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Letter to the Editor

The importance of retesting borderline results in COVID-19 diagnostics

Sir,

The first case of COVID-19 in the Republic of Serbia was registered on 6 March 2020 based on data from the Ministry of Health of the Republic of Serbia.[1]

RT-PCR (real-time reverse transcription polymerase chain reaction) is the gold standard for detection for many infective agents.[2] SARS-CoV-2 (human coronavirus 2019) comprises genes coding four basic structural proteins (E, M, N and S) and ORF1a and ORF1b genes coding two polyproteins. These genes represent targets for virus detection and the platform for RT-PCR test design.[3]

In response to the current COVID-19 pandemic have been developed many commercial tests to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). RT-PCR tests for SARS-CoV-2 are occasionally repeated when suspect false-positive or negative results according to the value of Ct.[4] False results have important implications for the management of COVID-19. Our attention is focused on additional verification of high values of Ct to confirm the obtained results.

It is known that magnetic extraction method used to isolate viral RNA provides an advantage over lysed samples, which we used in our study, in the sense that it provides a higher sensitivity. The 2019-nCoV test, following RNA isolation by lysis, in order to process a large number of samples, has lower sensitivity and an issue with questionable, borderline results, requiring a high level of skill in diagnostics. According to these facts we decided to pay special attention to borderline Ct values, to resolve borderline results. Retesting borderline values using the same, or a more sensitive method, helps delineate between positive and negative samples.[4]

From Jun 2021 to April 2022, 157998 samples were obtained for analysis for the presence of SARS-CoV-2 virus using the RT-PCR method. Samples were collected using nasopharyngeal swabs that were stored and transported in the media provided by the manufacturer (Sansure Biotech Inc., Changsha, China), under conditions prescribed by the manufacturer.[5]

To perform the 2019-nCoV tests, viral RNA was isolated from the samples using the reagent sample release reagent in an automatic extractor NATCH CS2 (Sansure biotech Inc.). Tests of two manufacturers were used to detect the SARS-CoV-2 virus: 2019-nCoV (Sansure Biotech Inc., Changsha, China) for testing and retesting, and xpert xpress SARS-CoV-2 test (Cepheid, Sunnyvale, CA, GeneXpert) only for retesting.[6] The results were considered positive when the CT value for one or both genes (N, RdRp) was less than or equal to 40, in the presence of internal control and regular sigmoid curve for 2019-nCoV test.[5] Based on manufacturer's instructions for the xpert xpress SARS-CoV-2 test is positive when the Ct values for N2 gene or for N2 and E genes are less or equal to 45.[6]

Of the 157998 samples analyzed by the RT-PCR method using 2019-nCoV tests, 22337 were unambiguously positive, while 614 borderlines positive ($40 \leq Ct \leq 42$ for N gene) samples were retested using xpert xpress SARS-CoV-2 tests, and same 2019-nCoV test, after using nucleic acid (DNA/RNA) extraction and purification kit (Sansure Biotech), to resolve any dilemmas arising from interpretation of the borderline values.

Out of the total of 614 samples processed using the reagent sample release reagent in which one of the target genes had been detected (N or ORF1ab), 299 borderline samples were retested with xpert xpress SARS-CoV-2 test, and 315 samples with 2019-nCoV test. Retested results were 187 positive samples from testing with xpert xpress SARS-CoV-2 test, and 153 were positive when we used 2019-nCoV test. After complete retesting 614 samples with borderline values, we obtained 340 positive results (55.37%).

As specified in manufacturers' protocols there are numerous factors affecting the outcome of RT-PCR testing.[5] Contamination is possible in some samples, as well as presence of many substances including numerous medications, which are not removed completely by this isolation method, and which could potentially act as inhibitors in the PCR reaction. Based on these results, we can conclude that special attention must be paid when interpreting borderline results of RT-PCR tests in situation of mass testing approaches at a time of a pandemic. It can be concluded that borderline results can be the result of low viral concentration, poor sampling or processing quality, and even possible contamination. The Ct value above the cut-off point cannot be a clear cut-off for positivity, when using fewer sensitive methods for RNA isolation, which excludes the possibility of the sample actually being positive. In the retest process, when there is a possibility for that, the use of tests of different characteristics is very important. This would benefit the healthcare community and potentially avoid risk of virus transmission in population without patient isolation, contact tracing, and outbreak declaration.

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