Comparative analysis of Real-Time PCR and Chemiluminescence for the detection of SARS-CoV-2

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Abstract

SARS-CoV-2 infection is capable of causing a multi-organ syndrome, mainly involving the lungs with Acute Respiratory Insufficiency. The easy access to a rapid diagnosis of COVID-19 is a key point to improve the management of SARS-CoV-2 infection to contain its spread. The aim of this study is to evaluate the use of chemiluminescence technology in the diagnosis of SARS-CoV-2. In particular, a retrospective analysis was carried out on nasopharyngeal swabs from oncology patients referred to the National Cancer Institute of Naples, comparing the results of the Elecsys® SARS-CoV-2 Antigen chemiluminescence assay with those obtained by molecular testing in Real Time RT-PCR. The concordance rate between the antigen test and Real Time RT-PCR was 70.83%. The false negative and false positive rates were 63.79% and 0%, respectively. Although the Elecsys® SARS-CoV-2 Antigen test showed a high specificity, it is not as sensitive as the molecular test which remains the reference method. Therefore, to compensate for the potential decrease in test sensitivity, negative results should be analysed together with more patient-related factors, such as history of exposure to COVID-19 and clinical symptoms, in order to guide the diagnosis and subsequent treatment of the patient. In the end, from a clinical point of view, the antigen test is useful for the identification of acute or early infection in a rapid and cost-effective way.

Introduction

In December 2019 in China a new virus, called SARS-CoV-2, was responsible for a series of pneumonia cases associated with high mortality [1,2]. The virus quickly spread worldwide, so much that on 11 March 2020, the World Health Organization declared a pandemic status [3,4]. In February-March 2020, the virus began to spread in Italy, first affecting the north of the country, and then spread throughout the country over the following months. SARS-CoV-2 infection is capable of causing a multi-organ syndrome, mainly involving the lungs with Acute Respiratory Insufficiency: a conspicuous percentage of subjects with COVID-19 disease required respiratory support in ordinary hospital wards or in Intensive Care Units [5,6]. The easy access to a rapid diagnosis of COVID-19 is a key point to improve the management of SARS-CoV-2 infection to contain its spread. The aim of this study is to evaluate the use of chemiluminescence technology in the diagnosis of SARS-CoV-2.

The diagnosis of COVID-19 is based on clinical symptoms, the epidemiological picture, laboratory and radiological procedures. Laboratory tests for COVID-19 primarily include molecular, antigen, and antibody tests. Molecular tests, such as reverse transcription polymerase chain reaction (RT-PCR), are considered the gold standard for diagnosing active infections. These tests detect the presence of viral RNA in respiratory specimens and are highly sensitive and specific [7]. Antigen tests, which detect viral proteins, offer quicker results but are generally less sensitive than molecular tests, making them more suitable for point-of-care testing in certain settings [8]. Lastly, antibody tests are used to detect past infections by identifying antibodies against SARS-CoV-2 in the blood. These tests are valuable for understanding the spread of the virus in populations and for epidemiological studies but are not typically used for diagnosing active infections [9-11].

The rapid evolution of testing technologies and methodologies has played a crucial role in managing the pandemic, guiding public health decisions, and improving patient outcomes. The easy access to a rapid diagnosis of COVID-19 is a key point to improve the management of SARS-CoV-2 infection to contain its spread. The aim of this study is to evaluate the use of chemiluminescence technology in the diagnosis of SARS-CoV-2.

Materials and Methods

The work carried out at the S.C. Laboratory Medicine of the National Cancer Institute of Naples IRCCS Fondazione Pascale. Molecular analysis for the detection of the SARS-CoV-2 genome was performed on nasopharyngeal swabs by automated extraction of the virus nucleic acids with MagNA Pure 24 instrumentation (Roche), followed by amplification on CFX96 (Bio-Rad) in real-time RT-PCR. At the same time, the antigen test was performed on the same swabs using the cobas e 801 immunoanalyzer (Roche).

The statistical analysis of the data was conducted using SPSS (Statistical Package for Social Science) software for Windows, version 28.0. Contingency tables were analyzed using the chi-square test (χ^2)

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to evaluate the concordance and discordance among the samples that tested positive, taking into account a CT value below and above 30 for the three genes examined, as well as the result of the antigen test. The analysis of the mean and standard deviation with respect to the concordance and discordance values between the two methods was carried out using the Student's t-test. Finally, the ROC curve was employed to relate the sensitivity and specificity of the antigen test compared to the molecular test. In all tests, p-values < 0.05 were considered statistically significant.

Results

From the molecular analysis performed by Real Time RT-PCR, among the 72 swabs analyzed, 58 were positive and 14 negative, with a percentage of 80.56% and 19.44% respectively (Figure 1).

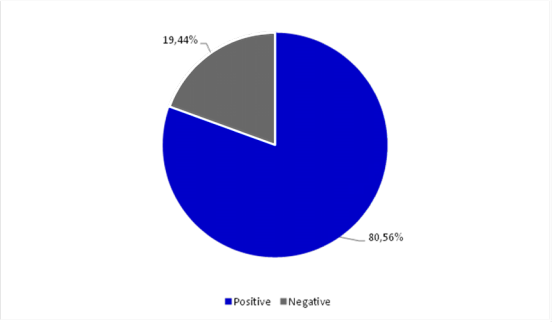

Figure 1: *positive-negative percentage with Real Time RT-PCR*

The samples analyzed with the antigen test resulted 37 positive and 35 negative, with a percentage of 51.39% and 48.6% (Figure 2).

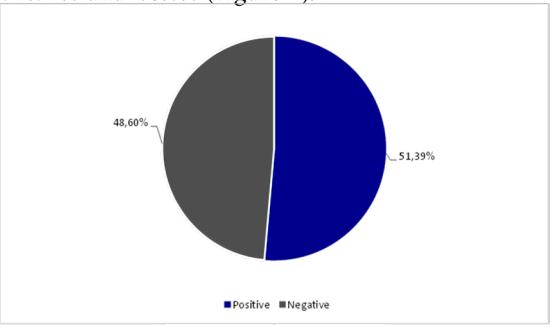

Figure 2: *Positive-negative percentage of antigen test*

By comparing the results obtained with the Real Time RT PCR and the chemiluminescent antigen analysis, we can note how the percentage of positive samples is higher in the molecular method (Figure 3).

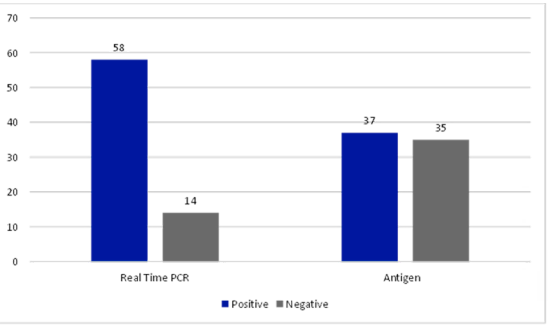

Figure 3: *the Real Time RT PCR and the chemiluminescent antigen analysis*

Moreover, a ROC curve was generated to relate the sensitivity and specificity of the antigen test to the molecular test, calculating the area under the curve (AUC) which was found to be 0.809 (Figure 4).

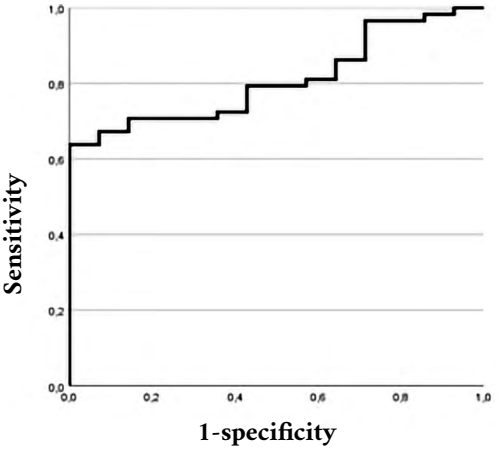

Figure 4: *ROC curve relating the sensitivity and specificity of the antigen test to the molecular test*

To evaluate relative sensitivity, we conducted a comparative analysis on positive samples, comparing the CT values of the RdRP/S, N, and E genes obtained through molecular analysis with the ICO values obtained using the chemiluminescence method (Tables 1, 2, 3). In contrast, relative specificity was assessed in all samples that tested negative by both Real-Time RT-PCR and the antigen test (Table 4).

CT RdRP/S gene	Number of samples	Number of antigen-positive	Relative sensitivity
CT <26	19	19	100%
CT <27	22	22	100%
CT <28	25	25	100%
CT <29	27	27	100%
CT <30	31	31	100%
CT <31	32	32	100%
CT <32	36	35	97,22%
CT <33	39	35	89,74%
CT <34	41	37	90,24%
CT <35	43	38	88,37%
Totale fino a ≤ 40	52	37	71,15%

Table1: *comparative analysis on positive samples between the CT values of the RdRP/S gene obtained by molecular analysis and the ICO values achieved with the chemiluminescence method in terms of relative sensitivity.*

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CT N gene	Number of samples	Number of antigen - positive	Relative sensitivity
CT <26	15	15	100%
CT <27	18	18	100%
CT <28	19	19	100%
CT <29	23	23	100%
CT <30	27	27	100%
CT <31	29	29	100%
CT <32	32	31	96,88%
CT <33	35	32	91,43%
CT <34	37	34	91,89%
CT <35	39	36	92,31%
Totale fino a ≤ 40	57	37	64,91%

Table 2: *comparative analysis on positive samples between the CT values of the N gene obtained by molecular analysis and the ICO values achieved with the chemiluminescence method in terms of relative sensitivity.*

CT E gene	Number of samples	Number of antigen-positive	Relative sensitivity
CT <26	22	22	100%
CT <27	24	24	100%
CT <28	27	27	100%
CT <29	32	32	100%
CT <30	32	32	100%
CT <31	35	33	94,29%
CT <32	39	36	92,31%
CT <33	42	38	90,48%
CT <34	43	39	90,70%
CT <35	45	39	86,67%
Totale fino a ≤ 40	57	37	64,91%

Table 3: *comparative analysis on positive samples between the CT values of the E gene obtained by molecular analysis and the ICO values achieved with the chemiluminescence method in terms of relative sensitivity.*

Number of negative molecular test	Antigen-positive	Antigen-negative	Relative specificity
14	0	14	100%

Table 4: *comparative analysis on negative samples between the results achieved by molecular analysis and the ICO values obtained with the chemiluminescence method in terms of relative specificity.*

Discussion

The ability to diagnose SARS-CoV-2 is an extremely important issue for governments and healthcare systems around the world. Being able to quickly and correctly identify a COVID-19 infection is essential both for managing the patient and for containing the spread of the virus. From the analysis of the results, we can observe that the percentage of concordance between the Elecsys SARS-CoV-2 Antigen test and the Real Time RT-PCR is 70.83%. Among the 58 samples tested positive with the molecular analysis, only 37 were positive with the antigen test, while 14 tested negative with the Real Time RT-PCR and 35 tested negative with the chemiluminescence method. The false negative rate is 63.79%, while no cases of false positivity occurred. From the ROC curve analysis we could see that the value obtained, calculating the area under the curve, is 0.809, indicating that the test is moderately accurate [10-12]. Eventually, we found that the sensitivity of the Elecsys SARS-CoV-2 Antigen test decreases with the increase in CT values; probably due to the decrease in the concentration of the virus below the detection limit of the test, as viral antigens are expressed only when the virus is actively replicating. Therefore, from a clinical point of view, the antigen test is useful for the identification of acute or early infection in a rapid and economically advantageous way [13-16].

The Elecsys® SARS-CoV-2 Antigen Test has a high specificity, although it is not as sensitive as molecular test that remains the reference method. Therefore, to compensate for the potential decrease in test sensitivity, negative results should be analyzed together with additional patient related factors, such as history of exposure to COVID-19 [17,18], clinical symptoms and results of additional tests, to help guide the diagnosis and subsequent treatment of the patient.

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Conflicts of Interest:
The authors declare no conflict of interest.

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