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Rapid assessment of the careGENE N-CoV RT-PCR Kit

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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.

This assessment examined the careGENE N-CoV RT-PCR Kit REF: MNC-N10082 IFU-MNC-EN/ Rev D (Effective Date 2020.04.14). The assay is used for the *in vitro* qualitative detection of SARS-COV-2 in viral RNA samples extracted from human respiratory tract specimens by real-time PCR systems. The first (screening) test is termed Pan-CoV and detects a region of the E gene common to members of subgenus Sarbecoviruses, including SARS-1, MERS and SARS-CoV-2. A second (confirmatory) test, termed N-CoV, is specific for SARS-CoV-2 and targets the RdRPP2 gene.

The Pan-CoV (E gene) / N-CoV (RdRPP2) coronavirus targets are detected in the FAM channel, the sample extraction and amplification efficiency internal control (IC) is detected in the ROX channel. The limit of detection of the assay is quoted as 5 copies / μL in IFU-MNC-EN/ Rev.D.

The assessment panel

The assessment sample-panel comprised 194 negative and 32 SARS-CoV-2-positive respiratory clinical specimens. The specimens had been tested using the validated in-house PHE PCR assay targeting orf1ab and confirmed using a different commercial kit that targeted orf1ab as well as the E and N genes of SARS-CoV-2.

A serial dilution of a single extraction of titrated SARS-CoV-2 virus was performed and additional positive control material representing high, medium and low titres of SARS-CoV-2 was tested with the screening and confirmatory assays.

Performing and analysing the assay

Real-time PCR was performed upon an Applied Biosystems™ 7500 Fast Real-Time PCR System following the cycling and fluorescence acquisition parameters detailed in the careGENE N-CoV RT-PCR Kit REF: MNC-N10082 IFU-MNC-EN/ Rev D. Ten microliters of nucleic extracts from clinical samples were used in each real-time PCR reaction, with a final volume for 20 µL. Samples were processed in batches of 88 with appropriate negative, internal and positive controls. Results of real-time PCR testing were verified as valid if the designated control wells achieved the defined criteria in the careGENE N-CoV RT-PCR Kit REF: MNC-N10082 IFU-MNC-EN/ Rev.D. The samples were interpreted as ‘Positive’, ‘Negative’, ‘Potential positive’ (to retest), or ‘result invalid’ (to retest) dependent upon the presence and value of a Ct in either/or the FAM or ROX channels.

Samples that gave false-negative results were re-tested using the careGENE assay. The sample status was defined according to the PHE COVID-19 assay. A further check, post-assessment, was performed using a different commercial assay.

Results

Compared with results from the PHE COVID-19 in-house real-time PCR assay the following was found:

Screening of 194 known negative samples with the Pan-CoV E-gene assay returned negative results for all. However, for 35 samples the IC (internal control) failed. Of these, 29 were resolved on repeat testing with a careGENE kit (from a different lot number), and one sample was not resolved as it contained insufficient human material in the sample for this test. Five samples did not have sufficient extracted material remaining for the additional testing to resolve the IC status.

Of 32 known positive samples, 24 were detected with the careGENE Pan-CoV E-gene assay as true-positives and 8 gave ‘false-negative’ results. For the same 32 SARS-CoV-2 positive samples, the N-CoV assay, which is specific for SARS-CoV-2 (RdRPP2), showed 21 true-positive results and 11 false-negative results.

Six known positive samples gave false-negative results in both the screening (Pan-CoV) and confirmatory (N-CoV) assays.

Additionally, to explore the specificity of the N-CoV assay, we tested a subset of 136 negatives (chosen from the larger set of negative samples tested using the Pan-CoV

assay) for which residual sample remained available. All 136 negative samples tested as valid and negative in the N-CoV SARS-CoV-2 specific assay (RdRPP2).

Pan-CoV screening assay (E-gene):

	Samples (n=)	True positive	False positive	True negative	False negative	Invalid test
careGENE N-CoV RT-PCR Kit REF: MNC-N10082	226	24	0	188	8	6

The Pan-CoV screening E-gene assay demonstrated the following assay performance:

Sensitivity: 75% (56.6 to 88.54%; 95% CI)
Specificity: 100% (98.1 to 100%; 95% CI)

N-CoV SARS-CoV-2 specific assay (RdRPP2):

	Samples (n=)	True positive	False positive	True negative	False negative
careGENE N-CoV RT-PCR Kit REF: MNC-N10082	168	21	0	136	11

The N-CoV SARS-CoV-2 specific assay demonstrated the following assay performance:

Sensitivity: 65.6% (46.8 to 81.4%; 95% CI)
Specificity: 100% (97.3 to 100%; 95% CI)

Three samples for a 3-step dilution series positive for SARS-CoV-2, were found positive using the careGENE N-CoV RT-PCR Kit in both the screening and confirmatory assays.

Report date

A version of the report was distributed by PHE's COVID-19 Incident Virology Cell on 15 July 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: https://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
3 July 2020	01.00	None – new document