analyte to be detected)	SARS-CoV biomarker (e.g. RNA, protein/antigen(s) specific for acute e.g. first week after onset of symptoms /current infection (assumption that SARS-CoV-1 is not circulating).	SARS-CoV-2 only biomarker (e.g. RNA, protein/antigen) specific for acute and subacute e.g. first two weeks after onset of symptoms/current infection.	Analytes associated with current infection are highest priority (days 1 through 8) as they detect the most infectious cases. On this basis, immunoglobulin-based tests would not be an acceptable analyte.
Analytical sensitivity/Limit of detection	equivalent to 10^6 genomic copies/mL or Ct \approx 25-30	equivalent to 10^4 genomic copies/mL or Ct \approx >30	Variable population characteristics are expected, which will result in variable clinical sensitivity and specificity. Therefore, a limit of detection (LOD) that is based on anticipated viral loads in patient specimens and associated infectivity is critical to anticipate clinical utility. Reports in literature are variable: copies/reaction, copies/mL but most often cycle threshold (Ct) values. Correlation between viral load and transmissibility is not entirely clear - some reports cite inability to culture virus < 10^6 ^{4,5} . Therefore, we propose a POC test that can consistently detect the most infectious patients (e.g. LOD 10^6) in order to

			interrupt transmission. Test developers should use well characterized reference material and international standards, when available, to determine limits of detection.
Analytical specificity	Assay detects all SARS-CoV-2 viral strains and does not cross react with common interfering substances or other human coronaviruses (except SARS-CoV-1) or any other common human diseases, especially those presenting with similar signs and symptoms of COVID19 (e.g. influenza A, B, RSV , malaria, dengue) ⁶	Assay detects all SARS-CoV-2 viral strains and does not cross react with common interfering substances or other human coronaviruses or any other common human diseases especially those presenting with similar signs and symptoms of COVID-19 (e.g. influenza A, B, RSV, malaria, dengue) ⁶	
Sensitivity	≥ 80%	≥90%	The targets are for the estimated true sensitivity and specificity; therefore, the lower bound of confidence intervals should ideally equal or exceed the target. Determination of sensitivity and specificity should be against an approved/authorised by a stringent regulatory authority (SRA), molecular based COVID-19 assay ⁷ .Product assessment of clinical specificity must include patients/samples with other human coronaviruses and pathogens in differential diagnosis for presenting signs/symptoms. For both sets of criteria, at low prevalence, PPV is <50%, and would require a second test for confirmation; however,
Specificity	≥ 97%	>99%	

			negative predictive value (NPV) is high. When prevalence increases to 10-20%, acceptable criteria for PPV increases to >78-89% and NPV remains high (95-98%).
Type of analysis	Qualitative (yes/no), semi-quantitative or quantitative	Not applicable	
Interpretation	Visual manual and/or hardware reader (proprietary or smart phone application)	Visual manual read or digital readout via smartphone application reader with connectivity	
Sample type	Nasopharyngeal, oropharyngeal swab (or wash) nasal swab (anterior nares or mid-turbinate), nasal wash, sputum	Anterior nares, saliva/oral fluid, sputum	Specimens that are easier to collect and associated with lower risk of aerosols are preferred i.e., saliva/oral fluid. Ideally the test can meet LOD requirements in an upper and a lower respiratory tract specimen.
Sample collection device	Compatible with an existing swab material e.g. flocked	Compatible with multiple swab materials; self-collection or no swab required e.g. saliva	
End user profile	Trained staff in health care facilities	Trained staff in health care facilities or community level (lay person) or self-administered.	
Training needs (including sample collection, test procedure, results interpretation, QC and biosafety)	0.5 days with instructions for use and quick reference guide (s)	2 hours with instructions for use and quick reference guide (s) including through smart phone application(s) to ensure ongoing compliance and up-to-date training.	
Test procedure			
Sample preparation steps Need to process the sample before performing the test	1	0	

Reagents reconstitution Need to prepare the reagents before utilization	Reconstitution acceptable if very simple to do.	All reagents ready to use.	
Sample minimum volume	single swab and minimal extraction buffer/diluent.		
Need to transfer a precise volume of sample	Acceptable if autofill or graduated volume markings on sample transfer device is provided	No, or limited to a number of drops.	Addition of drops is not considered 'precise' volume requirement.
Number of timed steps (use of different reagents/incubation steps)	≤ 3	1, with the potential for digitally guided workflows and built-in timers to reduce user errors on timed steps.	
Time to result	≤ 40 minutes	≤20 minutes	Expect patients would wait for results.
Sample stability pretesting	30 minutes (dry, not refrigerated, 1035°C); 2-4 hrs (dry, refrigerated (4-8°C); 8 hrs (refrigerated (48°C) in generic preservative); several days frozen in generic preservative (min -20°C).	i) Test compatible with both dry and preserved samples; ii) 3 hours (dry, not refrigerated (10-40°C); 8 hrs (dry, refrigerated (4-8°C); 24 hrs refrigerated in generic preservative and months frozen in preservative.	Accept tests that have to be done immediately as the point is to do this at the point of care but the ideal would be options for immediate stability, with refrigeration and freezing and that are compatible with both dry and preserved sample types.
Result validity stability	Fixed reading time.	Stored image or 6 weeks.	
Invalid rate	<2% invalid results with correct use by operator.	≤0.5% invalid results with correct use by operator.	
Additional characteristics			
Operating conditions	15-35°C; 25-80% relative humidity up to 1500m.	10-40°C; 25-90% relative humidity up to 3000m.	Ideally tests could support conditions in tropical countries.
Test kit stability and storage conditions	12 months ⁴ at 430°C; tolerates brief periods > 40°C; humidity 75%+ 5% any associated equipment must meet or exceed these requirements.	18-24 months at 4-40°C; tolerates freezing and brief periods > 45°C; humidity 75%+ 5%.; any associated equipment must meet or exceed these requirements.	Expect real time stability data to support shelf life requirements will not be available at the time of product release, but manufacturers should be challenged to meet targets that match what is realistic for supply chains in low- and middle-income countries.

Stability of the kit once opened	30 mins for single use test after opening the pouch.	1 hour for single use test after opening the pouch.	
Specimen capacity & throughput	≥5/hr per operator.	≥10/hr per operator.	Assume that individual tests can be run in parallel - probably not more than 5 per hour at a time feasible per operator.
Safety precautions	Standard respiratory sample collection safety precautions recommended, and all materials are free of components with a GHS classification H (particularly H350, H340, H360) ⁸	Tests that minimize the need for biosafety requirement are strongly preferred e.g. with a self-sample collection device with virus inactivation.	
Quality Control	Internal control (for sample flow/migration) is an area or region within the individual testing device; positive control and negative control sold separately; external quality assessment material compatible; calibration control for reader, if applicable.	Internal control is an area or region within the individual testing device; lyophilized positive control and negative (full process) control provided in the kit; external quality assessment material compatible; calibration control for reader, if applicable	
Remote connectivity capacity	Not required for reader independent tests; If device-based: Remote export of data possible.	Test is compatible with readers and other data capture devices; internal memory to store results even if power cut and with the ability to report to country health information management systems using an onboard unique identifier or other personal data protection safeguard, linking the test to the user (e.g., QR codes, 2-D barcoding, etc.)	
<p>1 POCT refers to decentralized testing that is performed by a minimally trained healthcare professional near a patient and outside of central laboratory testing and test results are generally made available within a single clinical encounter.</p> <p>2 WHO Clinical management of COVID-19, Interim Guidance, May 2020; https://www.who.int/publications/i/item/clinical-management-of-covid-19 (accessed 29 July, 2020)</p>			

3 Lee S, Kim T, Lee E, Lee C, Kim H, Rhee H, et al. Clinical Course and Molecular Viral Shedding Among Asymptomatic and Symptomatic Patients With SARSCoV2 Infection in a Community Treatment Center in the Republic of Korea. *JAMA Intern Med.* doi:10.1001/jamainternmed.2020.3862, 2020.

4 Van Kampen J, van de Vijver D, Fraaij P et al. Shedding of infectious virus in hospitalized patients with coronavirus disease-2019 (COVID-19): duration and key determinants. doi: <https://doi.org/10.1101/2020.06.08.20125310> medRxiv preprint

5 Wölfel, R., Corman, V.M., Guggemos, W. et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* **581**, 465–469 (2020). <https://doi.org/10.1038/s41586-020-2196-x>

6 minimum 6 months shelf-life remaining when product arrives at point of use.

7 Instructions and requirements for Emergency Use Listing (EUL) submission: In vitro diagnostics detecting SARS-CoV-2 nucleic acid and rapid diagnostics tests detecting SARS-CoV-2 antigens. https://www.who.int/diagnostics_laboratory/200609_final_pqt_ivd_347_instruction_ncov_nat_and_ag_rdt_eul.pdf?ua=1

8 Global Harmonized System of Classification and Labelling of Chemicals: H350 may cause cancer, H340 may cause genetic defects; H360 may damage fertility of the unborn child

2. Diagnostic or confirmatory test for acute or subacute SARS-CoV-2 infection (e.g. during the first 2 weeks after symptom onset).

A highly accurate diagnostic test for SARS-CoV-2 infection is needed to guide rapid action for isolation and clinical care. Results of such a test would also guide decisions about the need for contact tracing, antiviral therapy for COVID-19 or alternative management for test negative patients; for monitoring the impact of public health interventions; and targeting and monitoring outcomes of experimental/research interventions, such as on drug efficacy. This test would need to be sensitive and specific enough to be used alone for diagnosis and may also serve as second-line test to confirm results of a point of care test (TPP # 1). Furthermore, tests could be used for repeat assessment throughout the period of viral shedding.

To achieve the performance requirements, it is expected that this assay will require instrumentation and may or may not be usable outside laboratory settings. High-throughput laboratory-based instruments and low-throughput near-patient solutions are both needed and are covered in this TPP.

Regarding programmatic suitability, the profile’s acceptable test kit stability and shelf-life characteristics will not meet the needs of many tropical countries where distribution is challenging and cool storage (< 30°C) is not consistently feasible. Test developers are strongly encouraged to achieve desirable characteristics to maximize access, particularly in remote settings with hot climates.

Capacity for multiplexing and sample pooling are important clinical and practical features to link with this test profile but are not included in this first version. Nonetheless, assays adapted and validated for these purposes would be attractive.

2. Test for diagnosis or confirmation of acute or subacute SARS-CoV-2 infection, suitable for low or high-volume needs		Notes
Intended Use	To detect the presence of virus component(s) to diagnose or confirm acute and subacute SARS-CoV-2 infection e.g. first two weeks since onset of symptoms in suspected cases or contacts (of probable or confirmed COVID-19 patients) with or without symptoms.	Terms acute and subacute to emphasize that this is a test that should work through the period of viral shedding but not to detect patients in the recovery phase of illness.

Target Population/patient	Patients with acute or subacute respiratory symptoms or fever or other suspicious symptoms (anosmia, diarrhoea) and either having had a known contact with a probable or confirmed COVID-19 patient or living in an area of a cluster or community transmission and symptomatic, pre-symptomatic or asymptomatic close contacts. Suspected COVID-19 cases requiring confirmation (positive triage test but low PPV) or exclusion (negative triage test but low NPV) of COVID-19 infection.		
Key Feature	Acceptable	Desirable	Notes
Target use setting - low -and high-volume settings	High-volume needs: reference laboratories or ideally in district hospitals or mobile laboratories by laboratory technicians with appropriate training in sample collection, biosafety and in the use of the test. These labs can of course also serve low-moderate needs.	Low-volume needs/non-laboratory settings: outpatient clinics, emergency units at the point of care or near patient by health care workers or laboratory technicians with appropriate training in sample collection, biosafety and in the use of the test.	Anticipate that laboratory capacity will be required for higher throughput diagnostic testing demands. For low throughput diagnostic test needs, the setting can be outside of laboratories and near patient or point of care.
Target molecule (analyte to be detected)	Must have at least one target specific for SAR-CoV-2 RNA or protein/antigen.	Not applicable.	
Analytical sensitivity/Limit of detection	Equivalent to 10 ³ genomic copies per mL in any respiratory tract specimen type.	Equivalent to 10 ² genomic copies/mL in upper and lower respiratory tract specimens and stool.	Test developers should use well characterized reference material and international standards, when available, to determine limits of detection
Analytical specificity	Assay detects only circulating SARS-CoV-2 viral strains; no interference due to interfering substances		Cross reactivity with SARS-CoV-1 could be acceptable as this virus not currently circulating
Sensitivity	≥95%	≥98%	To be determined on target population against reference standard. The targets are for the estimated true sensitivity and specificity; therefore, the lower bound of confidence
Specificity	≥99%	≥99%	

			intervals should ideally equal or exceed the target.
Type of analysis	Qualitative (info sufficient to inform clinical decision making)	Qualitative and quantitative based on analyte detected	
Interpretation	Qualitative (positive/negative) with patient identification capacity	Qualitative and quantitative, e.g. CT values and amplification curves, with patient identification capacity	
Sample type	Any of the following: swabs – nasopharyngeal, oropharyngeal nasal; washes - oropharyngeal, nasal, bronchoalveolar; sputum	Sample types amenable to self-collection and/or easy to collect: saliva/oral fluid, stool; inactivated samples	
Sample collection device	Compatible with an existing swab material e.g. flocked	Compatible with multiple swab materials including self-collection devices or no swab required e.g. saliva	
Test kit format	A kit that is compatible with range of standard extraction methods (if applicable), and includes all required reagents, controls and needed consumables to perform the assay (excluding sample collection and sample transport preservative)	<u>Low volume</u> (non-laboratory-based testing): closed system with all necessary materials for sample collection, reagents for RNA extraction (if applicable), sample preparation and detection on-board <u>High volume</u> (laboratory based): self-contained kit that includes sample collection, RNA extraction (if applicable), reagents, controls and needed consumables to perform the assay	
End user profile	Laboratory technician	Laboratory technician or trained health worker not requiring very specialized knowledge	
Training needs (includes test procedure,	3 days	1 day with online modules	

interpretation of results, quality control, troubleshooting)			
Test procedure			
Sample preparation steps (Need to process the sample prior to performing the test)	High volume (lab): Benchtop preparation & transfer of sample. Low volume (nonlab): inactivation step and transfer step	Automated on-board sample preparation within cartridge	
Reagents reconstitution Need to prepare the reagents prior utilization	High throughput (lab): Yes	No	
Need to transfer a precise volume of sample	Yes	No	
Specimen volume	The minimal sample volume required to reach clinically relevant sensitivities and ideally would allow for repeat testing	Not applicable	
Time to result	< 4 hours (half day)	< 45 mins	
Additional characteristics			
Operating conditions	Operation between 10 °C and 35 °C at an altitude up to 2500 meters; ability to tolerate extremely low relative humidity to condensing humidity. Able to function in direct sunlight and low light; able to withstand dusty conditions	Same, plus operation between 10 °C and 40 °C at an altitude up to 3000 meters preferred.	
Additional characteristics			
Sample transport	Compatible with one or more preservative/viral transport medium; stable for at least 12 days at 2-8°C in triple packaging; > 12 days at 70°C	Preparation that stabilizes specimens removing need for cold chain and triple packaging	
Test kit stability and storage conditions	12 months ¹ , stable between 4-10°C, 70% humidity; 3000 meters altitude; Indicator of instability or expiration	18-24 months, stable between 4-40°C (no cold chain), 90% humidity; 3000 meters altitude; Indicator of instability or expiration	Expect real time stability data to support shelf life requirements will not be available at the time of product release, but manufacturers

			should be challenged to meet targets that match what is realistic for supply chains in low- and middle-income countries.
Stability of the kit once opened	30 days	60 days	
Specimen capacity & throughput	High volume: can test between 50-150 patient samples in 4 hours. Low volume (non-lab): 1-4 patients per 45 mins	High volume - Can test between 200-500 patient samples in 4 hours; random access option. Low volume (non-lab): 6 patients in 45 mins	
Safety precautions (includes sample collection)	In all cases, PPE for sampler (gloves, gown, mask). High volume/lab-based test: laboratory BSL-2 or equivalent. Low volume/nonlab-based test: good ventilation; easy decontamination of instrument surfaces	Patient provides sample and includes inactivation step (not heat based) or sample enters closed system, which removes biosafety concerns and can follow universal precautions; easy decontamination of instrument surfaces	
Quality Control	Positive control and negative control provided in the kit or are sold separately. If applicable, RNA extraction control	Sample adequacy control and internal extraction control integrated into testing system	
Remote connectivity capacity	Export of data to USB possible with proprietary or 3rd party instrument	Yes, direct electronic data exportation via LAN or WiFi and Bluetooth, possible	
Need for additional equipment	Assay compatible with off-the shelf equipment only, e.g. PC and at least the two most commonly available thermocyclers with thermocycler-specific CT cut- off values for assay determined	Instrument that runs integrated self-contained assay; highly desirable is open diagnostic platform instrument that runs integrated assays from a range of developers worldwide.	
Need for maintenance	Daily preventive maintenance can be performed by laboratory staff in <30 minutes; self-check alerts operator to instrument errors or	Routine preventive maintenance no more than 30 minutes 1x per week; 2-year maintenance and replacement option or maintenance	

	warnings; annual maintenance conducted by industry professional under maintenance contract and replacement option	conducted by onsite trained personnel in less than 1 hour; or ability to calibrate remotely or no calibration needed.	
Waste/disposal requirements	Standard biohazardous waste disposal or incineration of consumables, no high temperature incineration required	Small environmental footprint; recyclable or compostable plastics for test cartridges and other materials after decontamination, no incineration required	
Manufacturing	ISO 13485: 2016 compliant	WHO or stringent regulatory authority (SRA) emergency use listing/authorization or WHO Prequalification or other stringent regulatory authority approval	
Accessibility	To maximize accessibility, all product manufacturers should have the capability to rapidly scale-up production and offer the product at a cost that allows broad use, including in low- and middle income countries		
1 - minimum 6 months shelf-life remaining when product arrives at point of use.			

Appendix C: Table of secondary evidence sources identified within this review as potential sources of information on diagnostic test accuracy

Author, DOI, site of publication	Title	Review type as reported	Date of last literature search update	Index test(s)
Cochrane reviews				
Dinnes et al. DOI: 10.1002/14651858.CD013705 Cochrane Library	Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection	'Systematic review'	25 May 2020	Any rapid antigen or molecular-based test for diagnosis of SARS-CoV-2 meeting the following criteria: <ul style="list-style-type: none"> - portable or mains-powered device - minimal sample preparation requirements - minimal biosafety requirements - no requirement for a temperature-controlled environment - test results available within 2 hours of sample collection
Reviews by national/international health technology assessment agencies				
(In progress, expected publication date 20 Oct 2020) EUnetHTA https://eunethta.eu/rcrot02/	What is the diagnostic accuracy of molecular methods that detect the presence of the SARS-CoV-2 virus in people with suspected COVID-19	'Rapid Collaborative Review'	14 August 2020	Any molecular assay based on nucleic acid amplification tests, such as RT-PCR or isothermal RNA amplification methods, that is designed to detect the presence of SARS-CoV-2 infection in people with suspected COVID-19.

Jarrom et al. DOI: 10.2139/ssrn.3631281 MedRxiv See also: Health Technology Wales https://www.healthtechnology.wales/wp-content/uploads/2020/05/EAR025-COVID19-diagnostics-report-v2.6.pdf	The Effectiveness of Tests to Detect the Presence of SARS-CoV-2 Virus, and Antibodies to SARS-CoV-2, to Inform COVID-19 Diagnosis: A Rapid Systematic Review	'Rapid systematic review'	4 May 2020	Any test to detect the presence of SARS-CoV-2, or antibodies to SARS-CoV-2, in people suspected of having recent or ongoing infection
Other systematic reviews				
Subsoontorn et al. DOI: 10.1101/2020.07.09.20150235 MedRxiv	The diagnostic accuracy of nucleic acid point-of-care tests for human coronavirus: A systematic review and meta-analysis	Systematic review with meta-analysis	16 June 2020	Index tests for inclusion in systematic review not pre-specified. Review aimed to identify nucleic acid point of care tests. Studies identified within the review considered the following technologies: RT-LAMP, RCA, RT-iiPCR, RT-RPA, RT-RAA, SAMBA, CRISPR diagnosis
Axell-House et al. DOI: 10.1016/j.jinf.2020.08.043 J of Infection	The Estimation of Diagnostic Accuracy of Tests for COVID-19: A Scoping Review	'Scoping review'	19 June 2020	All NAATs
Yang et al. DOI: 10.1101/2020.06.25.20139931 MedRxiv	Evaluation on the diagnostic efficiency of different methods in detecting COVID-19.	'Meta-analysis'	25 March 2020	Index tests for inclusion in systematic review not pre-specified. Studies identified within the review considered the following tests: ePlex Panther Fusion, Simplexa, Cobas, Xpert Xpress, RT-LAMP

Böger et al. DOI: 10.1016/j.ajic.2020.07.011 American Journal of Infection Control	Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19	'Systematic review with meta-analysis'	April 2020	Index tests for inclusion in systematic review not pre-specified. Various forms of PCR, different samples, different gene targets. (Also looked at antibodies and chest-CT)
Kim et al. DOI: 10.1148/radiol.2020201343 Radiology	Diagnostic Performance of CT and Reverse Transcriptase Polymerase Chain Reaction for Coronavirus Disease 2019: A Meta-Analysis	'Meta-analysis'	3 April 2020	Index tests for inclusion in systematic review not pre-specified. Studies identified within the review considered RT-PCR with respect to varied targeting and sample sites.
Pang et al. DOI: 10.3390/jcm9030623 Journal of Clinical Medicine	Potential Rapid Diagnostics, Vaccine and Therapeutics for 2019 Novel Coronavirus (2019-nCoV): A Systematic Review	'Systematic review'	6 February 2020	Index tests for inclusion in systematic review not pre-specified. Studies identified within the review considered RT-LAMP, RT-iiPCR and a one-step rRT-PCR assay
La Marca et al. Reprod Biomed Online DOI: 10.1016/j.rbmo.2020.06.001	Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays	'Systematic review'	15 May 2020	Review includes reference to molecular tests. However, 'For the main objective of this review, all original studies reporting on the sensitivity and/or specificity of antibodies against SARS-CoV-2 were included in the analysis.'
Systematic reviews examining sample sites				
Czumbel et al. DOI: 10.3389/fmed.2020.00465 Frontiers in Medicine	Saliva as a Candidate for COVID-19 Diagnostic Testing: A Meta-Analysis	'Meta-analysis'	25 April 2020	Saliva specimens with PCR diagnostics

Appendix D: Reproduction of FIND results from independent evaluation of 22 manual SARS-CoV-2 molecular tests⁽²⁰⁷⁾

	Company	Product name	Gene target	Verified LOD (copies / reaction)	Avg Ct (lowest dilution 10/10)	Clinical sensitivity (50 positives)	Clinical specificity* (100 negatives)	PCR platform**	Supplier recommended Ct cut-off
1.	altona Diagnostics	RealStar® SARS-CoV-2 RT-PCR Kit 1.0	E	1–10	35.45	92% (95%CI: 81, 97)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	None; any signal can be considered positive
			S	1–10	35.99	92% (95%CI: 81, 97)	100% (95%CI: 96, 100)		
2.	Atila BioSystems Inc.	Atila iAMP COVID-19 Detection (isothermal detection, 'OMEGA' amplification method)	ORF1ab	50–100	N/A	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	BioRad CFX96 deep well	Any signal is considered positive (isothermal)
			N	1–10	N/A	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
3.	Beijing Wantai Biological Pharmacy Enterprise Co. Ltd	Wantai SARS-CoV-2 RT-PCR Kit	ORF1ab	1–10	36.20	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	≤40

			N	1–10	37.12	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
4.	BGI Health (HK) Co. Ltd	Real-time Fluorescent RT-PCR kit for detection 2019-nCoV (CE-IVD)	ORF1	1–10	32.43	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	Roche LightCycler 480	≤38
5.	bioMérieux SA	ARGENE® SARS-COV-2 R-GENE®[b]	N	10–50	36.44	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	Any signal considered as positive
			RdRP	10–50	32.44	96%[a] (95%CI: 87, 99)	100% (95%CI: 96, 100)		
6.	Bioneer Corporation	AccuPower® SARS-CoV-2 Real-Time RT-PCR Kit	E	10–50	35.85	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	<38
			RdRP	10–50	36.18	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
7.	Boditech Med. Inc.	ExAmplar COVID-19 real-time PCR kit (L)	E	10–50	34.9	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	≤42

			RdRP	50–100	33.46	90% (95%CI: 79, 96)	100% (95%CI: 96, 100)		
8.	CerTest Biotec S.L.	VIASURE SARS-CoV-2 Real Time PCR Detection Kit	ORF1ab	10–50	35.16	98% (95%CI: 90, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	<40
			N	1–10	35.46	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
9.	DAAN Gene Co. Ltd of Sun Yat-Sen University	Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)	ORF1	1–10	38.76	100% (95%CI: 93, 100)	96%* (95%CI: 90, 98)	Roche LightCycler 480	≤40
			N	1–10	36.97	100% (95%CI: 93, 100)	98%* (95%CI: 93, 99)		
10.	EUROIMMUN AG	EURORealTime SARS-CoV-2[c]	ORF1ab/N	1–10	37.88	100% (95%CI: 93, 100)	98%* (95%CI: 93, 99)	Light Cyclor 480 II	Any signal considered positive

11.	GeneFirst Ltd	The Novel Coronavirus (2019-nCoV) Nucleic Acid Test Kit	ORF1	1-10	35.45	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	BioRad CFX96 deep well	≤37.0 positive; 37-40 indeterminate; >40 negative
			N	1-10	36.72	98% (95%CI: 90, 100)	100% (95%CI: 96, 100)		
12.	KH Medical Co. Ltd	RADI COVID-19 Detection Kit	S	1-10	37.94	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	≤40
			RdRP	10-50	36.74	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
13.	PerkinElmer Inc.	PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay[c,d]	N	1-10	39,43	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	BioRad CFX96 deep well	≤42
			ORF1	1-10	38,99	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
14.	Primerdesign Ltd	Coronavirus COVID-19 genesig® Real-Time PCR assay[c]	RdRP	1-10	36.7	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	LightCycler 480	Any signal regarded as positive

15.	QuantumDx	QuantuMDx SARS-CoV-2 RT-PCR Detection Assay	Orf1, N, S	1–10	36.8	100% (95% CI: 92, 100)	100% (95% CI: 96, 100)	BioRad CFX96 deep well	≤40
16.	R-Biopharm AG	RIDA®GENE SARS-CoV-2 RUO	E	1–10	37.99	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	None; any signal can be considered positive
17.	Sansure Biotech Inc.	Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing)[e]	ORF1	10–50	35.16	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	Thermofisher Quantstudio 5	≤40
			N	10–50	34.96	100% (95%CI: 93, 100)	95%* (95%CI: 89–98)		
18.	SD Biosensor Inc.	STANDARD M nCoV Real-Time Detection Kit	E	1–10	37.43	100% (95%CI: 93, 100)	97%* (95%CI: 92, 99)	Roche LightCycler 480	≤41
			ORF1	1–10	36.99	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)		
19.	Seegene Inc.	Allplex™ 2019-nCoV Assay	E	1–10	33.3	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96	≤40

			N	1–10	36.74	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
			RdRP	1–10	34.73	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
20.	Shanghai Kehua Bio-Engineering Co. Ltd	KHB Diagnostic kit for SARS-CoV-2 Nucleic Acid (Real-time PCR)	ORF1	1–10	30.39	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	More than two targets detected and curve is of S shape
			N	1–10	32.95	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
			E	1–10	31.72	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
21.	ThermoFisher Scientific	TaqPath™ COVID- 19 CE-IVD RT-PCR Kit[f]	ORF1ab; S protein; N protein	1–10	NA	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	Quantstudio 5	Not Applicable (Automated software interpretation)
22.	Vela Diagnostics	ViroKey™ SARS- CoV-2 RT-PCR Test[c]	RdRP	10–50	30.95	94% (95%CI: 84, 98)	100% (95%CI: 96, 100)	BioRad CFX96 deep well	≤40

			ORF1	1–10	35.57	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)		
	Tib Molbiol/Roche Diagnostics	ModularDx Kit SARS-CoV (COVID19) E-gene (Tib Molbiol) + LightCycler Multiplex RNA Virus Master (Roche)	E	1–10	33.34	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	Roche LightCycler 480	Define the cut-off 2–4 cycles higher than observed Cp value for 10 copies
<p>* Clinical specificity: Further investigation is needed to determine if apparent false positives are truly false positives or whether they are due to a false negative reference standard result</p> <p>** PCR platform: All products were evaluated on a PCR platform recommended by the supplier, listed in this table. Each test can be performed on other PCR systems, detailed in the product's instructions for use.</p> <p>[a] The two false negative samples tested positive with the second PCR (PCR 2) that targets E gene of SARS, SARS-COV-2 and/or SARS-like coronaviruses.</p> <p>[b] Samples for both analytical and clinical analyses were from already-extracted specimen, therefore the methods varied from those recommended by the supplier as the internal control was not included.</p> <p>[c] Samples for both analytical and clinical analyses were from already-extracted specimen, therefore the methods varied from those recommended by the supplier as the internal control was added to the master mix.</p> <p>[d] Evaluation procedure varied from recommended protocol. In order to achieve the recommended sample input volume, a 2.5 fold dilution of the samples was used.</p> <p>[e] Sansure claims a lower LOD of 6.4 cp/rxn, which has been independently verified.</p> <p>[f] Evaluation procedure varied from recommended protocol as source material was already-extracted RNA; extracted MS2 control was added directly to the master mix.</p>									

Appendix E: RADTs undergoing independent evaluation by FIND as of 19 Sept 2020⁽²⁰⁸⁾

Company	Assay	Country of manufacturer	Interpretation of test	Regulatory status	Evaluation status
Coris BioConcept	COVID-19 Ag Respi-Strip	Belgium	Visual	CE-IVD	Complete for 2 countries
SD BIOSENSOR, INC.	STANDARD Q COVID-19 Ag Test	Rep. of Korea	Visual	Brazil; CE-IVD	Complete for 2 countries
Shenzhen Bioeasy Biotechnology Co., Ltd	Bioeasy 2019-nCoV Ag Fluorescence Rapid Test Kit (Time-Resolved Fluorescence)*	China	Reader	CE-IVD	Complete in 1 country
RapiGEN, Inc.	BIOCREDIT COVID-19 Ag	Rep. of Korea	Visual	Brazil; Philippines; CE-IVD	Ongoing
SD BIOSENSOR, INC.	STANDARD F COVID-19 Ag FIA	Rep. of Korea	Reader	Brazil; CE-IVD	Ongoing
Abbott Rapid Diagnostics	Panbio COVID-19 Ag Test	Rep. of Korea	Visual	CE-IVD	To start
Bionote, Inc.	NowCheck COVID-19 Ag Test	Rep. of Korea	Visual	CE-IVD	To start
Boditech Medical, Inc.	iChroma COVID-19 Ag Test	Rep. of Korea	Reader	CE-IVD	To start
Edinburgh Genetics, Ltd	ActivXpress+ COVID-19 Antigen Complete Testing Kit	UK	Visual	CE-IVD	To start
Green Cross Medical Science Corp.	GENEDIA W COVID-19 Ag	Rep. of Korea	Visual	CE-IVD	To start
JOYSBIO (Tianjin) Biotechnology Co., Ltd	SARS CoV 2 Antigen Rapid Test Kit (Colloidal Gold)	China	Visual	CE-IVD	To start

*Note that this fluorescence-based test differs from a colloidal gold antigen test of the same name that was withdrawn by the company

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