

THE DETECTION OF SARS-COV-2 IN NASOPHARYNGEAL SAMPLES USING AN ANTIGENIC TEST

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ABSTRACT

Objectives: SARS-CoV-2 virus detection on nasopharyngeal specimens to infected individuals was a challenge for the COVID-19 pandemic outbreak. The aim of the study was to compare the performance of antigenic detection of SARS-CoV-2 in nasopharyngeal samples using an immunochromatographic method with molecular detection via qRT-PCR. **Materials and Methods:** 47 nasopharyngeal exudates were collected from suspected COVID-19 cases. The samples were performed both with the qualitative immuno-chromatographic method for S protein detection in the SARS-CoV-2 structure, fluorescent labelled anti-protein S antibodies and by qRT-PCR test for the qualitative detection of the screening gene E and the specific ORF1ab region of the RNA-SARS-CoV-2. **Results:** There was a good correlation between the positive antigen tests and the positive PCR assays measured through threshold cycle ORF1ab region (Ct orf). A better correlation was obtained between the antigen test results and the positive qRT-PCR tests when including patients with Ct orf below 25. **Conclusions:** Antigen testing is helpful for symptomatic people, especially during infectious period. A positive test has a high predictive value for infection, while a negative antigen test result via immuno-chromatography must be confirmed by a qRT-PCR test.

Key words: SARS-CoV-2; immunochromatography; qRT-PCR; nasopharyngeal exudates

INTRODUCTION

Since reporting the first human infection case caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, humanity has experienced a new episode of alert. Researched data suggested that the viral spread and the disease severity are different from what was known for SARS-CoV [1]. These aspects are important for differentiating the different types of coronavirus by the diagnostic tests used. Thus, the specificity of diagnostic tests becomes as important as their sensitivity [2].

Among the diagnostic tests, the most

reliable method is the viral gene detection via qRT-PCR [3]. The "gold standard" for diagnostic detection of SARS-CoV-2 is qRT-PCR technique which identifies nucleotide sequences of SARS-CoV-2 RNA present in the respiratory samples. Results are provided within 12 - 24 hours and up to 48 hours in case of numerous requests and require reagents and laboratories with special equipment as well as highly qualified personnel [4]. To reduce the time from symptom onset to diagnosis and general costs, several antigen detection laboratory techniques have been introduced [5].

Therefore, rapid antigen tests were developed, being easy-to-use, offering rapid results at low costs [6,7]. When used in samples from early stages of illness or with high viral loads, FIAs are highly selective and can reach astonishingly high sensitivities. The results of a new FIA automated antigen detection techniques in samples from Covid-19 patients are shown here. The study aims at comparing the performance of antigenic detection of SARS-CoV-2 in nasopharyngeal samples using an FIA method to molecular detection by qRT-PCR assay.

MATERIAL AND METHODS

The antigen test and SARS-CoV-2 qRT-PCR were used to test all nasopharyngeal samples used in this prospective study, obtained from suspect COVID-19 cases. In order to evaluate antigenic test utility, we compared and analysed results of the antigenic test and of the qRT-PCR, based on positive and negative values and Ct orf value, respectively.

Ct orf means the threshold cycle for orf1ab, ie the point at which the fluorescent signal generated by the accumulation of amplicons resulting from the amplification of the gene sequence corresponding to the target region orf1ab (Open Reading Frame) rises above the background level and becomes detectable (Ct - threshold cycle). The orf1ab target region was used to specifically discriminate SARS-CoV-2.

Current studies suggest that Ct orf value bellow 25 means a high SARS-CoV-2 load which is a hallmark for active phase of infection [8]. The antigenic test was done using immunochromatographic test by an immunofluorescence technique. The gold standard for detecting SARS-CoV-2 is the molecular detection via qRT-PCR assay.

The study was conducted in the Netconsult Medical Center, Iasi City, Romania, between April and November 2020 on 51 nasopharyngeal exudates collected from suspicious COVID-19 cases. Of the 51 samples collected, a number of four samples were excluded from the statistical processing in which we did not obtain the amplification of the internal control during two successive RT-PCR tests on different RNA extracts. We explained this situation by the presence in these four samples of inhibitors of the PCR reaction.

Written informed consent and free choice were complied with, for both RT-PCR and antigen test in the same sample, as well as for this comparative study data collection. Patient's privacy and confidentiality were ensured, complete and understandable information about the tests were provided. The subjects were encouraged to ask questions and answers were provided during the consent process. Patients were not charged. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Scientific Council/Ethics Board of the Netconsult SRL, Iasi, Romania (No 2/23 March 2020).

All patients had symptoms suggesting the SARS-CoV-2 infection and were tested within 24 hours after onset. According to staging of the COVID-19 all study patients were in stage 2 [9]. After collection, the exudate buffers were immersed in 3 mL virus transport medium (VTM) and refrigerated at 4°C until processing. The equipment used in qRT-PCR testing was Montania® 4896 Real-Time PCR Instrument (Anatolia Geneworks, Turkey). For viral RNA extraction, we used QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) in a volume of 200 mL per sample

(nasopharyngeal exudate discharged into VTM). We performed qRT-PCR testing with Bosphore® Novel Coronavirus (2019-nCoV) Detection Kit v2 (Anatolia Geneworks®, Turkey) for qualitative detection of the screening gene E and the specific ORF1ab region (Open Reading Frame gene region) of the RNA-SARS-CoV-2 structure using Taq DNA Polymerase in a reaction volume of 25 mL and with a 35-cycle amplification protocol [10]. The method has a detection limit of 4.1 copies/rxn for E genes and 12.9 copies/rxn for the ORF1ab region.

Samples that displayed an exponential growth curve and a cycle threshold value (Ct) <35 were considered positive, while no Ct value indicated a negative result (non-numeric value). For each sample with positive result in the qRT-PCR test, the Ct value was marked down. In addition, the antigenic detection was performed via a qualitative immuno-chromatographic method using Fluorecare® SARS-CoV-2 Spike Protein Test Kit [11]. The test detected S protein in the SARS-CoV-2 structure using dye-labelled/fluorescent-labelled anti-protein S antibodies. Optical intensity emitted by the protein S complex - fluorescent-labelled anti-protein S antibodies from the test line was compared to the control line and expressed in S / CO units. Following manufacturer's instructions, we used a 100 mL volume of sample for antigenic testing (nasopharyngeal exudate discharged into VTM) to which was added a 100 µL sample treatment solution. The test provides results within 15 minutes and was considered positive if the number of S/CO units 1.0 and negative result if the number of S/CO units < 1.0, respectively. The result values were also charted. In processing the data for statistical purposes,

the antigenic test results were compared to the threshold cycle ORF1ab region (Ct ORF) obtained via qRT-PCR. Ct orf was used as main benchmark with antigenic test results, being specific for SARS-CoV-2 virus. The more than two-thirds of the genome comprises ORF1ab encoding orf1abpolyproteins, while the remaining third consists of other structural proteins including gene E (envelope). The gene E is present in all Sarbecovirus, including SARS-CoV-2. On this regard, in order to evaluate antigen test specificity, we also included Ct E values. According to the instructions of the Bosphore® Novel Coronavirus (2019-nCoV) Detection Kit regarding the interpretation of the results: a) in case of a sample in which Ct orf1ab value is obtained without Ct E genes, extraction and amplification are repeated and, if the repeated result remains the same, the sample is positive for SARS-CoV-2; b) for the test in which Ct is obtained only for the E gene, extraction is repeated, and if the new result remains the same, it will be confirmed by additional tests to differentiate Sarbecoviruses and SARS CoV-2. None of the two situations appeared in the tested group.

Statistical analysis included sensitivity, specificity, positive and negative predictive values, the accuracy of the CoV-2 antigen rapid detection test versus qRT-PCR. The MedCalc version 14.8.1 software was used for statistics. The descriptive characteristics of the groups' variables were expressed as mean values and standard deviations (SD). T-test corrected for equal variances was applied in order to assess the significant differences between the study groups.

Spearman's correlation coefficient among study variables was calculated. For each sample tested, we evaluated the relationship

between the Ct value determined via qRT-PCR and the number of S/CO units obtained via the antigen test.

RESULTS

Out of 47 analysed samples, 40 were positive via qRT-PCR testing for SARS-CoV-2 RNA (85%). The median Ct value of qRT-PCR-positive samples was 21.75 (mean 22.8±6.1) for the ORF1ab region and 21.23 (mean 22.0±6.1) for E gene. In table I are figured the main features of the study group divided through qRT-PCR results.

TABLE I: The main characteristics of the study groups; data are given as mean SD or counts

Data groups	qRT-PCR-pos	RT-PCR-neg	P
No subjects	40	7	-
Age (y)	36.7 ± 19.1	42.6 ± 24.2	0.47
M/F ratio	1	1.3	-
Ct orf	22.8 ± 6.1	-	-
Ct E	22.0 ± 6.2	-	-
Ag level (S/CO)	2.2 ± 2.16	0.2 ± 0.04	0.02*
Ag pos	23	-	-
Ag neg	17	7	-

Significance level: P < 0.05. qRT-PCR: Real Time Reverse Transcription Polymerase Chain Reaction, No: number, y: years, M: male, F: female, Ct orf: Threshold cycle ORF1ab region, Ct E: Threshold cycle E gene, Ag: antigen, pos: positive, neg: negative

TDA had an overall sensitivity of 57.5% (95%CI; 40.89% to 72.96%), a specificity

of 100.0% (95%CI; 59.04% to 100.00%) and a positive predictive value of 100.0% (95%CI; 85.18% to 100.0%). According to WHO data, antigenic tests have a superior utility in high viral load cases (Ct ≤ 25 or > 10⁶ genomic virus copies/mL) (12). In this study, by reporting the results obtained according to the number of amplification cycles (cut-off 25), a statistically significant correlation was obtained regarding the average antigen value in patients with Ct orf ≤ 25 versus Ct orf >25 (3.1 versus 0.24, P = 0.0004) (table II).

TABLE II: The patient's Ct orf characteristics in the study groups; data are given as mean SD or counts

Data groups	Ct orf < 25	Ct orf 25.01-34.99	P
No subjects	27	13	-
Age (y)	33.4 ± 16.6	43.5 ± 22.6	0.12
Ct orf	19.3 ± 3.6	30.3 ± 2.5	<0.0001
Ag level (S/CO)	3.1±2.7	0.2±0.07	0.0004

Significance level: P < 0.05. Ct orf: Threshold cycle ORF1ab region, Ct E: Threshold cycle E gene, No: number, y: years, Ag: antigen

For the 23 concordant positive samples, the median Ct value was 19.0 (mean 19.2±2.4) for the ORF1ab region and a median of 18.5 (mean 18.4±2.8) for E gene. On samples with discordant results, the median Ct value was 29.5 (mean 27.8±6.2) for the ORF1ab region and the median 28.5 (mean 26.9±6.2) for E gene. There is a significant difference between the mean in

concordant and discordant groups ($P < 0.0001$). Using diagnostic test 2 x 2 table for TDA versus qRT-PCR results, for antigenic test we obtained a sensitivity of 85.2% (95%CI; 66.3% to 95.8%), a specificity of 100.0% (95%CI; 75.3% to 100.00%), a positive predictive value of 100.0% (95%CI; 85.2% to 100,0%) and a negative predictive value of 76.5% (95%CI; 50.1% to 93.2%), table III.

TABLE III: Antigen test results

Ag results	Ct orf < 25	Ct orf 25.01-34.99
Ag pos	23	0
Ag neg	4	13

Ag: antigen, Ct orf: Threshold cycle ORF1ab region, pos: positive, neg: negative

The statistical relationship among study variables was tested via Spearman's correlation analysis, results being reported in table IV.

TABLE IV: Spearman's correlation coefficients among study variables (in group of subjects with Ct orf < 25, n = 27)

	Ag level	Ct orf	Ct E
Ag level (S/CO)	-	-0.645*	-0.628*
Ct orf	0.645*	-	0.968
Ct E	0.628*	0.968*	-

*Significance level: $P < 0.001$. Ct orf: Threshold cycle ORF1ab region, Ct E: Threshold cycle E gene, Ag: antigen

DISCUSSION

To stop the spread of SARS-CoV-2 and to prevent overburdening the molecular detection capacity of SARS-CoV-2, WHO recommended prioritising testing for early

diagnosis and protection of vulnerable people [3]. The guide advocates for the use of TDAs with sensitivity 80% and specificity 97%, respectively, when qRT-PCR is not available or when late results lower their clinical utility. TDA could be useful the first 5-7 days after infection onset, characterized by high viral load: Ct 25 and contagiousity.

TDA can also be used to prioritize qRT-PCR testing of negative TDA patients in confirmed COVID-19 outbreaks and of asymptomatic non-quarantined contacts in case of negative results [8, 12]. In our study, the TDA of SARS-CoV-2 S protein had high specificity and sensitivity in samples with high viral load ($Ct \leq 25$). The current study results are comparable to those conducted by Lambert-Niclot et al. [13]. In addition, our results are comparable to those mentioned in other studies, even though the overall diagnosis sensitivity was lower [13, 14, 15]. A published study used the same method of identifying SARS-CoV-2 antigens, as in the present study, in patients either symptomatic or in close contact with COVID-19 cases, revealed a lower sensitivity, with a similar specificity [16].

Our results could be explained by a lower viral load present in patients with negative results at antigenic test that can be influenced by a subclinical evolution. It is possible for these patients to have a longer time between the time of infection and the onset of symptoms, when performing the test. In these cases, the optimal time to detect the presence of antigen in the samples performed could be exceeded. The cycle threshold in qRT-PCR SARS-CoV-2 tests may be considered as a potential marker for disease severity in patients with COVID-19 illness [17]. Labs are expected to communicate the Ct value when reporting test results, for it shows the viral load. We

considered that Ct might help stratifying the risk of a contagiousness different degree in COVID-19 patients, which calls for treatment and patient isolation. The lack of standardization for Ct values across RT-PCR platforms makes result comparison among different tests difficult. So far, the usage of Ct value was not validated by the clinical trials to guide the management of COVID-19 cases. Although this study provided evidence for using Fluorecare® SARS-CoV-2 Spike Protein Test Kit as antigen detection test, it has several limitations. A limitation could be generated by the lack of information related to the the time elapsed from the infectious contact and the performing of the test. Even the patients were tested immediately after symptoms onset, there could be a variable time between these moments. Another limitation is related to the antigenic test that was performed after unloading the collection buffer in the transport medium, but the inset kit allow the use of diluted sample in VTM. However, the results obtained are consistent in terms of specificity and sensitivity in patients with Ct orf 25, the overall sensitivity was lower comparing with official data for this test. It could suggest that in cases with low viral load, the test is not sensitive.

REFERENCES

1. Perlman S, Netland J. Coronaviruses post-SARS. Update on replication and pathogenesis. *Nat Rev Microbiol.* 2009;7:439–450. doi:10.1038/nrmicro2147
2. Hurjui IA, Hurjui RM, Pînzariu GM, Cojocaru T, Popovici D, Neamțu A. Clinical utility of tests, markers and proteomics in salivary functional exploration. *Romanian Journal of Oral Rehabilitation.* 2022;14(3): 105-114.
3. World Health Organization (WHO). Laboratory Guidelines for the Detection and Diagnosis of COVID-19 Virus Infection. *Paho.* 2020 July: 1-10. <https://iris.paho.org/handle/10665.2/52458>
4. Nalumansi A, Lutalo T, Kayiwa J, et al. Field Evaluation of the Performance of a SARS-CoV-2 Antigen Rapid Diagnostic Test in Uganda using Nasopharyngeal Samples. *Int J Infect Dis.* 2021;104:282-286. doi: 10.1016/j.ijid.2020.10.073.
5. Chaimayo C, Kaewnaphan B, Tanlieng N, et al. Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand. *Virology*

CONCLUSIONS

In medical practice, antigen tests could be successfully used for their high specificity, for being relatively inexpensive and for largely, currently offering results within approximately 15 minutes. Although more research with higher numbers is needed, the FIA Ag test's great performance results imply that it could be used in situations when RT-PCR is unavailable or expensive.

Due to their high sensitivity for detecting infective patients, FIA Ag tests may play a key role in future “test-out” methods, such as the early release of suspected cases from self-isolation or the reduction of quarantine time for confirmed cases.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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- J. 2020;17:177. doi: 10.1186/s12985-020-01452-5.
6. Candel FJ, Barreiro P, San Román J, et al. Recommendations for use of antigenic tests in the diagnosis of acute SARS-CoV-2 infection in the second pandemic wave: attitude in different clinical settings. *Rev Española Quimioter.* 2020;33(6):466–484. doi: 10.37201/req/120.2020
 7. Hurjui IA, Hurjui RM, Hurjui I, Pînzariu GM, Cojocaru T, Goriuc A, Haisan A, Popovici D, Stefanescu O, Ciurcanu OA. algogenic tissue factors and their roles in oro-facial pain. *Romanian Journal of Oral Rehabilitation.* 2022;14(4): 142-149.
 8. World Health Organization (WHO). Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. *Interim Guid.* 2020 Sept. <https://apps.who.int/iris/handle/10665/334253>
 9. Cordon-Cardo C, Pujadas E, Wajnberg A, et al. Staging of a New Disease. *Cancer Cell.* 2020; 38(5):594–597. doi: 10.1016/j.ccell.2020.10.006.
 10. Bosphore Novel Coronavirus (2019-nCoV) Detection Kit v2. Anatolia Diagnostics and Biotechnology Products Inc. 2020.
 11. SARS-CoV-2 Spike Protein Test Kit (Fluorescence Immunoassay). Shenzhen Microprofit Biotech Co. Ltd. 2020.
 12. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. *Clin Infect Dis.* 2020;71(10) : 2663–2666. doi:10.1093/cid/ciaa638
 13. Lambert-Niclot S, Cuffel A, Le Pape S, et al. Evaluation of a rapid diagnostic assay for detection of Sars-CoV-2 antigen in nasopharyngeal swabs. *J Clin Microbiol.* 2020; 58(8):e00977-20. doi: 10.1128/jcm.00977-20
 14. Porte L, Legarraga P, Vollrath V, et al. Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *Int J Infect Dis.* 2020; 99:328–333. doi: 10.1016/j.ijid.2020.05.098
 15. Scohy A, Anantharajah A, Bodéus M, et al. Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis. *J Clin Virol.* 2020;129: 104455. doi:10.1016/j.jcv.2020.104455
 16. Salvagno GL, Gianfilippi G, Pighi L, et al. Real-world assessment of Fluorecare SARS-CoV-2 Spike Protein Test Kit. *Advances in Laboratory Medicine / Avances en Medicina de Laboratorio.* 2021; 2(3):409-412. doi:10.1515/almed-2021-0041
 17. Magleby R, Westblade LF, Trzebucki Scohy A. Impact of Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load on Risk of Intubation and Mortality Among Hospitalized Patients With Coronavirus Disease 2019. *Clinical Infectious Diseases.* 2020;73(11):e4197–e4205, doi: 10.1093/cid/ciaa851.