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Evaluation of the *artus*® Prep&Amp UM RT-PCR for detection of SARS-CoV-2 from nasopharyngeal swabs without prior nucleic acid eluate extraction

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ABSTRACT

Here we describe a retrospective clinical evaluation of the QIAGEN *artus*® SARS-CoV-2 Prep&Amp UM RT-PCR assay that detects SARS-CoV-2 RNA without the need for a nucleic acid eluate extraction procedure. Using Roche SARS-CoV-2 RT-PCR on the cobas® 8800 platform as a reference standard, a total of 225 confirmed SARS-CoV-2 positive and 320 negative nasopharyngeal swabs in viral transport media, were used to evaluate the *artus*® assay. Using the RT-PCR cycle threshold as a semi-quantitative marker of viral load, an assessment of over 370,000 SARS-CoV-2 RT-PCR positive results was used in the design of the reference positive specimen cohort. The viral load of all reference positive specimens used in the evaluation was a unique and accurate representation of the range and levels of SARS-CoV-2 positivity observed over a 13-month period of the COVID-19 pandemic. The *artus*® RT-PCR detects the presence of SARS-CoV-2 RNA, an internal control, and the human RNase P gene to ensure specimen quality. The diagnostic sensitivity of *artus*® was 92.89% with a specificity of 100%. To assess the analytical sensitivity, a limit of detection was performed using the 1st WHO NIBSC SARS-CoV-2 international standard, recording a 95% LOD of 1.1×10^3 IU/ml. The total invalid rate of specimens was 7.34% due to a lack of detectable RNase P (C_t >35). The *artus*® SARS-CoV-2 Prep&Amp UM RT-PCR assay is a new rapid RT-PCR assay, which may be considered to produce acceptable levels of diagnostic sensitivity and specificity whilst potentially halving the laboratory processing time.

1. Introduction

The speed and accuracy of diagnosis for any infectious pathogen is essential to limit the spread of infection and target appropriate treatment. Low assay sensitivity or delays with reporting test results can prevent timely isolation of infected individuals and reduce the effectiveness of contact tracing [1]. At the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, limitations with laboratory reagents and consumables were experienced globally [2,3], exposing how unprepared the world was to face this new global diagnostic challenge. Scientists and biotechnology companies responded en masse, first increasing their production of laboratory consumables, and later, developing new assays dedicated to the rapid detection of SARS-CoV-2.

New technologies to identify SARS-CoV-2 have had varied success with hundreds of *in vitro* diagnostics (IVD) receiving emergency use authorization by both the U.S. Food and Drug Administration [4] and the UK Medicines and Healthcare products Regulatory Agency (MHRA) [5]. Rapid antigen point-of-care tests (RAT) have been an important tool for the early detection of SARS-CoV-2, reporting high specificity but with varied interpretations of sensitivity (48.9-76.8%) [6–8]; a statistic heavily dependent on viral load, the test cohort, and the operative performing the test [8]. New rapid laboratory tests such as reverse-transcription

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loop-mediated isothermal amplification (RT-LAMP) assay (both Direct and extracted RNA formats) also received emergency use authorisation in the UK, although much wider reported interpretations of diagnostic sensitivity (34% to 99%) [9–15] raises questions regarding methods and/or interpretation of such studies. The use of spiked samples and duplicate/triplicate testing possibly accounting for some of the variability between reports [9–13,15].

The inconsistences in published results highlight the importance of proper guidance when validating/assessing new molecular procedures for the detection of SARS-CoV-2 [16]. Although the reported sensitivities of some rapid detection methods may be lower than usually accepted for clinical diagnostic tests, the ability to self-test (RAT), the speed at which individual results can be produced and the ability to detect high viral loads provide the main justifications for their use. The introduction of guidelines [16] from the UK government regarding the reporting of studies designed to properly assess the clinical performance of SARS-CoV-2 detection tests is welcomed and necessary to inform the scientific community of what is expected and acceptable.

Here we establish the clinical performance of the *artus*® Prep&Amp UM (QIAGEN, Hilden, Germany) RT-PCR for the rapid detection of SARS-CoV-2, using an RT-PCR reference standard with the highest reported detection of SARS-CoV-2 available to the Manchester University NHS Foundation Trust (MFT) and a sample set highly representative of the range of viral loads observed during a significant proportion of the SARS-CoV-2 pandemic. We explore the hypothesis that acceptable levels of diagnostic sensitivity and specificity can still be achieved whilst bypassing the time consuming, reagent-dependent, and costly nucleic acid extraction and purification process.

2. Materials and methods

2.1. Observed range of SARS-CoV-2 positive C_t values over a substantial proportion of the pandemic

To determine the most representative cohort of clinical specimens to assess *artus*® RT-PCR, all SARS-CoV-2 RT-PCR results, from multiple platforms, were collected from the MFT laboratory information management system between the 01/04/2020 and 30/04/2021. To ensure the most accurate assessment of *artus*®, the Roche SARS-CoV-2 RT-PCR on the cobas® 8800 platform (Roche, Basel, Switzerland) was used as the reference standard, as the platform was considered to be the most sensitive SARS-CoV-2 detection platform at the MFT [5]. Sample inactivation and cobas® RT-PCR was performed as previously described [11]. To enable an accurate reflection for the distribution of positive SARS-CoV-2 results, the C_t values for the ORF1ab gene from first-time positive specimens were used to map the distribution of viral loads received during the aforementioned 13-month pandemic period.

The distribution of C_t values was plotted for first-time positive patients. Positive results were grouped within a single numerical C_t value and the number of specimens recorded within each C_t value expressed as a percentage of the total first-time positive results. Specimens used to assess the clinical sensitivity of *artus*® were chosen with a viral load and range representative of the first-time positive distribution.

2.2. Clinical specimen cohort to determine the diagnostic sensitivity and specificity

A total of 545 clinical nasopharyngeal swabs (NPS) collected in Remel® VTM were tested using the *artus*®, comprising of 225 confirmed SARS-CoV-2 positive and 320 negative specimens. All specimens tested using the *artus*® were unlinked and anonymised prior to blind testing. Of 225 positive NPS specimens, 193 were stored at -80°C prior to testing and 32 stored at ambient temperature and tested within 72 h of receipt. Positive and negative frozen NPS were chosen randomly between March and April 2021 based on ORF1ab C_t detection values. All negative specimens were stored at -80°C prior to testing with the *artus*® assay. As only a limited number of *artus*® tests were available to the MFT, specimen numbers were in concordance with the guidance supplied by the MHRA Target Product Profile (TPP) for point-of-care SARS-CoV-2 detection tests [17].

2.3. Artus® *SARS-CoV-2 Prep&Amp UM PCR*

To ensure that viable virus was inactivated prior to testing, 50µl of specimen was placed in a MicroAmp® Optical 96-Well Reaction Plate (Thermo Fisher) and held at 95°C for 1 min on an Applied Biosystems 9700 Thermal Cycler (Thermo Fisher) as per Public Health England (PHE) SARS-CoV-2 heat inactivation protocol [18]. Specimens were cooled to 4°C prior to testing. Inactivated specimens were processed as per manufacturer's instructions for use (IFU) [19]. Both negative and positive (AMPLIRUN® - Vircell, Grenada, Spain) extraction controls were used for each run of the *artus*® protocol. Amplification was performed on an ABI 7500 Fast system (Thermo Fisher). Detection of SARS-CoV-2 RNA, an RNase P human sampling control (HSC) and the Internal Control was performed using the three dye layers FAM, VIC and Cy5, respectively. Results were analysed using ABI 7500 Fast system software (Thermo Fisher) and C_t values determined using a baseline-corrected normalised reporter (ΔRn) threshold of 0.15. The validity of test runs were determined as per Table 1 and result interpretation as per Table 2. Any reference positive specimens that produced a negative SARS-CoV-2 result using the *artus*® assay was tested using the CDC assay and repeat tested on the cobas® platform. Additionally, six specimens for each of the of Alpha, Beta, Gamma, Delta and Omicron SARS-CoV-2 variants were tested, all with previous C_t values between 24 and 30.

2.4. Multiplex RT-PCR using the centre for disease control and prevention (CDC) 2019-nCoV N1&N2 assay with Beta-2-Microglobulin (β2M) as endogenous control

As the cobas® SARS-CoV-2 RT-PCR platform does not include an endogenous control, SARS-CoV-2 negative specimens that recorded a HSC C_t result >35 with the *artus*® assay were tested using the CDC Real time SARS-CoV-2 N1&N2 RT-PCR assay [20]. Briefly, specimens were inactivated as previously described [11] and extraction (DSP Virus/pathogen mini-kit, QIAGEN, Hilden, Germany) performed on the QIAsymphony (QIAGEN) as per manufacturer's instructions. RT-PCR reactions consisted of 1.5µl of both the 2019 nCoV N1 and N2 primer/probe mix

Table 1
Run validity criteria for the artus® SARS-CoV-2 Prep&Amp UM PCR.

Control type	Detection in FAM dye (SARS-CoV-2)	Detection in VIC dye (HSC)	Detection in Cy5 dye (IC)	Interpretation
AMPLIRUN® SARS-CoV-2 whole genome RNA control	Ct ≤39.00	Indifferent	Indifferent	Run validated
	Ct ≥39.00 or No C_t	Indifferent	Indifferent	Run invalidated
Manufacturer Positive Control	Ct ≤39.00	Indifferent	Indifferent	Run validated
	Ct ≥39.00 or No C_t	Indifferent	Indifferent	Run invalidated
No template control and Negative extraction control	Ct ≥39.00 or No C_t	C_t ≥35.00 or No C_t	Yes	Run validated
	Any other combination with amplification in FAM or VIC dye		Indifferent	Run invalidated

Table 2
Sample validity criteria and result interpretation for the artus® SARS-CoV-2 Prep&Amp UM PCR.

Detection in FAM dye (SARS-CoV-2)	Detection in VIC dye (HSC)	Detection in Cy5 dye (IC)	Interpretation
C_t ≤39.00	Indifferent	Indifferent	SARS-CoV-2 detected
C_t >39.00 or No C_T	C_t ≤35.00*	Indifferent	SARS-CoV-2 NOT detected
C_t >39.00 or No C_T	C_t >35.00 or No C_T	Yes	Invalid
C_t >39.00 or No C_T	C_t >35.00 or No C_T	No	sample

* Analysis 2 interpretation performed with the Human sampling control interpreted positive at C_t <40.

Table 3
List of molecular controls used to determine SARS-CoV-2 variant detection and the analytical specificity of the artus® SARS-CoV-2 Prep&Amp UM assay.

Zeptometrixs Respiratory Pathogen panel 1[1]	https://www.zeptometrix.com/media/documents/PINATRPP-1.pdf
Zeptometrixs Respiratory Pathogen panel 2[1]	https://www.zeptometrix.com/media/documents/PINATRPC2-BIO.pdf
NIBSC 15/130. Clinical Virology Multiplex I: Immunodeficiency panel working reagent[1]	https://www.nibsc.org/documents/ifu/15-130-xxx.pdf
NIBSC 13/168. Human measles MVI/Moscow.RUS/0.88[1]	https://www.nibsc.org/documents/ifu/13-168-xxx.pdf
Speciated and untyped extracts[1]:	Coxsackie B virus, M. tuberculosis, M. intercellulare, M. avium, M. avium-intercellulare, M. africanum, M. gordonae, M. szulgai, Pneumocystis jirovecii, Streptococcus pneumoniae ATCC119619, Streptococcus pyogenes ATCC19615, Klebsiella pneumoniae and Haemophilus influenza ATCC11331
Previously identified SARS-CoV-2 Variants of Concern[2]	Alpha, Beta, Gamma, Delta and Omicron

[1] All listed specificity controls produced a negative SARS-CoV-2 result using the artus® RT-PCR assay.
[2] All SARS-CoV-2 variants tested with the artus® RT-PCR (C_t 24-30) produced a positive result.

(Integrated DNA Technologies, Inc.), 0.5 µl of TaqMan® Human β2M endogenous control (Thermo Fisher), 5µl of TaqPath™ 1-Step RT-qPCR MM (ROX) (Thermo Fisher), 6.5µl molecular grade water and 5µl of eluate. RT-PCR was performed on the 7500 Fast PCR system as previously described [20]. A nuclease-free non-template control (NTC), a negative extraction control and a SARS-CoV-2 whole genome RNA positive control (Vircell) were included on each run. Results were analysed using the ABI 7500 Fast system software and C_t values determined using a ΔRn threshold of 0.15. As per the CDC N1N2 IFU [20], C_t <40 were considered positive for both SARS-CoV-2 and the endogenous control. Reference positive specimens that produced a negative SARS-CoV-2 result with the *artus*® were tested using the CDC assay and repeat tested on the cobas® platform. Any false positive reference specimens were removed from the sample cohort and final analysis.

2.5. Time to result

The time taken from receipt of inactivated specimen to final result was taken for each of the SARS-CoV-2 detection methods used in the study. Results regarding specimen turnaround times relate to batches of 96 specimens. Measurements were taken from the receipt of 96 inactivated specimens and included any necessary manual processing (e.g. centrifugation/uncapping) up to the completion of all finalised results. The turnaround time for the cobas® 8800 was measured on nine occasions, the CDC N1N2 assay measured twice and the artus protocol measured three times, each recorded on separate days. The average (mean) time was calculated for each method (supplementary I).

2.6. Analytical sensitivity

The analytical sensitivity of *artus*®, CDC 2019 nCoV N1N2 and cobas® SARS-CoV-2 were determined using a dilution of the 1st WHO International Standard for SARS-CoV-2 (NIBSC 20/146) [21]. A doubling dilution series of NIBSC 20/146 was created from an initial concentration of log 7.7 IU/ml to log 1.99 IU/ml using VTM as diluent. A Limit of Detection (LOD) was performed using multiple replicates per analysis. The number of replicates used for the cobas® and the *artus*® were as follows: 2 at log7.7, 6 at log6.7, 6 at log5.7 and 20 replicates for all other dilutions. The LOD for the CDC assay was taken from a previous in-house study and followed the same dilution series but used 8 replicates per dilution rather than 20 from log4.7 to log1.99. The LOD for the *artus*® assay was performed twice, once at the beginning of the study and once at the end. Probit analysis was performed in SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp) software to determine 95% LOD in IU/ml.

2.7. Analytical specificity

Table 3 lists the molecular controls used to assess the analytical specificity and variant detection of the *artus*® RT-PCR.

3. Results

3.1. C_t value distribution from first-time positive patients over a 13-month period of the pandemic

More than half a million results were downloaded from the laboratory management system over the 13-month period of the pandemic. A total of 374,606 valid swab samples were tested using the cobas® SARS-CoV-2 assay, with 357,977 recording a negative result and 16,629 recording a valid positive result. It was calculated that 2654 specimens were repeat positives within 90 days, leaving a total of 13,975 first-time positives over the specified period. The cobas® SARS-CoV-2 assay positive (ORF1ab) C_t distribution and the representative sample set used to assess *artus*® is displayed in Fig. 1.

3.2. Sensitivity and specificity of the artus® SARS-CoV-2 Prep&Amp UM RT-PCR

Using *artus*®, 209 specimens were recorded as positive from a total of 225 cobas® SAR-CoV-2 positive specimens (Table 4). From the 16 reference positive specimens that failed to produce a positive result with the *artus*®, 14 (7.3%) specimens were previously stored at -80°C and two (6.3%) were stored at ambient temperature. Seven of the frozen *artus*® false negative specimens recorded an ORF1ab C_t of >35, 5 between 34-35, 3 between 33-34 and one at 32.48 (Table 5). The two false negative results recorded from specimens stored at ambient temperature had ORF1ab C_t values of 34.3 and 38. Each false negative result produced a β2M C_t <40 using the CDC assay. *artus*® demonstrated a diagnostic sensitivity and specificity of 92.89% and 100%, respectively (Table 4).

Analysis 1: Adopting the criteria stipulated in the manufacturers IFU (Table 2), a total of 40 specimens were considered invalid with the *artus*®, amounting to a specimen quality failure rate of 7.34% for the entire cohort of specimens and a reference negative failure rate of 12.5% (Table 4). Importantly, within the cohort of reference positive specimens, no invalid results were recorded.

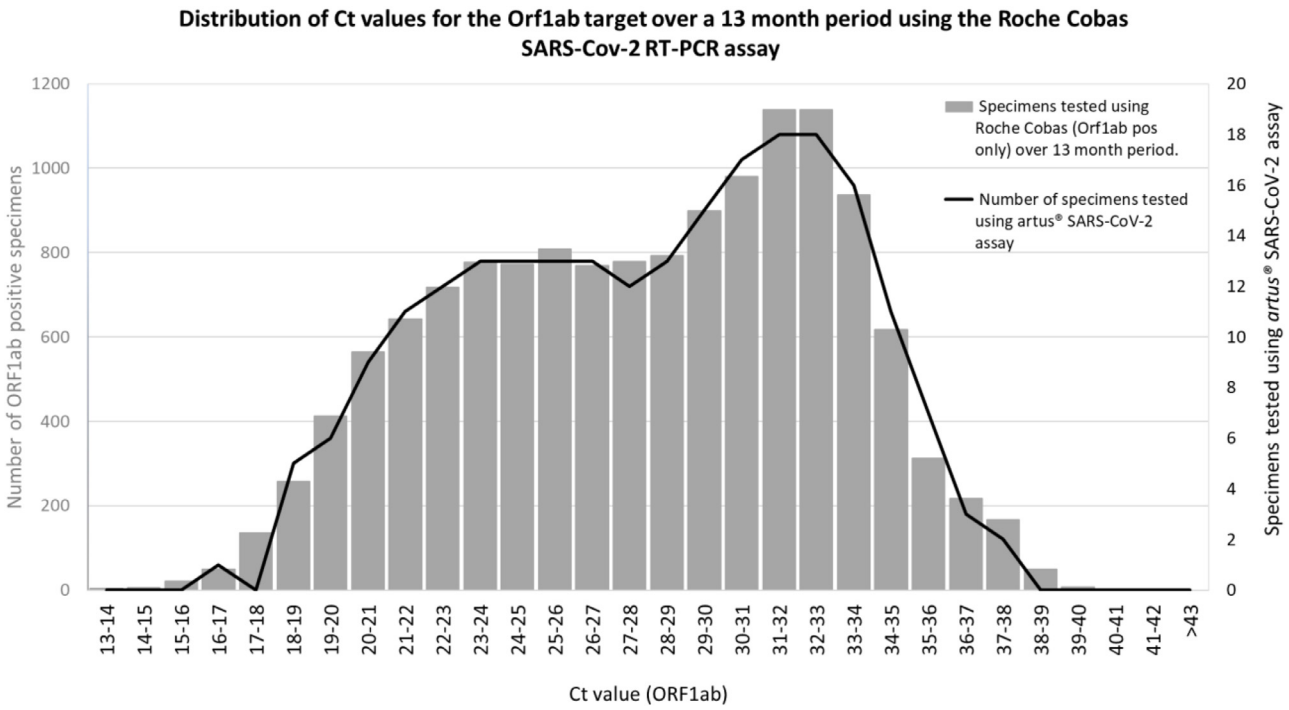

Fig. 1. C_t values for first-time positive patients using the Roche Cobas 8800 RT-PCR platform overlayed with a mirrored percentage distribution of specimen numbers tested using the artus® SARS-CoV-2 assay.

Table 4
Analysis 1: Diagnostic sensitivity and specificity of the artus® SARS-CoV-2 Prep&Amp UM RT-PCR compared to Orf1ab detection using the Roche Cobas platform; Number of invalid specimens recorded with the Human Sampling Control cut-off set at C_t 35.

	Orf1ab RT-PCR Positive[1]	**Orf1ab RT-PCR Negative**
artus® **Positive**	209	0
artus® **Negative**	16	280
	Value	**95% CI**
Sensitivity	92.89%	88.71% to 95.88%
Specificity	100.00%	98.69% to 100.00%
Invalids – artus® human sampling control >35 C_t	**All specimens (n545)**	**From negatives only (n320)**
	40 (7.34%)	40 (12.5%)
Number of artus® invalid specimens with β2M C_t of >40	17 (3.12%)[2]	17 (5.31%)[2]

[1] Three of the randomly chosen Orf1ab reference positive specimens were considered as false positives and removed from the study (Orf1ab Ct 30.01, 33.04 and 36.0) after confirmed repeat negative results on the cobas® assay and the CDC assay. Substitute specimens within the same Ct range were included and the mirrored Ct distribution range was unaffected.

[2] Six of the 17 specimens produced a HSC Ct value between 35 and 40 with the artus® assay. The remaining 23 invalid specimens all recorded a β2M Ct value of <40, with 15 recording a HSC between Ct 35 and 40.

Analysis 2: Table 6 displays the number and percentage of invalid specimens if a C_t cut-off employed for the *artus*® was equivalent to the RNase P target within the CDC 2019 nCoV N1N2 assay.

3.3. Turnaround times and analytical sensitivity and specificity

Table 7 displays the results for the analytical LOD performed on all assays used in the study, along with the average turnaround time for each protocol. The turnaround time using the *artus*® protocol was substantially quicker requiring only 1:42:00 in comparison to the cobas® SARS-CoV-2 reference standard, timed at 3:18:00.

Combined use of the ORF1ab and E genes targets on the cobas® platform produced the lowest LOD. From the two LOD analyses of the *artus*®, an average 95% LOD of approximately 1100 IU/ml was observed.

Table 3 displays the results for the analytical specificity of the *artus*® RT-PCR.

4. Discussion

RT-PCR remains the gold standard for the detection of most viral pathogens but requires the purity of a nucleic acid eluate extraction procedure to produce reliable results. This evaluation describes a rapid direct RT-PCR that produced a high level of diagnostic sensitivity and specificity without a lengthy extraction procedure, which can often account for half the process time. Direct-PCR without eluate extraction is not a new technique and it has been shown to be used reliably for pathogens in pure culture [22]. However, due to the nature of clinical specimens often containing an abundance of human material/PCR inhibitors, it is rarely used as a diagnostic tool for molecular testing [23].

Table 5
Positive results using the artus® SARS-CoV-2 Prep&Amp UM RT-PCR compared to the reference standard. Specimen numbers categorised by C_t value and prior storage.

Cobas® Orf1ab C_t Value	No. of Orf1ab C_t positive Frozen specimens tested	No. of specimens positive using the artus® SARS-CoV-2 Prep&Amp	No. of reference positive ambient specimens tested[1]	No. of specimens positive using the artus® SARS-CoV-2 Prep&Amp	Total positive reference specimens tested	Total artus® positives
16.77	1	1			1	1
18-19	4	4	1	1	5	5
19-20	6	6			6	6
20-21	8	8	1	1	9	9
21-22	9	9	2	2	11	11
22-23	10	10	2	2	12	12
23-24	11	11	2	2	13	13
24-25	11	11	2	2	13	13
25-26	11	11	2	2	13	13
26-27	11	11	2	2	13	13
27-28	11	11	1	1	12	12
28-29	11	11	2	2	13	13
29-30	13	13	2	2	15	15
30-31	14	14	2	2	16	16
31-32	16	16	2	2	18	18
32-33	16	15	2	2	18	17
33-34	13	10	2	2	15	12
34-35	9	5	2	1	11	6
35-36	5	1	2	2	7	3
36-37	2	1			2	1
>37	1	0	1	0	2	0

[1] 32 ambient temperature positive specimens were independently chosen to equally cover the range of Ct values from Ct 18 to 36.

Table 6
Analysis 2: Invalid specimen numbers for the artus® assay when using a validity C_t result of <40 for the Human sampling control assay, comparable with RNase P assay in the 2019 nCoV_N1 and N2 protocol.

	All specimens (n545)	From negatives only (n320)
Invalids - artus® human sampling control <40 C_t[1]	19 (3.49%)	19 (5.9%)
Number of artus® invalid specimens with β2M C_t of >40	17 (3.12%)	17 (5.31%)

[1] Adopting the Ct <40 cut-off for the artus® HSC, an additional 21 specimens would be deemed valid from the 40 invalid specimens. Using this criterion, only 19 of the negative specimens would be considered invalid amounting to a total HSC failure of only 3.49%; the adjustment not affecting the overall specificity of the artus® assay.

Table 7
Analytical 95% limit of detection for all diagnostic assays used in the study, including average time taken to result for each protocol used.

Assay	Target	95% LOD IU/ml	95% CI IU/ml	Time to result[2]
Cobas® SARS-CoV-2 (Extraction on Cobas 8800 closed system)	**Orf1ab**	367.7	208.7 - 1343.5	3:18 hours (n9)
	E gene	212.0	142.0 - 518.7	
	Orf1ab and E gene	149.1	88.9 - 895.5	
CDC 2019 nCoV_N1 and N2 with β2M (Extraction on QIAsymphony)	**N1 and N2**	677.8	381.4 - 4313.8[1]	7:09 hours (n2)
artus® SARS-CoV-2 Prep&Amp UM RT-PCR				
LOD 1	**N1 and N2**	995.4	657.3 - 2678.5	1:42 hours (n3)
LOD 2	**N1 and N2**	1184.2	894.4 - 2310.2	

[1] The CDC 2019-nCoV N1 and N2 assay produced an LOD with much wider confidence limits due to the reduced number of replicates tested.
[2] Average time taken to final result from receipt of 96 inactivated specimens.

Interestingly, the distribution of C_t values for the 13,975 SARS-CoV-2 positive patients was not a typical bell-curve distribution but instead a plateau in positive patient numbers between C_t 23 and 29 and a peak between C_t 31 and 33 was observed (Fig. 1). We can speculate that the reason for this may be due to the viral trajectory of SARS-CoV-2, typified by a characteristic rapid short-lived peak of viral replication (mean C_t 23-28 for mild to severe symptoms [24,25]) followed by a slow decrease in viral RNA levels over several days or weeks [24–29]. As the MFT performs testing for SARS-CoV-2 on hospitalised patients, new admissions, asymptomatic staff and community outbreaks, the range of viral loads and the stage at which infection is detected, will be broad. Therefore, it is hypothesised that a significant proportion of the first-time positive specimens in our study are likely to be sampled outside the relatively short-lived peak viral load. Furthermore, it is not possible to determine to what degree we are observing viral loads from two independent patient characteristics, symptomatic and asymptomatic. One study of 97 positive participants showed symptomatic patients had an increased peak viral load compared to asymptomatic patients (Ct 23.3 vs 30.7) [26]. Notwithstanding the reasoning behind the distribution, we believe the cohort of specimens used in this evaluation was highly representative of the viral load distribution that would be encountered in a real-world laboratory scenario, allowing a more accurate assessment of diagnostic sensitivity.

With regards to the desired and acceptable criteria stipulated by the MHRA in the TPP for Laboratory-Based SARS-CoV-2 Viral Detection test [30], the *artus*® assay performed well as a rapid SARS-CoV-2 detection assay, with an analytical sensitivity of 1100 IU/ml, a diagnostic sensitivity of 93%, and a diagnostic specificity of 100%, whilst also providing a concurrent assessment of test and specimen quality. Clinically, the three molecular targets used in the *artus*® RT-PCR supply the desired set of results when assessing the quality of any diagnostic PCR result; the level of human nucleic acid allowing clinicians to better interpret specimen quality and consider whether retesting is an appropriate course of action [31,32]. However, not all rapid assays that detect SARS-CoV-2 provide this scope of results. Rapid tests such as the Optigene LAMP assay does not provide an internal control or an reverse transcriptase control [33], nor does it provide a endogenous control to assess sample quality [34,35], not dissimilar to the RAT or Roche reference assay used in this study [36].

At first glance, the number of invalid results produced by the *artus*® assay interpretation was concerning. The 7.34% of specimens deemed invalid by the *artus*® (analysis 1) may reflect the overall quality of NPS received by our laboratory; the invalid interpretation conceivably reducing the number of false negatives and demonstrating an improved level of accuracy rather than test failure. However, it may also be that a proportion of these invalid results are due to a number of technical reasons, such as a reduced nucleic acid purity with the *artus*® chemical extraction process, specimen freeze thaw, RNA degradation over time, or a possibility that the HSC C_t assay cut-off for the *artus*® protocol is overly stringent.

Exploring the contention that the HSC C_t is too stringent, analysis 2 (Table 6) used the C_t cut off (<40) instructed within the CDC N1N2 SARS-CoV-2 assay [20] for the same RNase P target. This adjustment greatly decreased the number of overall invalids to only 3.5%, comparable to the invalid number of specimens recorded using the β2M target (3.12%).

One of the main limitations of this study was the lack of a comparable reference test. As no molecular test run at the MFT has all three of the RT-PCR targets in the *artus*®, a combination of assays was required to assess molecular targets to ensure the most stringent and fair comparison. However, the use of two platforms for an index test led to inconsistencies between levels of C_t cut-off when assessing specimen quality, a discrepancy, we believe, mitigated by the additional analysis made for the endogenous control.

With regards to the practicality of the assay, the Remel® VTM used in this study contains a protein element that can form a precipitate during the inactivation procedure (95˚C for 1 min). The physical precipitate did not seem to directly affect the results of the assay but may become more relevant with the implementation of automated testing and the potential of pipette tip blockage. However, the formation of precipitate may be mitigated by a change in inactivation procedure or use of an alternative VTM.

Further studies are required to assess if the extraction procedure used in this assay can be applied to other molecular techniques. It is accepted that eluate extraction procedures will remain best practice but dependant on the objective, along with continuous improvements to the extraction chemistry, there may be a place for non-eluate extraction within the molecular laboratory.

Consent for publication

All authors gave their consent for publication.

Availability of data and materials

All the data for this study will be made available upon reasonable request to the corresponding author.

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QIAGEN supplied the *artus*® SARS-CoV-2 Prep&Amp UM RT-PCR kit for evaluation. No other form of funding received.

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2022.100098.

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