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EVALUATION OF THE ANALYTICAL VALUE OF SARS-CoV-2 ANTIGEN TEST IN RELATION TO Ct-VALUES OF RT-qPCR IN PATIENTS SUSPECTED OF COVID-19

ЕВАЛУАЦИЈА НА АНАЛИТИЧКАТА ВРЕДНОСТ НА SARS-CoV-2 АНТИГЕНСКИ ТЕСТ ВО ОДНОС НА Ct ВРЕДНОСТИТЕ НА РТ-ПВР КАЈ ПАЦИЕНТИ СУСПЕКТНИ ЗА КОВИД-19

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Abstract

Introduction. COVID-19 pandemic threatens global human health. Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) is a reference test for identification of acute SARS-CoV-2 infection, but it is associated with results delay. There is a need of fast and reliable tests which can improve the efforts of controlling the transmission of SARS-CoV-2.
Aim. The aim of this study was to determine the analytical value of the rapid SARS-CoV-2 Ag-test in relation to the Ct values of the RT-qPCR.
Methods. The study group comprised outpatients suspected for COVID-19, sampled twice, first for the routine RT-qPCR, and second for SARS-CoV-2 antigen testing. The results obtained by the rapid antigen test (Panbio™ COVID-19) were evaluated in relation to Ct values of the SARS-CoV-2 E-gene, obtained by RT-qPCR Allplex 19-nCoV multiplex assay platform.
Results. SARS-CoV-2 prevalence, based on RT-qPCR, was 50.8% (186/366). Specificity of the Panbio™ COVID-19 Ag Rapid Test was 100%. Test sensitivity was 73.8%. Restricting RT-qPCR to Ct-values<30 increased test sensitivity to 91.2%.
Conclusion. The findings underscored the epidemiological value of the rapid Ag-test since it reliably identifies contagious SARS-CoV-2 infected individuals who actively spread the virus in the community.

Keywords: COVID-19, RT-qPCR, rapid SARS-CoV-2 Ag test, sensitivity, specificity

Апстракт

Вовед. Здравјето на луѓето, на глобално ниво, е загрозено поради пандемијата со КОВИД-19. Референтен тест за идентификација на акутна инфекција со SARS-CoV-2 е РТ-ПВР, но ова тестирање е поврзано со доцнење на резултатите. Од тука произлегува потребата од брзи и сигурни тестови кои ќе помогнат во контрола на ширењето на SARS-CoV-2.
Цел. Да се одреди аналитичката вредност на SARS-CoV-2 антигенски тест преку споредба со Ct-вредностите добиени со РТ-ПВР.
Методи. Испитувана група беа амбулантски пациенти суспектни за КОВИД-19, од кои беа земени примероци, прво за рутинско РТ-ПВР тестирање и второ за SARS-CoV-2 антигенски тест. Резултатите добиени од брзиот антигенски тест (Panbio™ COVID-19) беа компарирани со Ct-вредностите на Е-генот добиени со мултиплекс РТ-ПВР (Allplex 19-nCoV assay).
Резултати. Преваленцата на SARS-CoV-2, заснована на РТ-ПВР, изнесуваше 50,8% (186/366). Специфичноста на брзиот Panbio™ COVID-19 Ag тест беше 100%. Сензитивноста на тестот изнесуваше 73,8%. При ограничување на Ct-вредностите на РТ-ПВР на <30 сензитивноста на тестот се зголеми на 91,2%.
Заклучок. Резултатите ја потенцираат епидемиолошката вредност на брзиот антигенски тест кој со сигурност ги детектира инфицираните лица со SARS-CoV-2 кои се заразни и активно го шират вирусот во заедницата.

Клучни зборови: КОВИД-19, РТ-ПВР, Брз SARS-CoV-2 Аг тест, сензитивност, специфичност

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel corona virus, emerged in December 2019 in Wuhan, China [1], and within a few months had spread worldwide. To date, 89.9 million have been infected with SARS-CoV-2, and 1.9 million have died from coronavirus disease 2019 (COVID-19) [2]. In this pandemic situation, early diagnosis of infectious patients is especially important for implementation of relevant epidemiological measures for discontinuation of the SARS-CoV-2 transmission chain. Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) is a reference test for identification of

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acute SARS-CoV-2 infection and it is routinely used in clinical practice [3]. Despite its high sensitivity and specificity, RT-qPCR test typically takes 4-5 h for results and requires specialized laboratory equipment and skilled technicians. Therefore, the need of inexpensive, reliable tests for detection of SARS-COV-2 was recognized by the WHO [4]. Lateral Flow Assay (LFA)-based point of care tests (POCT) for rapid antigen detection seems to be a good choice. They do not require special equipment or specially trained staff and generate results within 20 minutes [5]. Considering short turn around times, this testing system enables expanding of the testing and therefore detection of a larger number of contagious people. However, the diagnostic value of the rapid tests should be based on comparing the test results with the results obtained by the RT-qPCR as a gold standard. There are rapid SARS-CoV-2 Ag detecting tests with a different specificity and sensitivity [6-9].

Aim

The aim of this study was to determine the analytical value of the Panbio™ COVID-19 Ag rapid test in relation to the Ct values of the SARS-CoV-2 E-gene, obtained by RT-qPCR Allplex 19-nCoV multiplex assay platform, in outpatients suspected for COVID-19.

Material and methods

During the one-month period, from 1st to 30th of December 2020, a total of 366 outpatients visited the COVID-19 testing center, situated at the Institute of Respiratory Disease in Children, Skopje, RNM. Patients were referred by their general practitioners (GPs) due to high suspicion of COVID-19 aiming to be PCR tested to detect SARS-CoV-2 infection.
Eighty-four of them were sampled twice, first for the routine RT-qPCR testing, using a combined throat/nasopharyngeal swab, and second for SARS-CoV-2 antigen testing, using additional nasopharyngeal swab.

Diagnostic tests
RT-qPCR

PCR was conducted in a certificated clinical laboratory situated at the Institute of Respiratory Diseases in Children, Skopje, RNM. After collection, swabs were transferred into 2 ml PBS (Dulbeco's Phosphate Buffered Saline, Sigma, Life Science) and transported to the laboratory which is located within 2 min of walking distance from the sampling location. All specimens were processed in biosafety level-2 (BSL-2) facilities with full personal protective equipment. Nucleic acid extraction, RT-qPCR and results interpretation were performed according to the instructions of the manufacturer. Briefly, RNA was isolated and purified using the STARMag 96 ProPrep extraction kit (Seegene, South-Korea) on an automatic nucleic acid extractor SEEPREP 32 (Seegene South Korea). Amplification was performed in a single tube assay using the Allplex 19-nCoV multiplex platform which targets three SARS-CoV-2 genes [envelope gene (E) of *Sarbecovirus*, RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes which are specific of SARS-CoV-2], according to the manufacturer's instructions (Seegene, South Korea). Amplification and detection were performed on a CFX-96 real-time thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The conditions consisted of 1 cycle of 20 min. at 50 °C, 15 sec. at 95°C and followed by 45 cycles of 15 s at 94 °C, 30 s at 58 °C. The results were interpreted with Seegene Viewer data analysis software, in which the threshold Cycle (Ct) was automatically determined, and a positive result was defined as amplification of any of the three SARS-CoV-2 genes, within the cut-off values $\leq$40.

LFA (Lateral Flow Assay)

The Panbio™ COVID-19 Ag rapid test device by Abbott (Lake Country, IL, U.S.A) is a membrane-based immunochromatography assay which detects the nucleocapsid protein of SARS-CoV-2 in nasopharyngeal samples. Collected swabs were transferred into dedicated sample collection tubes containing a sampling buffer and transported to the same laboratory where the RT-qPCR was conducted. All samples were analyzed within a maximum of 30 minutes after collection, during which time the samples were kept at ambient temperature. Collected samples were subsequently processed in a level 2 biosafety cabinet. Test results were recorded after 15 min. of assay initiation by two independent observers (blinded to each other and to the PCR results). Intensities of the test bands were compared to the control bands and designated as "++" if the test and control bands intensity were similar or "+" if the test band intensity was weaker than the control band.

Results

During December 2020, a total of 366 outpatients were RT-qPCR tested because of high suspicion of Covid-19. According to the results interpreted by Seegene Viewer data analysis software (in which a positive result is defined as amplification of any of the three SARS-CoV-2 genes within the cut off $\leq$ 40) 50.8% (186/366) of all tested samples were recorded as positive. In 16.1% of all positive samples (30/186), test result indicated amplification of only one or two genes. In these cases (previously categorized as inconclusive results) the most often detected was N gene with mean Ct-value equal to 38.01 (35.49-39.5).
Of all double tested patients (n=84), 31 were tested positive by both test methods-RT-qPCR and rapid antigen (Ag) detecting test, with mean Ct-value of the E

gene 22.27 95% CI [20.52-24.02] (Figure 1). According to the intensity of the test band compared to the intensity of the control band, 20 of them were designnated as ''++'', and the corresponding mean Ct-value of the E gene was 19.6 95% CI [17.97-21.23]. Eleven had test band intensity weaker than the control band and were designated as ''+'' with corresponding mean Ct value of the E gene equal to 27.1 95% CI [24.84-29.37] (Figure 1).

Discrepancy between both test methods was observed in 11 cases.

Six cases tested positive by RT-qPCR with amplifycation of only one or two genes (N gene was detected in all six cases with mean Ct-value of 38.4, and in two cases, the E gene was detected along with the N gene with mean Ct-value of 35.4) were tested negative by the rapid antigen (Ag) test.

In addition, 5 cases tested positive with RT-qPCR by amplifying all 3 genes within the cut-off values, were also tested negative by the rapid Ag test. These cases had a corresponding mean Ct value of the E gene of 30.3 (29.17-32.30) (Figure 1).

All specimens tested negative by the rapid Ag test (n=42) were also tested negative by the RT-qPCR.

When the RT-qPCR was used as a reference, the antigen test diagnosed SARS-CoV-2 infection status with sensitivity of 73.8% (31/42), and specificity of 100% (42/42) (Table 1).

False negative Ag test results were observed in subjects with high RT-qPCR Ct-values (including inconclusive results), reflecting low viral levels in nasopharyngeal material. When defining RT-qPCR Ct positivity on a cut-off Ct-value of 30, Ag test sensitivity increased to 91.2% (31/34) (Table 1).

Table 1. Sensitivity and specificity of the antigen detection test in comparison with RT-qPCR

		Antigen test			
		Negative	**Positive**	**Sensitivity**	**Specificity**
RT- qPCR	Negative	42	0		100%
	Positive	11	31	73.8%	
Ct < 30	Negative	42	0		100%
	Positive	3	31	91.2%	

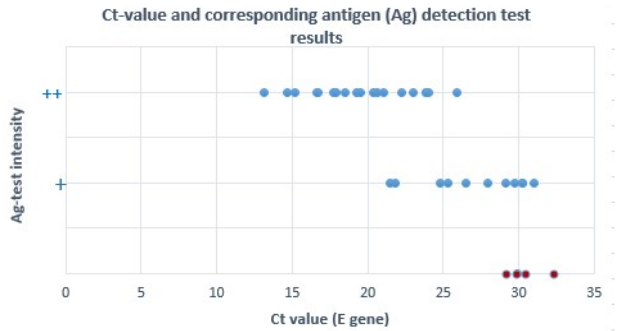

Fig. 1. Ct-value and corresponding antigen (Ag) detection test results

Cycle threshold (Ct) value of the E-gene and corresponding antigen (Ag) detection test results [blue circles positive (n=31), red circles negative (n=5) for each RT-qPCR positive sample with amplification of the all tree genes (n=36)].

Intensities of the test bands were compared to the control bands and designated as "++" if test and control bands intensity were similar or "+" if the test band intensity was weaker than the control band.

Discussion

In this study the Panbio™ COVID-19 Ag rapid test by Abbott (Lake Country, IL, U.S.A) was compared with the Allplex 19-nCoV multiplex platform RT-qPCR as a confirmatory test. Both different testing methods were performed in the same settings, and the samples for the two tests were collected at the same time, as it is recommended by WHO [4]. The tested population was outpatients highly suspected for COVID-19, and this could be the explanation for the high percentage of positivity (50.8%). The positive samples with no amplification of all three SARS-CoV-2 genes (inconclusive results) always corresponded to high Ct-values (the most often detected was N gene with mean Ct-value 38.01). In this context, Bhattacharya and colleagues [10] stated that the inconclusive results were probably due to different analytical sensitivity of individual viral gene PCR and were probably more subject to stochasticity which can result in positive results in only one or two targets especially at low viral load levels.

According to data of this study, Panbio™ COVID-19 Ag rapid test, has 100 specificity and overall, 73.8% sensitivity compared to Allplex 19-nCoV RT-qPCR. The manufacturer reported sensitivity of 93.3%, which is probably resulted from testing individuals with symptoms for less than seven days in high-endemic settings in Brazil [11]. In another study with cohort of 257 patients, the overall sensitivity was 73.3%, and 86.5% among individuals with symptoms for less than seven days [12]. Gremmeles and colleagues reported sensitivity of 72.6% and 81.0% in community-dwelling mildly symptomatic subjects in a medium- and high-endemic area [13].

In this study, the rapid Ag detecting test reliably identified SARS-CoV-2 infected individuals with Ct-values lower than 30 cycle by RT-qPCR. The overall positive samples by Ag detecting test had a mean Ct value of the E gene equal to 22.27. The intensity of the test

bands correlated with the Ct values of the RT-qPCR. Those with test band intensity similar to the control band had a corresponding mean Ct-value of the E gene equal to 19.6, and those with test band intensity weaker than the control band had a corresponding mean Ct value of the E-gene equal to 27.1 (95% Confidence Interval, CI: 24.8-29.4). Hence, this study demonstrates that the Panbio™ COVID-19 Ag rapid test has limit of detection of viral antigen near to the viral load which corresponds to 30 Ct value of the E gene detected by Allplex 19nCoV RT-qPCR. On the other hand, there are studies which undoubtedly revealed that high viral RNA load was independently associated with shedding of infectious virus [14,15]. Using cycle threshold (Ct) values as a quantitative measure for viral RNA load, Bulland and colleagues [16] reported that infectious virus could not be isolated from diagnostic samples when Ct values were above 24. These reports point out that from an epidemiological point of view most important is to detect persons with SARS-CoV-2 RNA load associated with spreading of infectious viruses. Furthermore, they recommend the use of quantitative viral RNA load assays as a part of test-based strategies for infection prevention and control measurements.

False negative Ag test results were observed in subjects with high RT-qPCR Ct-values (including inconclusive results), reflecting low viral levels in nasopharyngeal material. Intending to single out clinically significant cases, as well as in accordance with the results from previously mentioned viral culture studies [14-16], the lowering of Ct cut-off to 30 cycles increased the sensitivity of the rapid antigen test to 91.2%. Hence, the results suggest that Panbio™ COVID-19 Ag rapid test can detect SARS-CoV-2 infected individuals who are infectious and can potentially transmit the virus.

Conclusion

The results underscore the epidemiological value of the Panbio™ COVID-19 Ag rapid test. Positive samples indicate persons who are highly contagious, and this should be taken into consideration when implementing strategies aiming to prevent the spread of the virus in the community. Despite the lower sensitivity comparing to RT-qPCR, these quick and inexpensive tests should be especially helpful for low income countries where the availability and cost of RT-qPCR tests are limiting factors.

Conflict of interest statement. None declared.

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