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Reporting of RT-PCR cycle threshold (Ct) values during the first wave of COVID-19 in Qatar improved result interpretation in clinical and public health settings

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Abstract

Introduction. The cycle threshold (Ct) value in real-time PCR (RT-PCR) is where a target-specific amplification signal becomes detectable and can infer viral load, risk of transmission and recovery. Use of Ct values in routine practice is uncommon.

Gap Statement. There is a lack of routine use of Ct values when reporting RT-PCR results in routine practice.

Aim. To automatically insert Ct values and interpretive comments when reporting SARS-CoV-2 RT-PCR to improve patient management.

Methodology. Routine Ct values across three different RT-PCR platforms were reviewed for concordance at presentation and clearance in patients with COVID-19. An indicative threshold (IT) linked to viral clearance kinetics was defined at Ct30 to categorize Ct values as low and high, reflecting high and low viral loads respectively.

Results. The different gene targets of each platform showed high correlation and kappa score agreement (P<0.001). Average Ct values were automatically generated with values ≤Ct30 reported as positive and >Ct30 as reactive; interpretive comments were added to all reports. The new reporting algorithm impacted on: physician interpretation of SARS-CoV-2 results; patient management and transfer; staff surveillance; length of stay in quarantine; and redefinition of patient recovery.

Conclusion. Incorporation of Ct values into routine practice is possible across different RT-PCR platforms and adds useful information for patient management. The use of an IT with interpretive comments improves clinical interpretation and could be a model for reporting other respiratory infections. Withholding Ct values wastes useful clinical data and should be reviewed by the profession, accreditation bodies and regulators.

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Abbreviations: CAP, College of American Pathologists; CDC, Centers for Disease Control and Prevention; COVID-19, coronavirus infectious disease 2019; Crl, credible interval; Ct, cycle threshold; HCoV, human coronavirus; IT, interpretive threshold; NPS/OPS, nasopharyngeal / oropharyngeal Swab; RT-PCR, reverse transcriptase PCR; WHO, World Health Organization.

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INTRODUCTION

The World Health Organization (WHO) reported: clusters of pneumonia on 31 December 2019 in Wuhan, China; isolation of SARS-CoV-2 on 9 January 2020; definition of Coronavirus Infectious Disease 2019 (COVID-19); a Public Health Emergency of International Concern on 30 January 2020; a pandemic on 11 March 2020 [1]. WHO advised the use of reverse transcriptase PCR (RT-PCR) on combined nasopharyngeal/oropharyngeal swabs (NPS/OPS) for the diagnosis of COVID-19 and two negative RT-PCRs 24 h apart for defining recovery [2].

RT-PCR amplifies over 40–45 cycles, with the cycle threshold (Ct) value determined when the amplification signal exceeds that of controls. The Ct value is inverse to viral load and rises with clearance as more cycles are required to detect the virus. Reporting Ct values is not routine because of a perceived lack of platform to platform concordance and absence of a direct link with clinical outcome.

A positive result for SARS-CoV-2 is defined by the manufacturer-specified Ct values for one or more genes, with an inconclusive result reported where the combination of reactive genes fails to meet the definition. A negative result is defined by the absence of an amplification signal or cumulative values above predefine cut-offs.

In most infections, SARS-CoV-2 has a short latent period where the virus is undetectable for about 4 days, followed by a period of high viral load through consecutive pre-symptomatic and symptomatic phases lasting for another 4 days, and then prolonged viral RNA shedding [3–8]. The timing of transmission varies with the data source, with Ganyani reporting the generation interval (time between infection events in an infector–infectee pair) as 5.20 days [95% credible interval (CrI): 3.78–6.78] and 3.95 days (95% CrI: 3.01–4.91) for two separate outbreaks [9].

Patients with high viral loads (<Ct 20) present with symptoms which fall in both upper and lower respiratory tracts but higher loads can persist in the lower airways especially if the clinical condition deteriorates [10]. Low-level viral RNA can persist but transmission does not typically exceed 10 days from first PCR or symptom onset in mild cases and 20 days in severe cases [10–12]. Categorical reporting (positive or negative) with prolonged shedding infers patients are infectious when they are in recovery, leading to extended quarantine or exclusion from school.

Attempts to use Ct values as a surrogate of viral load and disease severity have been reported but not adopted. Wishaupt [13] showed low Ct values in common respiratory viral infections, including seasonal human coronaviruses (HCoV), were associated with hospitalization, increased oxygen requirement and longer stays. Spencer and colleagues categorized influenza with an arbitrary Ct threshold into low and high values to differentiate high and low viral loads respectively [14]. They confirmed that low Ct values were seen in younger patients (3–8 years) were more likely to have a primary infection, symptomatic elderly patients and within 2 days of symptom onset, indicating early-stage infection. A similar pattern was reported in children with *Bordetella pertussis*, with low values indicating high bacterial loads in unvaccinated children; bacterial loads were three orders of magnitude higher than in those vaccinated [15].

Ct values can estimate the period of infectivity. Bullard [11] found that SARS-CoV-2 could not be isolated after 8 days or >Ct24. The Centers for Disease Control and Prevention (CDC) [16] and La Scola [12] reported virus culture was restricted to the first 8 days of a symptomatic infection and Ct values <33. Wolfe [10] and van Kampen [17] reported viral culture correlated with high viral loads and by implication low Ct values, and that viral recovery in severe disease could be prolonged [17]. Time-based prediction of infectiousness using virus culture is more consistent than individual Ct thresholds, although it is likely to overestimate the period of infectivity for most people.

Reporting Ct values in respiratory virus infections faces regulatory and accreditation challenges that emphasize sensitivity but take no account of viral kinetics and transmission. Omitting these data deprives physicians of information that could impact patient management, a common directive in all viral respiratory guidelines such as the IDSA influenza guidelines [18]. At the start of this study in the first wave of the pandemic three reporting categories were used: positive, negative and inconclusive. A follow-up was requested where inconclusive.

In Qatar, which has a population of 2.8 million, three platforms were operational for SARS-CoV-2 detection, Roche, ThermoFisher and Cepheid, respectively amplifying two, three and two gene targets. Against the growing appreciation of the kinetics of viral replication in COVID-19, a review of their respective assay performance was undertaken to gauge if Ct values could be safely added to reporting and improve interpretation of SARS-CoV-2 results.

METHODS

RT-PCR assay platforms

All assays were validated in line with College of American Pathologists (CAP) accreditation standards and analysed for comparative amplification kinetics of each gene target. Two automated platforms used were: Roche cobas 6800 using the cobas SARS-CoV-2 Test amplifying E and orf1a/b genes (Roche); the Xpert Xpress SARS-CoV-2 amplifying the E and N genes (Cepheid). The manual

platforms used the EZ1 (Qiagen) and QIAsymphony (Qiagen) extraction processes with thermal cycling provided by the TaqPath PCR COVID-19 Combo Kit, amplifying the N, S and orf1a/b genes (Thermo Fisher Scientific), on ABI 7500 thermal cyclers (Thermo Fisher Scientific). SARS-CoV-2 results were reported by use of pre-determined gene cut-offs of the respective kit. Kit combinations were used for defining categories as positive, negative or inconclusive. A fourth category, reactive, was later defined using an indicative threshold (IT).

Thermal cycling Ct kinetics

A total of 173557 individual Ct values from 148066 patients across the Roche, Thermo-Fisher and Cepheid platforms were analysed for concordance using routine Ct values from the respective instruments. Sequential samples on the Roche and Thermo Fisher platforms were followed to observe the viral kinetics associated with viral clearance during recovery.

Indicative threshold for reporting Ct values

With reference to observed clearance on the respective platforms and publications linked to infectiousness, a threshold above and below which transmission was less or more likely was defined. The time taken to reach an average Ct value from initial detection to viral clearance was reviewed and that at day 14 (Ct28), in line with local practice, was used as a guide to the limit of infectiousness. In practice, a higher value, Ct30, was selected to increase the safety margin.

Change in reporting practice

Reporting was redesigned with reference to the IT. Individual gene Ct values were co-reported with the alphanumeric results through a flat file interface to a mainframe computer (Cerner). An average Ct value was automatically calculated and used to generate reporting categories. Kit cut-offs and instructions were used to define the combinations of gene target signals to be reported as positive, inconclusive or negative, with the fourth reactive category (>Ct30) defined by the IT. At auto verification, an interpretive comments table was automatically appended. The four categories were also interfaced in real time to the National Dashboard and used for setting the colour category change on Qatar's national COVID-19 track and trace EHTERAZ application.

Statistics

Spearman rank coefficient and kappa scores were used for comparison of Ct values across each platform with $P<0.05$ taken as significant.

RESULTS

Ct kinetics across thermal cycling

Assay correlation

Assay performances during the study were acceptable in internal and external proficiency panel assessments, in line with CAP accreditation standards.

The Ct values across the different gene targets for each of the three platforms showed good agreement as measured by Spearman correlation coefficient and kappa scores (Table 1), in keeping with acceptable performance during routine practice.

Rise in Ct value in follow-up swabs

Swabs 1–5 represented the cumulative number of swabs taken sequentially from the first swab to the last from patients who had follow-up swabs; not all patients had five swabs taken. The Roche and Thermo Fisher assays confirmed an early decline in viral load with the proportion of swabs <Ct30 falling from approximately 75% with first swabs to approximately 30% for immediate follow-up swabs (Fig. 1). A slower decline between the immediate follow-up and subsequent swabs was noted with the Thermo-Fisher assay, indicating a probable divergence of assay sensitivity between the two platforms during clearance.

Indicative Ct threshold

The lowest Ct value indicated the highest load of infectious virus detected by RT-PCR, and detectable viral RNA continued for some weeks, with steadily rising Ct values [19, 20]. Fig. 2 shows the number of days spent at or below each Ct value across the different platforms, with Ct28 equating to 14 days. The IT selected for defining the new reactive reporting category was set at Ct30 to arbitrarily build in an additional margin where the likelihood of transmission was deemed low for most recovering patients.

Reporting using the indicative threshold

The use of an IT of Ct30 allowed the use of four reporting categories: positive, reactive, inconclusive and negative. Interpretive comments based on the kinetics of SARS-CoV-2 replication and probable infectiousness were added at auto verification (Table 2).

Table 1. Correlation of gene targets across the Roche, Thermo Fisher and Cepheid RT-PCR* platforms

The two and three gene combination target sets from 148066 patients were compared across the three amplification platforms: Roche cobas 6800, Cepheid Xpert Xpress SARS-CoV-2 and Thermo Fisher TaqPath PCR COVID-19 Combo Kit. The genes amplified were: N=nucleoprotein, S=spike, E=envelope, orf1a/b.

Platform	Pearson correlation coefficient	*P*-value	Spearman correlation coefficient	*P*-value	Positive agreement (%)	Negative agreement (%)	Overall agreement (%)	Kappa	*P*-value
Thermo Fisher									
N protein-S protein	0.967	<0.001	0.974	<0.001	98.20	87.70	94.00	0.872	<0.001
N protein-ORF1ab	0.97	<0.001	0.974	<0.001	96.90	91.40	94.70	0.889	<0.001
S protein-ORF1ab	0.978	<0.001	0.984	<0.001	95.20	98.20	96.40	0.923	<0.001
Roche									
E protein orf1ab	0.995	<0.001	0.997	<0.001	100.00	84.90	94.80	0.88	<0.001
Xpert									
Xpert E-Xpert N	0.573	<0.001	0.721	<0.001	100.00	66.70	83.00	0.662	<0.001

Comments emphasized the need for repeat testing in an outbreak or post-contact and the need for clinical assessment of patients, irrespective of the Ct values.

Retesting of a subset of COVID-19 patients after reporting of a non-positive RT-PCR result

In a follow-up subset of patients initially reported with a non-positive result (reactive, inconclusive or negative), a positive (Ct ≤30) result was reported within 10 days in 5.2, 1.9 and 7.1% of those with reactive, negative and inconclusive results respectively (Table 3). High viral loads defined by a Ct <20 were reported in 0.2, 0.8 and 1.6% of cases respectively.

DISCUSSION

Thresholds for accepting Ct values as 'positive' used manufacturer recommndations that take account of stochastic and signal variation at low copy number in defining respective cut-offs. Selection of the IT used local and international definitions of infectiousness. The widely accepted 10 day period for removing transmission-based precautions was increased to 14 days to reflect local practice. While incubation periods vary, the kinetics of increasing load from the first positive RT-PCR were assumed to be

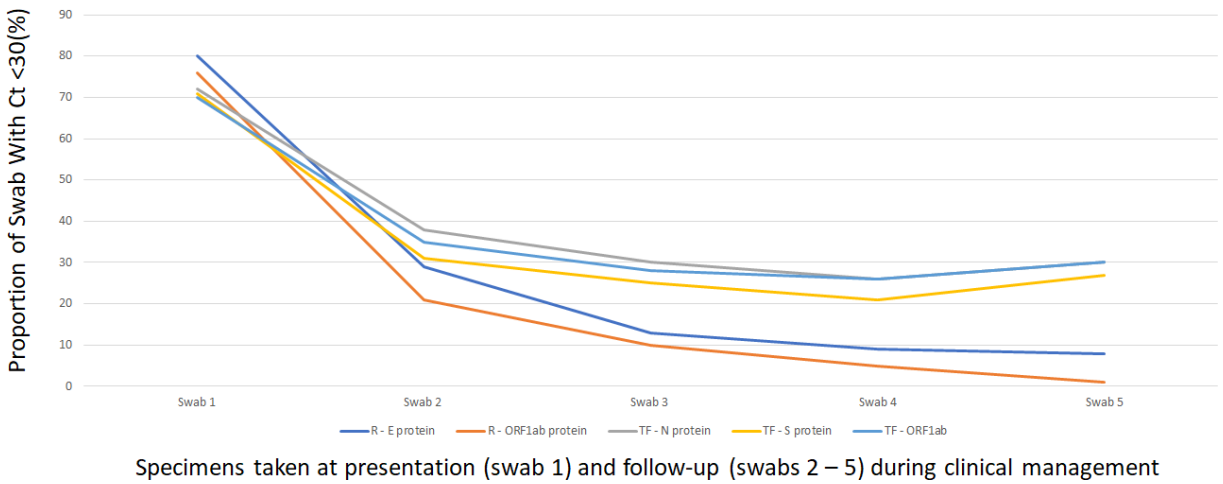

Fig. 1. Proportion of swabs with cycle threshold <Ct30 in sequential swabs taken from patients with confirmed COVID-19. Gene targets in the Thermo Fisher (N, S, orf1a/b) and Roche (E, orf1b/a) platforms with proportions <Ct30 in sequential swabs. Swabs 1–5 reflected the cumulative number of swabs taken from the first to the last where repeat testing was undertaken; not all patients had five swabs taken. Gene identifier: N=nucleoprotein; S=spike; E=envelope; orf1a/b. Company Identifier: R=Roche; TF=Thermo Fisher.

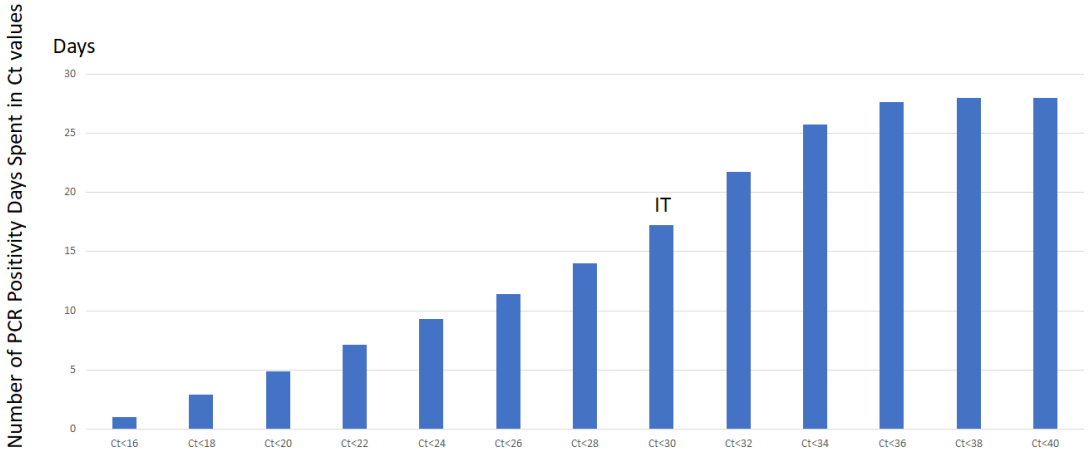

Fig. 2. RT-PCR positivity days by Ct value. Increasing cycle threshold values are seen during recovery in patients with COVID-19 with the indicative threshold (IT) of Ct30 reached after approximately 17 days.

uniform and this was observed over the first 14 days to Ct28 (Fig. 2). The IT was not designed as an absolute cut-off but a pivot point around which the stage of infection and likelihood of transmission could be inferred. While poor sample quality will impact Ct values, this is more likely at low viral loads in the post-infectious stage than at presentation. Interpretive comments allowed for this and encouraged follow-up testing where necessary; Table 3 confirms this need.

Including average Ct values in routine reporting was deemed possible based on the good correlation of Ct values across the respective test platforms. Physicians and public health teams were informed of the change in advance through an alert, which is common practice in the health sector in Qatar. The change was felt necessary because a 'positive' result can reflect viral RNA loads over a wide Log_{10} range, making categorical reporting of positive or negative open to misinterpretation. In the early stage of the first wave, all results were reported positive with an assumed equal risk of transmission and all patients were managed by a single protocol. Reporting Ct values with an IT and interpretive comments increased the scope for interpreting the result and allowed a more tailored approach to patient management, which is a key requirement of clinical guidelines.

Withholding Ct values stems from perceived lack of platform to platform concordance or specific outcomes linked to Ct values [21]. The approach described here does not define clinical thresholds or assign Ct values to outcomes. The IT categorized results around a Ct value based on the viral kinetics of clearance. This along with the interpretive comments allowed inference of the phase of infection and further risk of transmission. Since most RT-PCR platforms provide similar amplification protocols around 40 cycles, as demonstrated in Figs 1 and 2, the use of an IT is a pragmatic way to allow reporting across platforms. Correlation of new with existing platforms is now applied in our laboratory and confirms the expected concordance.

Table 2. Interpretive comments for enhanced reporting

Interpretive comments were grouped by reporting categories and appended to every report at result release.

Interpretive comments for NPS/OPS* swab results			
Positive (Ct≤30)†	Reactive (Ct>30)	Negative	Inconclusive
SARS-CoV-2 RNA detected – regard as potentially infectious up to 10 days from symptom onset or first PCR-positive; prolonged shedding seen in severe cases	In asymptomatic patients usually seen in recovery phase, which can be prolonged	SARS-CoV-2 virus RNA not detected – excludes COVID-19	In asymptomatic patients usually seen in recovery phase, which can be prolonged. Repeat if detected in pre-procedure screening
Low and high Ct values indicate high and low viral loads respectively	Repeat where clinical suspicion remains/in an outbreak/after contact with a case	Repeat where clinical suspicion remains/in an outbreak/after contact with a case	Repeat where clinical suspicion remains/in an outbreak/after contact with a case
Clinical assessment essential regardless of Ct value	Clinical assessment essential regardless of Ct value	Clinical assessment essential regardless of Ct value	Clinical assessment essential regardless of Ct value

*NPS/OPS, nasopharyngeal/oropharyngeal.
†Ct, cycle threshold.

Table 3. Follow-up of patients initially reported reactive, inconclusive or negative

Follow-up samples within 10 days from a sample reported as non-positive (reactive, inconclusive or negative) producing a positive result in keeping with the first non-positive sample being a probable early infection at the time of the initial presentation, often seen during an outbreak.

Category	Ct*<20	Ct <30
Reactive†	26/11291 (0.2%)	591/11291 (5.2%)
Negative‡	2515/328095 (0.8%)	6429/328095 (1.9%)
Inconclusive§	460/29104 (1.6%)	2066/29104 (7.1%)

*Ct, cycle threshold.
†Reactive: Ct >30.
‡Negative: all gene targets with no detectible values.
§Inconclusive: high Ct gene targets fail to meet the definition of a positive result as defined in the manufacturer's kit insert.

This approach could be applied to other respiratory infections. Curran reported Ct values for *Bordetella pertussis* where low Ct values were seen in unvaccinated children with whooping cough compared to those who had received the vaccine (mean Ct23 versus mean Ct33; $P>0.001$); interpretive comments were not reported [15]. These low CT *Bordetella* infections in pre-vaccinated children give rise to potentially life-threatening infections, which are associated with a significant transmission risk to unvaccinated staff and family.

While reactive and inconclusive reports most often are in keeping with a previous SARS-CoV-2 infection, in a small number of cases these and negative results are seen in acute early infection (Table 3). Reporting Ct values in these situations with interpretive comments provided clinicians with additional information concerning follow-up samples.

The introduction of Ct reporting saw a fall in enquiries to the specialist COVID-19 advisory call centre that had been established in Qatar for COVID-19 (J. Mitchel, pers. comm.). It also led to a reduction in enquiries from front-line clinical staff to virology (our pers. obs.). In addition, a microbiology advisory group providing interpretation advice to clinical teams was stepped down.

For patient admission and discharge policy the new reporting provided an objective categorization for assisting patient flow that was used for admission, interfacility transfer, and discharge from COVID and non-COVID designated hospitals respectively. This increased flexibility and improved efficiency eased the pressure on an at-times stretched service.

Use of Ct values also helped triage arrangements for quarantine. WHO guidelines for two negative RT-PCR results 24 h apart often extended the period of quarantine for confirmed infection beyond 14 days because of prolonged shedding and a reluctance to discharge patients with a categorically positive RT-PCR result [2]. Reporting Ct values with interpretive comments linked to the CDC time-based discontinuation of transmission precautions allowed a reduction of the quarantine period. While CDC guidelines advocated 10 days from symptoms or first positive PCR [16], the IT was aligned to a more precautionary 14 days. This provided a better metric of recovery and the number of recovered patients increased. The period of quarantine for patients with a reactive result was reduced from 14 to 7 days and allowed home self-isolation. This reduced the numbers in hotel quarantine or hospital isolation and improved patient well-being by avoiding or reducing hotel quarantine.

The policy also supported the country's track and trace application EHTERAZ (meaning PRECAUTION), which is installed on mobile phones used by individuals when leaving their homes. EHTERAZ defines four categories of health status by colour: red, RT-PCR positive – quarantine; yellow, RT-PCR reactive – quarantine or home-isolation; grey, suspected or exposed awaiting RT-PCR confirmation; green, no current restrictions. A gold halo was added to the green category for those fully vaccinated. The new reactive category allowed release of people from quarantine to home isolation with a change of status from red to yellow, a practice that was supported by a report from the Korean Centers for Disease Control that confirmed patients with ongoing raised Ct RT-PCR were not a transmission risk [22].

This approach has drawbacks. To properly address concordance between multiple platforms would need head to head comparisons with shared samples, which this study did not attempt but has been confirmed subsequently; this is now incorporated into our routine validation protocols. In addition, the premise that infectivity can be inferred safely from a Ct value may not be valid. A well-designed campus study in New Orleans, Louisiana, USA, found no difference in Ct-values between index cases who did or did not transmit, with five transmissions linked to index cases with Ct >30 [23]. It concluded that Ct values should be used for populations but not individuals. In another campus study from Boulder, Colorado, USA, the findings suggested 50% of PCR positive individuals were in the non-infectious phase, with only 2% carrying 90% of the virions, serving as viral 'supercarriers' and possibly superspreaders [24]; unpublished data during the second wave of the pandemic in Qatar supports this observation. A balance between the absolute categorical interpretation of a PCR positive result to confirm transmission risk, against a graded interpretation as reported here, needs to be addressed and increasingly is being resolved by the use of rapid antigen tests [25].

CONCLUSION

The use of Ct values and interpretive comments in routine reporting improved understanding of SARS-CoV-2 results and impacted on patient and public health management criteria. The current accepted practice of withholding Ct values should be reviewed by the profession, accreditation bodies and regulators.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study was approved by the Hamad Medical Corporation Institutional Review Board with waiver of informed consent.

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