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Journal of Infection and Chemotherapy 27 (2021) 1676–1677

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: www.elsevier.com/locate/jic

Letter to Editor

Does the E gene provide additional information in SARS-CoV-2 PCR?☆

ARTICLE INFO

Keywords
SARS-CoV 2
PCR
False positivity

To the Editor,

The concerns regarding the indication and interpretation of SARS-CoV-2 PCR tests raised by Otsuka et al. [1] seem to be truly significant. However, we had to notice that they initially used tests targeting the N gene, which is known to be fairly specific. In contrast, numerous other tests are based on a wider spectrum of target sequences, which might be a reason why the sources of diagnostic uncertainty might spread far beyond possible human or instrumental errors. Among the possible PCR targets the E gene of SARS-CoV 2 is considered to be the least specific and it shows significant sequence homology to other common coronaviruses (according to the LALIGN alignment software [2] the Sarbecovirus SARS-CoV, the Beta CoV 229E and OC43, Alpha CoV HKU1 and NL63: 93.5%, 50.2% and 58.3%, 54.2% and 53.4% respectively). Furthermore recently two mutations of the E gene were reported to compromise its detectability with certain commercially available diagnostic PCR tests [3,4]. In our laboratory we chiefly use the Seegene Allplex™ SARS-CoV-2 Assay (Seegene Inc., Seoul, Republic of Korea), which detects the E, N and RdRp (RNA-dependent RNA polymerase) and S genes. The software provided by the manufacturer (SARS-CoV-2 Viewer for Real time Instruments V3) suggests the interpretation „Presumptive positive" for a lone E gene positivity. After acquiring numerous lone E gene positive results, we decided to characterize their context.

With the consent of the Institutional Research Ethics Committee we performed a retrospective analysis involving all the diagnostic PCR results of airway samples between 1st January and 1st May 2021 fulfilling the following criteria: samples taken in our institution; all measurements done using the same setup (nucleic acid isolation on Seegene Nimbus or Starlet systems using STARMAG 96 × 4 Viral DNA/RNA 200C Kit, amplification with Allplex SARS-CoV 2 Assay on BioRad C1000 Thermal Cycler with CFX96 Real-Time System, Bio-Rad Laboratories Inc., Hercules, CA, USA); at least one sample with a lone E gene positivity; a minimum of three PCR results in total from the same patient. All materials were used according to the manufacturers recommendations. Kyplot 6.0 (KyensLab Inc., Tokyo, Japan) was used for statistics.

The number of presumptive positive results was 205, which accounted for 0.92% of the total 22186 samples measured. These were interpreted as „Indeterminate, repeated sampling needed". Only 24 patients met all inclusion criteria. Almost 80% of the lone E gene positive results of these patients occurred without any further positivity: in 37.5% as a final sign of the previous apparent infection, and in 41.7% without any positive results ever, in asymptomatic individuals. The target cycle threshold (Ct) values for the E gene were without exception above 37. Table 1 summarizes the results in detail.

La Scola et al. demonstrated that the E gene Ct value might be a useful predictor of infectivity, as coronavirus could not be cultured from any of the patient samples with an E gene Ct above 34 [5]. A study by Singanayagam et al. using the RNA-dependent RNA polymerase (RdRp) gene as a target resulted in similar, but not identical results, as virus could be cultured from 5 of 60 samples with a target Ct > 35 [6]. The difference might originate from that the PCR detects any compatible transcript, including mRNAs. The dynamic changes in the proportions of these are coherent with the stage of the infection, as we also detected previously [7], and the presence of the RdRp transcript might rather indicate active replication. According to these results, the lone E gene positivities with high Ct values in our asymptomatic patients might be of questionable clinical relevance. Furthermore, a large-scale epidemiological study involving over 9 million Wuhan inhabitants concluded that new infections could not be attributed to asymptomatic (not including pre-symptomatic) or re-positive (PCR positivity after previous recovery) individuals [8]. The clinical samples of each of these patients also turned out to be culture negative, indicating the lack of „viable" virus in the secretions of these individuals [8]. Of note, in the Wuhan study the N gene and the ORF1ab region (which includes the RdRp gene) were used as targets. We could speculate that the lone E gene positive samples of the ten asymptomatic patients in our analysis would fall into the non-infectious category in both the study of La Scola et al. (according to the >37 Ct values) and in the Wuhan study (as asymptomatics). The similarly high Ct values (each >37) of the nine post-positive samples also make their infectivity doubtful. However, we should highlight that the detected cycle numbers depend on the quality of sampling, as

☆ **Comment on:** Otsuka Y, Hagiya H, Nakano Y, Omura D, Hasegawa K, Yamada H, et al. A patient with human coronavirus NL63 falsely diagnosed with COVID-19; Lesson learned for the importance of definitive diagnosis. J Infect Chemother Off J Jpn Soc Chemother 2021. https://doi.org/10.1016/j.jiac.2021.05.001.

https://doi.org/10.1016/j.jiac.2021.08.017
Received 28 May 2021; Received in revised form 22 July 2021; Accepted 12 August 2021
Available online 16 August 2021

Table 1
Characteristics of airway samples with a lone E gene PCR positivity.

	Patient samples	Symptomatic infection	Age at testing	Gender	E gene	Lowest Ct after indeterminate	
	N (%/total)	N (%/group)	(mean ± SD)	(F/M)	Mean Ct (total range)	RdRp/S	N
Preceding positivity	1 (4.16%)	0	80	0/1	38.02	∞	37.95
After positivity	9 (37.5%)	8 (88.9%)	71.33 ± 15.04	6/3	37.97 (37.54–38.21)	N/A	N/A
Among positive results	4 (16.67%)	4 (100%)	64.5 ± 22.29	2/2	38.45 (37.75–39.83)	35.74	34.22
No known positivity	10 (41.67%)	0	54.4 ± 21.93	6/4	37.84 (37.47–39.26)	N/A	N/A

suggested by the over 15% of indeterminate results occurring in between clearly positive ones. These indicate that Ct values might provide valuable information for epidemiological or research purposes, but individual clinical decisions based on them could be hazardous.

Our results are consistent with the previous experimental and epidemiological studies, and might suggest that the detection of the E gene might not offer clinically relevant additional information if the test kit is capable of detecting multiple other target sequences. Furthermore, the „over-detection" of a near-faded viral presence, or probably even false positivity of any origin might in certain cases lead to repeated testing, hence unnecessary excess costs and diagnostic hesitancy without true clinical benefit. Targeted studies might be needed to clarify which set of target genes might be the most suitable for diagnostic, screening, and epidemiological purposes.

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