



Public Health  
England

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# **Rapid assessment of selected commercial molecular diagnostic tests for the laboratory detection of COVID-19 infections**

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## Executive summary

Presently, cases of COVID-19 disease are identified by the detection of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) in clinical samples taken from infected patients. The predominant methodology is the detection of specific regions of the viral genome using molecular diagnostic techniques.

As part of PHE's response to this incident, Nucleic Acid Amplification Test (NAAT) assays<sup>1</sup> were developed to detect the newly emerged virus (February 2020) and an assay for the RdRp<sup>2</sup> gene was selected by PHE in early-February 2020 and deployed to UK laboratories, including PHE regional laboratories, PHE collaborating laboratories, Devolved Administrations and several NHS laboratories.

Subsequently, an increasing number of commercial assays have become available. In February 2020, PHE invited manufacturers with market-ready assay kits to supply details of their diagnostics for assessment. Pathology service providers to the NHS were surveyed. The information received was used to guide a desktop review by PHE to select several assays from amongst those first-to-market based upon their format, existing supplies to NHS diagnostic providers as well as interest from stakeholders, including end-users.

PHE has undertaken rapid assessments of 11 commercially provided diagnostic tests for SARS-CoV-2 nucleic acid detection with further evaluations ongoing.

This report summarizes laboratory assessments of COVID molecular diagnostic tests undertaken by PHE and completed in the period February and April 2020.

The 11 commercial molecular assays for the diagnosis of COVID-19 all showed good agreement with the RdRp assay developed by PHE.

Wider use of these commercial tests in diagnostic laboratories will generate data on larger numbers of samples, including positive clinical materials. Such use may highlight performance issues that were not apparent in PHE's initial rapid assessments.

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<sup>1</sup> NAAT - Nucleic acid amplification test – a type of method used widely in molecular biology to make millions to billions of copies of small pieces of genetic material (DNA or RNA) rapidly, allowing a very small amount of target in a sample to be amplified into an amount large enough to be studied. This is a very sensitive type of test

Assay - An investigative (analytic) procedure in laboratory medicine or pharmacology, for example. Commonly referred to as a 'test'

<sup>2</sup> RdRp - RNA dependent RNA polymerase

# Molecular diagnostic assessments

## Introduction

Molecular diagnostic tests are the gold standard methods for identifying individuals with active viral infection, such as SARS-CoV-2 (the cause of COVID-19 disease), in their respiratory tract.

These methods rely on testing a clinical sample from a patient (as per PHE guidance for COVID-19: laboratory investigations and sample requirements for diagnosis<sup>3</sup>) for evidence that viral particles are present. These tests detect the presence of the virus by amplifying specific regions of the viral genome to a point where a signal can be detected.

At the start of the COVID-19 incident no CE marked commercial kits were available for virus detection. The overall strategy for laboratory testing for COVID-19 was therefore to provide first-generation in-house assays for public health laboratories as an interim measure and for gradual migration to a commercial alternative, appropriate to local equipment and procurement.

As per regulatory requirements, PHE received a derogation authorisation from the Medicines and Healthcare Products Regulatory Agency (MHRA), allowing the continued use of its non-CE marked assay for the RdRp gene for a period of up to 6 months, or until a suitable device is CE marked or there is a sufficient capacity of a CE-marked equivalent product available in the UK.

In parallel with the application for derogation from CE marking, PHE proactively commenced a programme of commercial kit assessments on the 3<sup>rd</sup> February 2020 to rapidly assess the functionality of a selection of commercially-available assays that were entering the market at that time, as either CE marked or research use only (RUO) kits.

The principal goal of these assessments was to rapidly assess the utility of selected commercial diagnostic kits. It was not to undertake basic research, nor to aid the development of diagnostic assays nor to collaborate with SME's seeking to secure research or expansion funding.

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<sup>3</sup> <https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/laboratory-investigations-and-sample-requirements-for-diagnosing-and-monitoring-wn-cov-infection>

## Inclusion criteria

From January 2020 onwards, PHE was approached by diagnostic kit manufacturing companies (SME's to large multi-nationals) with products at differing points with regard to market entry. PHE invited manufacturers with market-ready assay kits to supply details of their diagnostics for assessment, along with a signed contract agreeing, if selected, to an assessment and to wide distribution of the results.

Pathology service providers to the NHS were surveyed via email to determine existing modalities for the detection of viral nucleic acids already available for use within the sector.

This information was used to guide the selection of commercial assay suppliers whose assays were both:

- i) compatible with the existing equipment of the NHS
- ii) were available to the market.

Desktop review selected several assays from amongst those first-to-market with testing timetabled for 3-months commencing 3 February 2020 (Table 1).

The selection of complete, market-available kits was based upon their format, existing supplies to NHS diagnostic providers as well as interest from stakeholders, including end-users.

Assessment of the manufacturers kits determined that these devices detected Orf1ab, E gene, N gene and the RdRp viral genomic targets. Some kits sought a single target whilst others sought multiple targets.

## Methodology

Methodology described below details the approach taken for those assays assessed at the PHE Colindale site. Assessments using similar methodology are also undertaken at other PHE sites. Specific deviations from the described methodology are detailed in Table 1.

The sample test panel included 195 respiratory clinical specimens found negative for SARS-CoV-2 by the in-house PHE NAAT assay (Table 1). Statistical assessment of panel sizes determined that the true specificity of the test under assessment would be at least 98.1% if the measured specificity is 100% for these 195 negative samples.

The complete negative sample panel was used for 10 of the 11 specificity assessments.

The low availability of positive clinical material at the start of the COVID-19 incident limited the composition of sample panels for these rapid assessments. The first live viral strain isolated in the United Kingdom was therefore cultured, titrated and used to generate a dilution series of positive material. This enabled assessment of assay performance to detect viral nucleic acids from an extant clinical isolate, in a series of dilutions. This material was titrated and was tested by the RdRp gene NAAT assay in use at the time at PHE Colindale. The intention was not to find the end-point of detection, but to ensure that reasonable copy numbers could be detected and to identify rapidly any misperformance of the products assessed.

For 10 of the 11 evaluated assays, a limited sensitivity assessment used 3 dilutions of titrated stock material ( $4 \times 10^8$  PFU/ml) positive for SARS-CoV-2 (Table 1).

One assessment (Roche/TiB molbiol assay) examined 150 respiratory specimens negative for SARS-CoV-2 measuring the true specificity of the assay to be at least 97.8% based on this number of samples. During the same assessment, 15 SARS-CoV-2 positive respiratory specimens were tested due to increased availability (Table 1).

In parallel to ascertaining performance, an ease-of-use assessment was undertaken for all assays in this report. This analysis sought to describe user-relevant characteristics of these assays pertinent to their ready deployment for use as part of COVID-19 testing services and workflows.

This ease-of-use analysis was performed on the assumption that these tests would be performed by competent, laboratory-trained staff, with the appropriate support being available from commercial suppliers where necessary. No assessment was made regards costing or throughput as this information is dependent upon factors relating to local capacity.

These rapid assessments were undertaken by PHE to address the need to generate data to determine if these methods were considered to be fit for purpose of SARS-CoV-2 virus detection and were not complete and thorough assessments either of specificity against a range of viruses or of assay sensitivity, which manufacturers should provide.

Readers should be aware that wider use of these commercial tests in diagnostic laboratories will generate data on larger numbers of samples, including more positive clinical samples. Such use may highlight performance issues that were not apparent in PHE's initial rapid assessments.

## References

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## Results

Table 1 Molecular diagnostic assessments<sup>4</sup>

NAAT assay name	Assay target loci	Assay type and format <sup>5</sup>	Sample size (n) <sup>6</sup>	Diagnostic performance metric reported	Challenge with dilutions of positive material <sup>7</sup>	Assay ease of use <sup>8</sup>
Altona. RealStar SARS-CoV-2 Real Time-PCR Kit 1.0.	E gene S gene	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes Manipulations: Medium Interpretation: Moderate
AusDiagnostics Coronavirus and SARS-CoV-2 Real Time PCR typing assay (20619)	orf1ab region	Requires specific robotics/reader 20-well RT-PCR, Manufacturers liquid handling platform	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes Manipulations: Low Interpretation: Moderate
GeneFirst. Novel Coronavirus (COVID-19) Real-Time PCR	N gene orf1ab region	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	Positive control dilutions representing TCID <sub>50</sub> of 10 <sup>-5</sup> , 10 <sup>-4</sup> and 10 <sup>-3</sup> successfully detected	Commonality: Yes Manipulations: Low Interpretation: Moderate
genetic PCR solutions. 2019-nCoV MONODOSE dtec-RT-qPCR Test	Not stated	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes Manipulations: Medium Interpretation: Moderate
Genetic Signatures. EasyScreen™ SARS-CoV-2 Real Time kit (RP010-HT)	E gene N gene	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes Manipulations: Low Interpretation: Easy

<sup>4</sup> Costs not assessed

<sup>5</sup> Some require additional items such as nucleic acid extraction/liquid handling robotics for assay set up and/or dedicated NAAT instruments (Real-time PCR instruments).

<sup>6</sup> Input volume not detailed

<sup>7</sup> A dilution series of the SARS CoV-2/England/02/2020 virus strain was used as positive material. A virus stock of 4 x 10<sup>8</sup> PFU / ml virus was used to create 3 positive control dilutions ('High', 'Medium' and 'Low'). Note input volume to each assay varies and detection analysis is not comparative.

<sup>8</sup>Ease of use. This was described as consensus after subjective assessment by several experienced laboratory scientists of the following areas: 1. Commonality with existing equipment for high volume NAAT processes YES / NO 2. Manipulations: Does this assay require a perceived LOW / MEDIUM / HIGH number of pipetting and manipulation steps3. interpretation: Whether interpretation of the outputs of the assay is this process EASY (clear instructions or automated result calling by proprietary software), MODERATE (Requires some user input and validation) or EXTENSIVE (Requires significant user interpretation to produce result output)

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Liferiver. Novel Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Detection for 3 Genes)	E gene N gene orf1ab region	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes  Manipulations: Low  Interpretation: Moderate
NovaCyt / PrimerDesign. COVID-19 genesig® Real-Time PCR (Z-Path-COVID-19-CE)	RdRp gene	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes  Manipulations: Medium  Interpretation: Moderate
Randox. Extended Coronavirus Multiplex Array assay (EV4418) <sup>9</sup>	Not stated	Requires specific robotics/reader Block based PCR followed by DNA-DNA hybridisation, followed by camera reading	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: No  Manipulations: High  Interpretation: Moderate
Roche/TiBMolBio. Coronavirus LightMix® Modular SARS and Wuhan CoV E-gene assay (09155368001)	E gene	Requires specific robotics/reader RT-PCR, manufacturers liquid handling and RT-PCR platform	165	Sensitivity 100% (95%CI 78.2-100%) Specificity 100% (95%CI 97.57-100%)	100% detected	Commonality: Yes  Manipulations: Medium  Interpretation: Easy
Seegene. Allplex 2019-nCoV	E gene N gene S gene	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes  Manipulations: Low  Interpretation: Easy
Viasure/CerTest. SARS-CoV-2 Real Time PCR Kit (VS-NCO296T)	N gene orf1ab region	Reagent kit 96 well	195	Negative Percentage agreement 100% (195/195)	The 'High', 'Medium', and 'Low' dilutions of positive control material (derived from a 4 x 10 <sup>8</sup> PFU/ml virus stock) were successfully detected	Commonality: Yes  Manipulations: Low  Interpretation: Easy

<sup>9</sup> NAAT assay, not a real-time PCR assay, involved distinct and more numerous laboratory processes to other assays, and did not obviously fit existing diagnostic laboratory workflows.

Four assays required companion equipment for deployment. Three of these (from Roche, AusDiagnostics and Genetic Signatures) were based on real-time PCR and the required instrumentation could be integrated well into existing work flows.

The fourth assay that required companion equipment (Randox Extended Coronavirus Multiplex Array NAAT assay) was not based on real-time PCR. This assay required multiple additional manipulations and interventions and did not fit well with existing laboratory workflows.

## Conclusions

Within the context of these limited, rapid assessments, the commercial assays were each found equivalent to the in-house comparator method. Commercial assays targeting one or more of the conserved regions of the Orf1ab locus, or the E, N and S genes were specific against the assessment panel of known SARS-CoV-2 negative clinical specimens, as determined previously by the routinely used in-house SARS-CoV-2 assay, which targets the RdRp gene.

The workflows of most of the assessed NAAT assays were broadly comparable to assays routinely performed in diagnostic laboratories. Most could be operated on existing laboratory real-time PCR equipment.

However, several assays required companion equipment, some of which did not fit easily into workflows, and that ease of use is an important component of consideration for implementation.

Wider use of these commercial tests in diagnostic laboratories will generate data on larger numbers of samples, including positive clinical materials. Such use may highlight performance issues that were not apparent in PHE's initial rapid assessments.

Individual reports to summarize each completed assessment were issued as part of PHE's COVID-19 incident response through the PHE Incident Virology Cell for onward cascade. These reports have also been shared with the World Health Organization and are available at:

<https://www.gov.uk/government/publications/covid-19-phe-laboratory-assessments-of-molecular-tests>

## List of organisations participating in the Incident Virology Cell diagnostics group

Advisory Committee on Dangerous Pathogens
COVID-19 Genomics UK Consortium
Health and Social Care, Northern Ireland
Health Services Laboratories
NHS Blood and Transplant
NHS England and NHS Improvement
NHS Scotland
NHS Wales
National Institute for Biological Standards and Control
Public Health England
Public Health Agency, Northern Ireland
Public Health Scotland
Public Health Wales
The Doctors Laboratory
University of Glasgow
Viapath
University College Dublin, Ireland

## Disclaimer

PHE's assessments of commercial products for diagnosing COVID-19 infection have been carried out primarily for PHE's own use and under agreement; the reports of such assessments are shared solely for the readers' information; PHE does not in any way recommend any particular COVID-19 diagnostic assay or extraction platform; PHE shall not be responsible for any choice of COVID-19 diagnostic assay or extraction platform, and it is the testing laboratory's responsibility to ensure that any such assay or platform implemented has undergone the necessary verification and validation; and PHE shall not be liable, to the greatest extent possible under any applicable law, for any claim, loss or damage arising out of or connected with the use of this and related reports and any choice of COVID-19 diagnostic assay products or extraction platforms.

A position statement regarding COVID-19 tests evaluated by PHE is available at:

<https://www.gov.uk/government/publications/position-statement-regarding-covid-19-tests-evaluated-by-phe>