Public Health England

Protecting and improving the nation's health

Rapid assessment of Biomerieux ARGENE® SARS-COV-2 R-GENE® real-time detection kit

V01.00. Uncontrolled when printed or downloaded

About Public Health England

Public Health England exists to protect and improve the nation's health and wellbeing and reduce health inequalities. We do this through world-leading science, research, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. We are an executive agency of the Department of Health and Social Care, and a distinct delivery organisation with operational autonomy. We provide government, local government, the NHS, Parliament, industry and the public with evidence-based professional, scientific and delivery expertise and support.

Public Health England
Wellington House
133-155 Waterloo Road
London SE1 8UG
Tel: 020 7654 8000
www.gov.uk/phe
Twitter: @PHE_uk
Facebook: www.facebook.com/PublicHealthEngland

Prepared by NIS Laboratories, National Infection Service, Public Health England

Published September 2020
PHE publications
gateway number: GW-1596

PHE supports the UN
Sustainable Development Goals

Rapid assessment of Biomerieux ARGENE® SARS-COV-2 R-GENE® real-time detection kit

Introduction

The emergence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in humans and spread of the associated disease, COVID-19, has been declared a Public Health Emergency of International Concern by the WHO. In the UK, the deployment of a PHE in-house real-time PCR assay in PHE, PHE collaborating laboratories as well as in Devolved Administrations is being followed up with assessments of commercially developed and provided diagnostic tests for SARS-CoV-2 detection.

The assay and the assessment panel

This assessment examined the Biomerieux ARGENE® SARS-COV-2 R-GENE® Real-time detection kit (Product REF: 423720). The assessment was carried out following the instructions for use (IFU; Revision No. 055836 - 02 - 2020-06), with one exception; the IFU for 423720 requires the internal control (IC1) to be added to the supplied molecular grade water (W0) to create the reference Extraction Inhibition Control (IC1W0). In this assessment, no IC1 W0 was present on each extraction run, instead multiple viral transport media (VTM) aliquots containing IC1 were extracted and tested to determine IC1 values in replicate extractions.

The assay is intended for use for the *in vitro* qualitative detection of 2019 novel coronavirus (SARS-CoV-2) RNA by real-time PCR systems and is validated for use with nasopharyngeal swabs from upper respiratory tract specimens. The kit comprises 2 separate multiplex RT- qPCR assays, the second of which is designed to be used on a subset of samples that may require investigation subsequent to PCR1:

Multiplex PCR1: SARS-CoV-2 specific detects 3 targets simultaneously which are:

- N gene of SARS-CoV-2 at 530 nm (FAM)
- RdRp gene of SARS-CoV-2 at 670 nm (Cy5)
- internal control at 560 nm (VIC)

Multiplex PCR2: also detects 3 targets simultaneously which are:

- E gene of Sarbecovirus at 530 nm (FAM)
- human cellular control at 670 nm (Cy5)
- internal control at 560 nm (VIC)

The human cellular control (Cc) in Multiplex PCR2 detects a target for the hypoxanthine phosphoribosyltransferase 1 (HPRT1) gene and is used to validate the sample quality. The claimed limit of detection for SARS-CoV-2 using the assay with PCR1 and PCR2 is 0.43 TCID 50/mL, equivalent to 380 copies/mL.

The assessment sample-panel totalled 241 specimens. Nucleic acids were extracted using a modified extraction protocol (200 µL sample + 10 µL IC1 +790 µL of NucliSENS Lysis buffer) which, requires a greater volume of sample than the 150 µL used in the PHE extraction protocol.

The samples included 196 respiratory clinical specimens negative for SARS-CoV-2 and 45 respiratory clinical specimens positive for SARS-CoV-2, as determined by a validated in-house PHE PCR assay targeting the ORF-1ab of SARS-CoV-2. It also included 3 dilutions of cell-cultured material that was positive for SARS-CoV-2.

Performing and analysing the assay

Real-time PCR was performed on an Applied Biosystems™ 7500 Fast Real-Time PCR System and Applied Biosystems™ QuantStudio 5 Real-Time PCR System, following the cycling and fluorescence acquisition parameters detailed in the ARGENE® SARS-COV-2 R-GENE® Real-time detection kit (REF:423720). Ten microliters of nucleic acid extract were tested in each real-time PCR reaction, with a final volume of 25 µL. Samples were processed in batches of 24, 72 or 88 with appropriate; negative, internal and positive controls.

Results of real-time PCR testing were deemed valid if the designated control wells achieved the defined criteria in the ARGENE® SARS-COV-2 R-GENE® IFU. As per the IFU, thresholds were set on a per-reaction basis, placing thresholds above background noise and in the exponential phase of each amplification curve, generally corresponding to 5–10% of the sample's final fluorescence. In this assessment, samples and controls were assigned a cycle threshold value at which signal was detected above the background fluorescence, generally corresponding to >5% of the maximum fluorescence detected in any of the FAM, Cy5, or VIC channels.

For PCR1, the samples were then interpreted as either “SARS-CoV-2 detected”, “perform PCR2”, “SARS-CoV-2 NOT detected or below Limit of Detection” or “Invalid result or inhibition/poor extraction”, dependent upon the presence and value of a Ct in either and or the FAM, Cy5 or VIC channels.

For PCR2 the samples were interpreted as either “Sarbecovirus detected”, “Sarbecovirus NOT detected or below Limit of Detection”, “Cells NOT detected (test on new sample)” or “Invalid result (inhibition/poor extraction)”, dependent upon the presence and value of a Ct in either and or the FAM, Cy5 or VIC channels.

Results

During a challenge with cell-cultured positive material, all samples in a 3-step dilution series were found positive for SARS-CoV-2, in both PCR1 and PCR2.

Compared with results from the PHE COVID-19 in-house real-time PCR assay targeting ORF-1ab, the following was found during the testing of clinical samples:

PCR1	**Samples (n)**	**True positive**	**False Positive**	**True negative**	**False negative**	**Negative percentage agreement**
Biomerieux ARGENE® SARS-COV-2 R-GENE® Real-time detection kit (REF:423720)	241	43[#]	0	196*	2[Ω]	100% (196/196)

* Two samples were classed as invalid due to out of range Ct of the IC.
[Ω] Two nucleic acid extracts from SARS-CoV-2 positive patients were not initially detected using multiplex PCR1 but were subsequently detected upon re-testing. The sensitivity calculation given is as would occur in diagnostic setting.
[#] Two positive samples were positive for only a single target within multiplex PCR1; one in only the N-gene and the other was detected only in the RdRp gene.

The ARGENE SARS-CoV-2 PCR1 test demonstrated an assay performance of:

- sensitivity 95.6% (84.9 – 99.5%; 95% CI)
- specificity 100% (98.1 – 100%; 95% CI)

PCR2 (optional PCR)	**Samples (n)**	**True positive**	**False Positive**	**True negative**	**False negative**	**Negative percentage agreement**
Biomerieux ARGENE® SARS-COV-2 R-GENE® Real-time detection kit (REF:423720)	221	28	0	193*	0	100% (193/193)

*Eight samples were classed as invalid due to out of range Ct of the IC.

The ARGENE SARS-CoV-2 PCR2 (optional PCR) test demonstrated an assay performance of:

- sensitivity 100% (87.7 – 100%; 95% CI)
- specificity 100% (98.1 – 100%; 95% CI)

In this evaluation using the SARS-CoV-2 specific test (PCR1), 2 samples were classed as invalid due to the Ct of the IC >+/- 3 Cts of the extraction control.
In the generic test (PCR2), 8 samples were classed as invalid due to the Ct of the IC >+/- 3 Cts of the extraction control.

Report date

A version of the report was distributed by PHE's COVID-19 Incident Virology Cell on 25 September 2020.

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

Further information

Queries about our assessments of SARS-CoV-2 (COVID-19) diagnostics should be sent to labvalidation.cov@phe.gov.uk

Table of changes

Date	New version no.	Details of changes
	01.00	None – new document