Current Trends in Medical Diagnostic Methods

GAVIN PUBLISHERS

Research Article

Novel CE-Marked SARS-CoV-2 IVD Assay Solves the Problem for Genomic DNA Contamination

Velislava I. Ilieva[1]*, Edward M. Flynn[1], Jessica M. C. Marvin[2], Marcin Szynkiewicz[1], Catherine Morrissey[1], Rachel S. Hulme[1], Michael D. Risley[1], Joanne Mason[1]

[1]Yourgene Health, Citylabs 1.0, Nelson Street, Manchester, M13 9NQ, United Kingdom

[2]Qiagen, Skelton House, Lloyd St N, Manchester M15 6SH, United Kingdom

*Corresponding author: Velislava I. Ilieva, Yourgene Health, Citylabs 1.0, Nelson Street, Manchester, M13 9NQ, United Kingdom

Abstract

The COVID-19 pandemic created a worldwide need for rapid and accurate testing of individuals for the SARS-CoV-2 virus. One of the earliest Reverse Transcription Polymerase Chain Reaction (RT-PCR) test designs for detection of SARS-CoV-2 in clinical Nasopharyngeal/Oropharyngeal (NP/OP) samples was published by the United States Centre for Disease Control (US-CDC). The internal control RPP30 primers and probe target exon 1 of the human RPP30 gene and can therefore result in amplification of both complementary DNA (cDNA) and genomic DNA (gDNA). In the event of lack of RNA in the clinical samples, the risk of false negative results is increased. In order to support the global efforts against COVID-19, Yourgene Health developed the Clarigene™ SARS-CoV-2 test, a CE-marked real-time RT-PCR *in vitro* Diagnostic (IVD) assay for detection of SARS-CoV-2 RNA sequences in NP/OP samples. The Clarigene™ SARS-CoV-2 test incorporates re-designed RPP30 primer sequences which only detect human RPP30 RNA sequences, as opposed to RNA and gDNA, thereby serving as a reliable internal control for sample collection and RNA extraction, as well as verifying the performance of the RT-PCR. Here, we demonstrate the high accuracy (100% sensitivity, 100% specificity) and limit of detection (5 copies/reaction) of the Clarigene™ SARS-CoV-2 test. We also provide evidence for the optimised design of the RPP30 primers and show that the Clarigene™ SARS-CoV-2 test can identify individuals infected with the novel UK SARS-CoV-2 variant B.1.1.7. Since the emergence of the SARS-CoV-2 B.1.1.7 variant, associated with increased infectivity, we have extended our testing regime to include *in silico* and *in vitro* analysis of SARS-CoV-2 strains circulating in the current population to ensure the ability of Clarigene™ SARS-CoV-2 test to detect them.

Introduction

At the end of 2019 a novel zoonotic human virus emerged in the Chinese city of Wuhan, Hubei province [1]. The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) strain, causing Coronavirus disease 2019 (COVID-19), rapidly spread across China and the rest of the world, leading to declaration of worldwide pandemic by the World Health Organisation (WHO) on March 11, 2020. Since then, the SARS-CoV-2 strain has been heavily studied and great insights have been gained into its genetics, phylogeny and epidemiology [2-5]. A critical need for rapid and accurate testing emerged to support the public healthcare efforts to identify symptomatic and asymptomatic individuals, enable them to isolate and thus, reduce the spread of SARS-CoV-2.

Yourgene Health, an international molecular diagnostics company based in Manchester, UK, mobilised its expertise in early March 2020, in order to support the global efforts against COVID-19. We present here a novel high-throughput diagnostic test, Clarigene™ SARS-CoV-2 test, able to detect the presence of SARS-CoV-2 in NP/OP patient samples.

The US-CDC designed and made available a SARS-CoV-2 nucleic amplification assay early in the COVID-19 pandemic (https://www.fda.gov/media/134922/download). The assay incorporates RT-PCR internal control primers and probe targeting a region of exon 1 of the human RPP30 gene. However, the primers cannot distinguish between gDNA and reverse transcribed RNA, as they do not span the exon boundary [6]. In the event that gDNA is amplified in the absence of RNA (caused for example by a poor swabbing technique, failed RNA extraction, failed reverse

transcription step or a combination of all three) the conclusion could be drawn that a reaction has worked as intended and therefore would have detected the virus, if present. This has the potential to lead to false negative calls and in turn infected individuals would not be advised to self-isolate resulting in an increased transmission rate.

Clarigene™ SARS-CoV-2 test is a real-time RT-PCR assay that amplifies Nucleocapsid (N) gene and Envelope (E) gene RNA sequences from the SARS-CoV-2 genome. The Clarigene™ SARS-CoV-2 internal RPP30 control primers are re-designed to span the exon 1-exon 2 boundary, thus ensuring only RPP30 RNA is amplified.

Additionally, a novel variant of SARS-COV-2 was identified in the UK towards the end of 2020. Studies by Public Health England (PHE) revealed that the B.1.1.7 lineage is associated with higher transmission rate and is present in regions with higher incidence of COVID-19 in Southern England, but there is no evidence for increased fatality of patients [7]. The B.1.1.7 lineage contains 23 mutations (13 non-synonymous, 4 deletions and 6 synonymous) and was deemed a variant of concern by PHE on December 21. A significant impact on diagnostic tests targeting the Spike gene (S gene) of SARS-COV-2 was observed, because the accumulation of seven mutations in the region prevented detection of the S gene target in individuals positive for SARS-COV-2 infection [8]. Although, the Clarigene™ SARS-CoV-2 test does not target the S gene and therefore is not affected by these mutations, one of the mutations in B.1.1.7 is located in the N gene, which is a target in the Clarigene™ SARS-CoV-2 test. The impact on this variant was assessed and it was found that the Clarigene™ SARS-CoV-2 test successfully detects COVID-19 in the presence or absence of the C28977T (p.S235F) mutation in the N gene ensuring maintained detection levels with the emergent strain.

Clarigene™ SARS-CoV-2 test offers a CE-marked solution for SARS-CoV-2 detection with high specificity, sensitivity and fast turnaround time compatible with automation for high-throughput laboratory testing.

Materials and Methods

Clinical samples

Extracted NP/OP clinical samples were purchased from Tissue solutions (Glasgow, UK) and used to demonstrate accuracy of the Clarigene™ SARS-CoV-2 test as part of clinical validation for CE marked IVD test. The samples have been previously tested with 2019-nCoV real-time RT-PCR RdRp gene assay [9]. All samples were supplied with evidence for patient consent.

Re-design of RPP30 Reverse Primer

Primers and probes targeting N gene and E gene from the SARS-CoV-2 genome were adapted from Vogels, et al. [10], who investigated the sensitivity and specificity of publicly available SARS-CoV-2 primers and probe sets. The forward primer and probe sequences for the RPP30 internal control are the same as the US-CDC published sequences (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html). The reverse primer was re-designed to span the exon 1-exon 2 junction of the human RPP30 gene in order to distinguish between cDNA and gDNA sequences (Figure 1A). The Clarigene™ SARS-CoV-2 test is a multiplex real-time RT-PCR assay detecting the three targets in a single reaction well with each probe conjugated to a different reporter dye: FAM (N gene probe), SUN (E gene probe) and Cy5 (RPP30 probe) (Figure 1B).
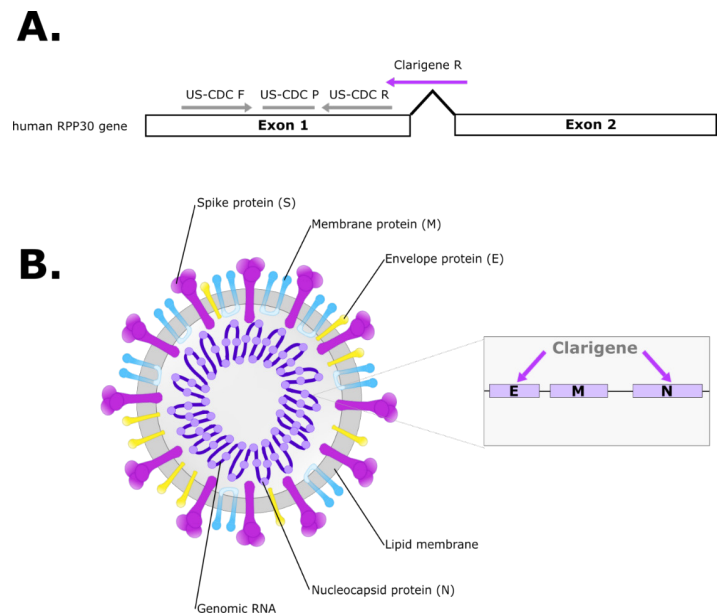

Figure 1: Clarigene™ SARS-CoV-2 test targets. A. Schematic representation of the human RPP30 gene and the location of the primer/probe sets. B. Schematic representation of the SARS-CoV-2 virus particle and Clarigene™ SARS-CoV-2 test targets.

To confirm the selectivity of the re-designed RPP30 primers and probe for cDNA, and to provide evidence that the US-CDC RPP30 primers and probe set cannot distinguish between gDNA and cDNA, human RPP30 gDNA (72.5 copies/µL) and cDNA (72.5 copies/µL) sample material was tested with both the re-designed and the US-CDC RPP30 primer and probe sets. The reaction setup followed the Clarigene™ SARS-CoV-2 test instructions for use (see 'Real-Time RT-PCR reaction setup' subsection for an overview). The experiment was carried out on a LightCycler 480 II (Roche Life Science, Penzberg, Germany) in a 96-well format (LightCycler® 480 Multiwell Plate 96, Roche Life Science, Penzberg, Germany).

Positive control

The Clarigene™ SARS-CoV-2 test is supplied with an exogenous positive control manufactured by Yourgene Health, containing equimolar concentration of N gene and E gene plasmids, diluted to 200 copies/µL (1000 copies/reaction).

Real-Time RT-PCR Reaction Setup

Reaction set-up followed the Clarigene™ SASR-CoV-2 instructions for use. Briefly, each reaction contained 11 µL RT-PCR master mix, 5.06 µL Primers and Probes mix, 0.44 µL of ROX reference dye and 5 µL of sample material. Samples were run in a 96-well format (MicroAmp™ Optical 96-Well Reaction Plate, Thermo Fisher Scientific, Waltham, USA) on QuantStudio 7 Flex (Thermo Fisher Scientific, Waltham, USA) with the programme settings displayed in Table 1. Signal acquisition is carried out by selecting the appropriate emission wavelength for the reporter dyes: FAM, VIC and Cy5.

Steps	Time	Temperature	Cycles
Reverse Transcription	5 min	52°C	1
Reverse Transcription Inactivation Stage	10 sec	95°C	1
Denaturation	5 sec	95°C	40
Annealing and Extension	30 sec	56°C	

Table 1: Clarigene™ SARS-CoV-2 test Real-Time RT-PCR reaction conditions.

SARS-CoV-2 lineage B.1.1.7

The ability of the Clarigene™ SASR-CoV-2 test to detect the mutant N gene (N^{mut}) in the recently-characterised B.1.1.7 lineage of SARS-CoV-2 [8] was assessed both *in silico* and *in vitro*. The Clarigene™ SASR-CoV-2 N gene primers and probe were mapped to the N^{mut} gene sequence using the Geneious software (8.1.9). *In vitro* analysis involved obtaining two gBlock Gene Fragments (Integrated DNA Technologies, Coralville, USA), one with the C28977T (p.S235F) variant and a wild-type N gene (N^{wt}). Both were tested in parallel at 5 copies/reaction, 50 copies/reaction and 500 copies/reaction. All samples were run in 4 replicates.

Performance Characteristics

Limit of Detection

The lowest concentration of SARS-CoV-2 viral targets at which the Positive Percent Agreement (PPA) is ≥95% was determined by serial dilution of synthetic RNA from SARS-related Coronavirus 2 (BEI, Manassas, USA). The tested target concentrations include 100 copies/reaction, 50 copies/reaction, 25 copies/reaction, 10 copies/reaction, 5 copies/reaction and 2.5 copies/reaction. Preliminary limit-of-detection experiment was also carried out by serial dilution of BEI synthetic RNA to target concentrations of 5 copies/reaction, 25 copies/reaction, 50 copies/reaction, 100 copies/reaction and 2000 copies/reaction.

Accuracy (Sensitivity and Specificity)

A cohort of 92 clinical samples, 46 positives and 46 negatives, was tested in a blinded study to determine the ability of the Clarigene™ SARS-CoV-2 test to accurately detect the presence of SARS-CoV-2 viral sequences in clinically derived sample material.

Repeatability

The robustness and repeatability of the Clarigene™ SARS-CoV-2 test was assessed by testing synthetic sample material at 3 copies/µL and human RNA, negative for SARS-CoV-2, at 0.25 ng/µL in thirty replicates per sample. The experiment was repeated three times in total over a 24-hour period.

Reproducibility

In total, 432 positive contrived samples (216 at 1 copies/µL and 216 at 3 copies/µL) and 216 negative synthetic samples were tested in order to demonstrate the inter- and intra-assay reproducibility of Clarigene™ SARS-CoV-2 test. Three operators used three reagents lots over a three day-period testing synthetic sample material at 1 copies/µL and 3 copies/µL, and human RNA, negative for SARS-CoV-2, at 0.25 ng/µL in eight replicates per sample.

Cross Reactivity

In silico analysis of the primer sequences contained within the Clarigene™ SARS-CoV-2 test was carried out using the Basic Local Alignment Search Tool (BLAST). Assay primers were tested for sequence similarity to a range of common respiratory pathogens (Table 2).

Respiratory Pathogen	*In silico* Analysis Result	*In vitro* Analysis Result
Human Coronavirus 229E	Negative	Negative
Human Coronavirus OC43	Negative	Negative
Human Coronavirus HKU	Negative	Negative
Human Coronavirus NL63	Negative	Negative
SARS Coronavirus	>80% homology (E gene target)	Inconclusive
MERS Coronavirus	Negative	Negative

Human Metapneumovirus	Negative	Negative
Parainfluenza Virus Type 1	Negative	Negative
Parainfluenza Virus Type 2	Negative	Negative
Parainfluenza Virus Type 3	Negative	Negative
Parainfluenza Virus Type 4	Negative	Negative
Respiratory Syncytial Virus A	Negative	Negative
Bordetella pertussis	Negative	Negative
Influenza A H3	Negative	Negative
Influenza A H1N1	Negative	Negative
Influenza A 2009	Negative	Negative
Influenza B	Negative	Negative
Enterovirus	Negative	Not tested
Rhinovirus 1A	Negative	Negative
Chlamydia pneumophila	>80% homology (N gene forward)	Negative
Haemophilus influenzae	>80% homology (N gene reverse)	Not tested
Legionella pneumophila	Negative	Negative
Mycobacterium tuberculosis	Negative	Not tested
Streptococcus pneumoniae	Negative	Negative
Streptococcus pyogenes	>80% homology (N gene probe)	Negative
Mycoplasma pneumoniae	Negative	Negative
Candida albicans	>80% homology (N gene probe)	Negative
Pseudomonas aeruginosa	Negative	Negative
Adenovirus Type 1	Not tested	Negative
Adenovirus Type 3	Not tested	Negative
Adenovirus Type 31	Not tested	Negative
Staphylococcus epidermis	Not tested	Negative
Pooled human nasal wash	Not tested	Negative

Table 2: List of common respiratory pathogens used for *In silico* and *In vitro* analysis of Clarigene™ SARS-CoV-2 test primers specificity.

In order to determine if the primers can specifically amplify only SARS-CoV-2 viral sequences *in vitro*, three respiratory specimen panels were purchased from ZeptoMetrix (Buffalo, USA), NATtrol Respiratory Verification Panel 2, NATtrol Blood Culture Identification 2 Panel and NATtrol Respiratory Pathogen Panel 1 and tested with Clarigene™ SARS-CoV-2 test.

Results

Re-design of RPP30 Reverse Primer

The Clarigene™ SARS-CoV-2 test RPP30 primers and probe exclusively amplified cDNA sequences of the human RPP30 gene (Figure 2). In contrast, the US-CDC primers and probe set amplified both cDNA and gDNA sequences of the human RPP30 gene.
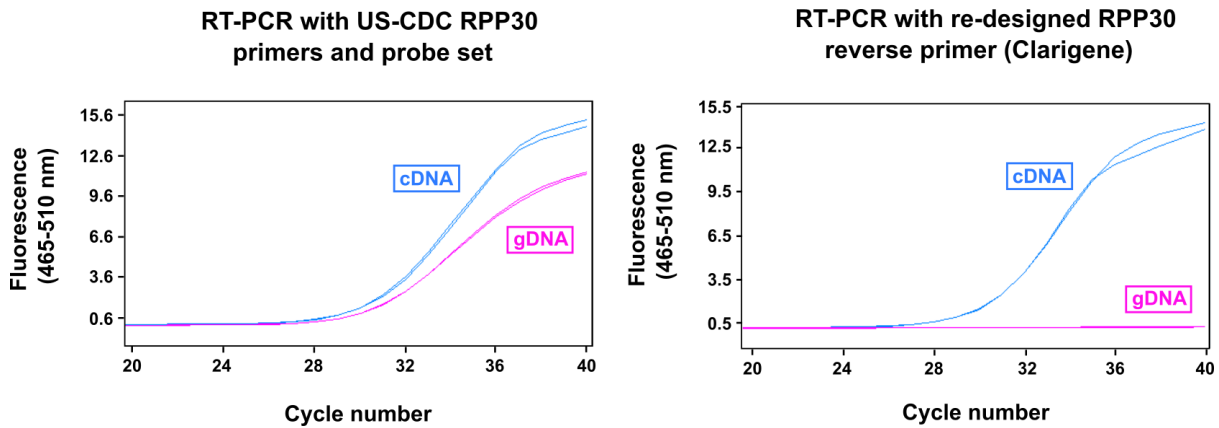
RT-PCR with US-CDC RPP30 primers and probe set

RT-PCR with re-designed RPP30 reverse primer (Clarigene)

Figure 2: Amplification of cDNA and gDNA sequences from the human RPP30 gene with US-CDC and re-designed primer/probe sets.

SARS-CoV-2 lineage B.1.1.7

The *in silico* analysis identified that the C28977T (p.S235F) mutation falls within the 5'-end of the N gene reverse primer (Figure 3). In vitro testing showed that the Clarigene™ SARS-CoV-2 test successfully detected N^{mut} gene of the B.1.1.7 lineage of SARS-CoV-2. The mean Ct values for N^{mut} at 5 copies/reaction, 50 copies/reaction and 500 copies/reaction were 36.19, 33.07 and 29.91, respectively. The mean Ct values for N^{wt} for 5 copies/reaction, 50 copies/reaction and 500 copies/reaction were 36.15, 32.99 and 29.95, respectively. A paired-sample t-test was conducted to compare the mean Ct between N^{wt} and N^{mut}. There was no significant difference in the scores for N^{wt} (mean: 33.03, standard deviation: 3.10) and N^{mut} (mean: 33.06, standard deviation: 3.14); t = 0.7559, p = 0.5286.

> SARS-CoV-2 N gene from B.1.1.7 lineage

tac gca gaa ggg agc aga ggc ggc agt caa gcc tct tct cgt tcc tca tca cgt agt
cgc aac agt tca aga aat tca act cca ggc agc agt agg gga act tct cct gct aga
atg gct ggc aat ggc ggt gat gct gct ctt gct ttg ctg ctg ctt gac aga ttg aac
cag ctt gag agc aaa atg ttt ggt aaa ggc caa caa caa caa ggc caa act gtc act
aag aaa tct gct gct gag gct tct aa

Figure 3: Portion of the SARS-CoV-2 B.1.1.7 lineage N gene. Clarigene™ SARS-CoV-2 N gene reverse primer sequence (underscore) overlaps with the C28977T (p.S235F) mutation (red) of the B.1.1.7 lineage.

Performance Characteristics

Limit of Detection

The analytical sensitivity of the Clarigene™ SARS-CoV-2 test was determined by testing synthetic SARS-CoV-2 RNA transcripts at varying concentrations. The results showed that the assay can detect SARS-CoV-2 targets at an input concentration of 5 copies/reaction 95% of the time (Table 5). Additionally, the amplification plot of the preliminary limit-of-detection is shown in Figure 4.
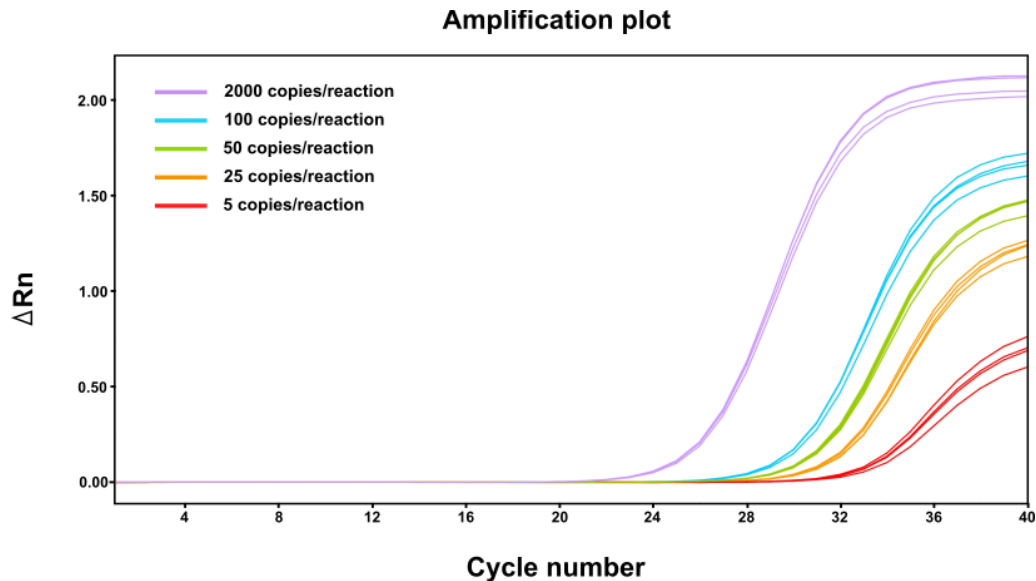
Figure 4: Amplification plot representative of a limit of detection run of the Clarigene™ SARS-CoV-2 test. The plot displays Envelope gene targets.

Accuracy (Sensitivity and Specificity)

The accuracy of the Clarigene™ SARS-CoV-2 test was assessed by testing 92 pre-extracted NP/OP clinical samples, which have been previously tested with a commercially available EUA approved test (see methods). The results showed 100% Positive Percent Agreement (PPA) for all 46 positive samples and showed 100% Positive Percent Agreement (PPA) for all 46 negative samples. Thus, the overall rate of agreement was determined to be 100% (Table 3).

	Positive result	**Negative result**	**Total**	
Positive sample	46	0	46	PPA=100%
Negative sample	0	46	46	NPA=100%
Total	46	46		
	Sensitivity=100%	Specificity=100%		

Table 3: Accuracy of the Clarigene™ SARS-CoV-2 test.

Repeatability

The repeatability of the Clarigene™ SARS-CoV-2 test was demonstrated by testing contrived SARS-CoV-2 positive (at 15 copies/reaction) and negative samples in thirty replicates three times over a 24-hour period. All samples (90 positive and 90 negative) were correctly identified, showing 100% overall agreement between all replicates.

Reproducibility

The reproducibility of the Clarigene™ SARS-CoV-2 test was determined by testing 24 replicates of SARS- CoV-2 samples at 1 copy/μL and 3 copies/μL, and 24 replicates of SARS-CoV-2 negative samples across three manufactured batches. As appropriate, variability was also assessed over three days and across three operators (n=216). The study output showed 100% PPA among all three operators, reagent lots and sample replicates. Despite the presence of one inconclusive result, which will be subjected to re-test criteria in a clinical setting, NPA of 100% was achieved for the negative synthetic samples (Table 4).

			Confidence Intervals
Limit of Detection		1 copies/ reaction	N/A
Reproducibility	PPA	100% (216/216)	98.31% - 100%
	NPA	100% (215/215)*	98.30% - 100%
Repeatability	PPA	100% (90/90)	95.98% - 100%
	NPA	100% (90/90)	95.98% - 100%
Accuracy	Sensitivity	100% (46 of 92)	92% - 100%
	Specificity	100% (46 of 92)	92% - 100%
	PPA	100% (46/46)	92% - 100%
	NPA	100% (46/46)	92% - 100%
*215/216 of the negative samples gave the expected diagnostic result. 1/216 of the negative samples gave an inconclusive result on day 1, batch 3, operator 3-however this would provide an "inconclusive" result and was omitted from the analysis. In an ordinary clinical setting this sample would be subject to a re-test.			

Table 4: Clarigene™ SARS-CoV-2 test performance characteristics.

Cross Reactivity

In silico analysis of the Clarigene™ SARS-CoV-2 test primers showed several instances of ≥80% sequence similarity with other respiratory pathogens. The cases where both forward and reverse primers were highly similar to a different pathogen were investigated in detail. The analysis showed that the distance between the forward and reverse primers in all instances is >70 kb, thus making potential amplification highly unlikely under standard assay conditions. Potential cross-reactivity with E gene sequences from SARS was detected.

Wet lab testing of several commercially available panels of pathogens from the same genetic family and other organisms likely present in a respiratory specimen were assessed with the Clarigene™ SARS-CoV-2 test. Amplification of the E gene target (<40 Ct) in the presence of SARS coronavirus was demonstrated, however, the N-gene target was not amplified, therefore this cross reactivity would lead to an inconclusive result. No additional cross reactivity was detected for any of the other pathogens tested (Table 2).

Target Concentration	Mean Ct (FAM™)	Mean Ct (VIC®)	Mean Ct (Cy5®)	Correct Calls	PPA (%)
100 copies/reaction	30.89	29.14	30.52	9/9	100%
50 copies/reaction	31.96	30.37	30.57	9/9	100%
25 copies/reaction	32.78	31.41	30.49	9/9	100%
10 copies/reaction	34.11	32.80	30.28	28/29	96.6%
5 copies/reaction	35.26	33.89	30.29	28/29	96.6%
2.5 copies/reaction	36.54	34.26	30.62	4/9	44.4%

Table 5: Limit of detection of the Clarigene™ SARS-CoV-2 test.

Discussion

One of the early developed tests in the COVID-19 pandemic was the US-CDC the 2019-nCoV Real-Time RT-PCR Diagnostic Panel, which targets two N gene regions of the SARS-CoV-2 genome and the human RPP30 gene in a singleplex format. The RPP30 targets serve as an endogenous control for verification of the assay performance. However, concerns have emerged regarding the primer design. Reports from the literature highlight that both gDNA and cDNA sequences will be amplified because the forward and reverse primers align to a region of exon 1 of the RPP30 gene [6,11]. This can prove problematic in instances where a negative test result has been reported in the absence of RNA in the patient sample. The high abundance of gDNA in clinical samples would allow the RPP30 control to produce a strong signal in such cases, thereby resulting in false negative results. This issue could be alleviated by the addition of a DNase I treatment step after RNA extraction. However, it would also extend the SARS-CoV-2 sample processing pipeline by adding an additional step in the workflow.

The Clarigene™ SARS-CoV-2 test is an easy to use multiplex assay which allows detection of all targets in a single reaction well, thus saving reagent cost and time. The re-designed internal control primers and probe set, ensure clear distinction between true negative and false negative results, arising from failed RT step, poor swabbing technique and failed extraction. Moreover, the studies presented here show the high analytical sensitivity, high accuracy, reproducibility and repeatability, as well as the high specificity of the assay targets (ensured by requirement of N gene and E gene target detection), summarised as Performance Characteristics (Table 4). The Clarigene™ SARS-CoV-2 test was also shown to successfully detect the novel SARS-CoV-2 variant which was identified in the UK in late 2020. We continue to monitor the emergence of SARS-CoV-2 strains within the population and perform adequate in silico and in vitro investigations to ensure that Clarigene™ SARS-CoV-2 test successfully detects COVID-19.

Contributions

All authors have equally contributed to this work.

References

1. Wu F, Zhao S, Yu B, Chen YM, Wang W, et al. (2020) A new coronavirus associated with human respiratory disease in China. Nature 579: 265-269.

2. Khailany RA, Safdar M, Ozaslan M (2020) Genomic characterization of a novel SARS-CoV-2. Gene reports 19: 100682.

3. Forster P, Forster L, Renfrew C, Forster M (2020) Phylogenetic network analysis of SARS-CoV-2 genomes. Proceedings of the National Academy of Sciences 117: 9241.

4. Rothan HA, Byrareddy SN (2020) The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun 109: 102433.

5. Sironi M, Hasnain SE, Rosenthal B, Phan T, Luciani F, et al. (2020) SARS-CoV-2 and COVID-19: A genetic, epidemiological, and evolutionary perspective. Infect Genet Evol 84: 104384.

6. Rosebrock AP (2020) Patient DNA cross-reactivity of the CDC SARS-CoV-2 extraction control leads to an inherent potential for false negative results. bioRxiv. 2020.05.13.094839.

7. Chand M, Hopkins S, Dabrera G, Allen H, Lamagni T, et al. (2020) Investigation of novel SARS-CoV-2 variant: VOC202012/01. Technical briefing 2.

8. Chand M, Hopkins S, Dabrera G, Allen H, Lamagni T, et al. (2020) Investigation of novel SARS-CoV-2 variant: 202012/01. Technical briefing 1.

9. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, et al. (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 25: 2000045.

10. Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, et al. (2020) Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. Nat Microbiol 5: 1299-1305.

11. Dekker RJ, Ensink WA, van Leeuwen S, Rauwerda H, Breit TM (2020) Overhauling a faulty control in the CDC-recommended SARS-CoV-2 RT-PCR test panel. bioRxiv.