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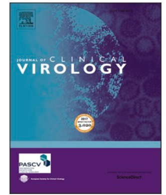
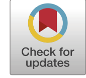


Short communication

Detection of SARS-COV N2 Gene: Very low amounts of viral RNA or false positive?



Francesca Falasca [b,1], Ilaria Sciandra [b,1], Daniele Di Carlo [a], Massimo Gentile [a,b], Alberto Deales [b], Guido Antonelli [a,b], Ombretta Turriziani [a,b,*]

[a] *Department of Molecular Medicine, Sapienza University, Rome, Italy*
[b] *Sapienza University Hospital "Policlinico Umberto I", Rome, Italy*

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ABSTRACT

Background: The detection of a low amount of viral RNA is crucial to identify a SARS-CoV-2 positive individual harboring a low level of virus, especially during the convalescent period. However, the detection of one gene at high Cycle threshold (Ct) has to be interpreted with caution.
In this study we address this specific issue and report our real-life experience.
Study design: A total of 1639 nasopharyngeal swabs (NPS) were analyzed with Xpert® Xpress SARS-CoV-2. Positive samples showing high Ct values (Ct>35) were concentrated by centrifugation and re-tested with Cepheid or other methods (RealStar SARS-CoV2 RT-PCR, Altona Diagnostics; GeneFinder COVID-19 Plus Real-Amp Kit, Elitech).
Results: 1599 (97.5%) negative samples, 36 (2.3%) positive samples and 4 (0.2%) presumptive positive samples were detected. In 17 out of 36 positive patients, very low viral RNA copies were suspected since positivity was detected at high Ct. We confirmed positivity for patients who showed both E and N genes detected and for patients with only N detected but with Ct <39. On the contrary, samples with only gene N detected with Ct values >39 were found negative. NPS taken 24 hours after the first collection confirmed the negativity of the 12 samples. Clinical data sustained these results since only 2 of these 12 patients showed COVID-19-like symptoms.
Conclusions: These data support our consideration that detection of the N2 gene at high Ct needs to be interpreted with caution, suggesting that collaboration between virologists and clinicians is important for better understanding of results.

1. Introduction

The timely diagnosis of COVID-19 cases and subsequent infection control are essential to prevent transmission in healthcare facilities and the community. Rapid SARS-CoV-2 testing, and particularly molecular assays, can have a considerable impact on the ability to make immediate decisions regarding management of the infected patient, including his isolation or the assessment of risk of transmission to healthcare workers performing invasive procedures on critically ill patients.

So far, nucleic acid amplification testing is still the gold standard for the diagnosis of SARS-CoV-2 in respiratory samples [1,2]. Despite the good performance of validated nucleic acid amplification assays, a risk of false-negative results still exists. The negativity of the assay may be due to inappropriate sample collection as well as to extraction/Real Time-PCR workflow and to sensitivity of the assays used. Concerning the latter, it is worth noting that the ability of molecular assays to detect SARS-CoV-2 infection can be limited by low amounts of viral RNA (e.g., early or late in COVID-19 disease).

Xpert® Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) is a rapid molecular diagnostic test utilizing real time RT-PCR technology to detect the nucleocapsid gene (N2 region of the N-gene) and envelope gene (E) in respiratory specimens. The limit of detection (LoD) was reported at 100 copies/ml. Studies have assessed the N2 target's high specificity and sensitivity for SARS-CoV-2 detection, and cases testing positive with only the N2 region have often been observed in samples containing very low viral RNA copies [3]. The detection of a low amount of viral RNA is crucial to identify a positive individual harboring a low level of virus, especially during the convalescent period. However, the

* Corresponding author at: Department of Molecular Medicine, Sapienza University, Viale dell'Università 33, 00185 Rome, Italy.
E-mail address: ombretta.turriziani@uniroma1.it (O. Turriziani).
[1] Equal contribution.

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detection of one gene at high Cycle threshold (Ct) introduces problems of interpretation, and the results should be handled with caution.

In this study we address this specific issue and report our real-life experience on the above assay

2. Methods

A total of 1639 nasopharyngeal swabs (NPS) were analyzed with Xpert® Xpress SARS-CoV-2 (Cepheid). The Xpert Xpress SARS-CoV-2 test provides positive results when a signal for the N2 region or signals for both nucleic acid targets (N2 and E) have a Ct within the valid range (<45 Ct) and endpoint above the minimum setting. A presumptive positive result is given when the SARS-CoV-2 signal for only the E nucleic acid target has been detected. Positive samples showing high Ct values (Ct>35) were concentrated by centrifugation (2 hours at 14000 rpm at 4 °C) and re-tested with Cepheid or other methods (RealStar SARS-CoV2 RT-PCR, Altona Diagnostics; GeneFinder COVID-19 Plus RealAmp Kit, Elitech).

3. Results

A total of 1639 NPS were analyzed with Xpert® Xpress SARS-CoV-2 from 01 April 2020 to 31 July 2020. One thousand five-hundred ninety-nine (97.5%) negative samples, 36 (2.3%) positive samples and 4 (0.2%) presumptive positive samples were detected. In 17 out of 36 positive patients, very low viral RNA copies were suspected since positivity was detected at high Ct. Specifically, in 14 patients only the N2 gene was detected with Ct between 38.0 and 43.4 (Table 1), while in 3 samples the E and N genome regions were both amplified with high Ct (Table 1: Pt 2, gene E 45 Ct and gene N 38.9; Pt 9 gene E 37.6 and gene N 42 Ct; Pt 16 gene E 41.7 and gene N 40.1 Ct). To confirm positivity, samples were re-tested with Cepheid and other methods (Altona or Elitech), after 10-fold virus concentration.

Surprisingly, we confirmed positivity for Pt 2, Pt 9 and Pt 16, which showed both E and N genes detected and for patients with only N detected but with Ct <39 (Pt 7 and Pt 8). On the contrary, samples with only gene N detected with Ct values >39 were found negative. NPS taken 24 hours after the first collection confirmed the negativity of the 12 samples. Notably, only 2 of these 12 patients were admitted to hospital with COVID-19-like symptoms; the rest were screened preoperatively or prior to admission to hospital wings, as recommended in order to identify possible asymptomatic infections.

Table 1
Interpretation of Ct values and results after nasopharyngeal swab concentration.

Samples	Ct Gene E	Ct Gene N	Interpretation	Result after virus concentration
Pt 1		42.1	positive	**negative**
Pt 2	45.0	38.9	positive	positive
Pt 3		42.1	positive	**negative**
Pt 4		42.3	positive	**negative**
Pt 5		42.2	positive	**negative**
Pt 6		41.9	positive	**negative**
Pt 7		38.3	positive	positive
Pt 8		38.0	positive	positive
Pt 9	37.6	42.0	positive	positive
Pt 10		42.2	positive	**negative**
Pt 11		41.6	positive	**negative**
Pt 12		42.9	positive	**negative**
Pt 13		41.7	positive	**negative**
Pt 14		42.7	positive	**negative**
Pt 15		43.4	positive	**negative**
Pt 16	41.7	40.1	positive	positive
Pt 17		42	positive	**negative**

4. Discussion

The qualitative real time PCR (qRT-PCR) assay is being widely used for detection of SARS-CoV-2 infection. However, the problem of qRT-PCR with inaccurate results is increasingly reported. Studies have shown that a significant number of SARS-CoV-2 false negative samples is inevitably due to low viral load in patients' throats and to the limited sensitivity of PCR technology. PCR is easily affected by sample inhibitors, poor amplification efficiency, and less precision in low-concentration samples [4].

False negative results may compromise the timely diagnosis, early treatment, prevention of transmission, and assessment of discharge criteria [5]. For all these reasons, several publications have focused their attention on false negative results [6,7]. However, false positive results have been observed, even if less frequently, due to contaminations of commercial primers/probe sets or poor test specificity [8,9].

Katz et al. reported false-positive reverse transcriptase polymerase chain reaction screening for SARS-CoV-2 in the setting of urgent head and neck surgery and otolaryngologic emergencies [10]. The implications for the patients were significant, including delay of urgent surgery and transfer to COVID-19-designated units.

A false positive result may have several effects: delay of correct diagnosis in patients with breathing difficulties or other symptoms; administration of inappropriate treatment; wasteful consumption of personal protective equipment; reduction in healthcare-workers leading to uncareful management of patients; unnecessary stress in isolated individuals including family members.

Finally, epidemiological analysis could be distorted due to false results.

In this analysis we found 17 SARS-CoV-2 positive samples with high Ct value. Surprisingly, when we re-tested the samples after viral concentration with Cepheid or other methods, only 5 samples were confirmed positive: 3 samples with both the E and N genes detected and 2 samples with only the N gene detected with Ct<39. In this context, a critical analysis and a careful evaluation of the test results is fundamental to minimize the false positive rate in order to achieve better management of patients. Based on our experience, we suggest suspecting false positive results when only the N gene is detected with Ct values >39. For all 12 patients, NPS collected 24 hours after the first negative NPS were still negative, supporting the idea that the low positivity detected in the first samples was not due to a recent infection. Alternatively, the results may be due to a late stage of infection.

In this situation, serology testing could aid in discriminating true positivity, especially in a final phase of infection. IgM and IgG antibodies to SARS-CoV-2 were tested in only two individuals and no antibodies were found in either. However, since these individuals were asymptomatic and their anamnesis did not report any previous contact with SARS-CoV-2 positive patients, the detection of only N2 gene at very high Ct (>39) suggests a false positive result.

These data support our consideration that detection of the N2 gene at high Ct needs to be interpreted with caution and highlight the importance of virologist-clinician collaboration for better understanding of results.

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Declaration of Competing Interest

The authors report no declarations of interest.

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FF and IS design the study, interpreted data and drafted the article. DDC and MG acquired the data; GA, AD and OT revised and approved the final version.

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