Rapid Diagnosis of SARS-CoV-2 Pneumonia on Lower Respiratory Tract Specimens

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Abstract

Background: The ongoing pandemic of SARS-CoV-2 requires the availability of accurate and rapid diagnostic tests, especially in some clinical settings like emergency and intensive care units. The objective of this study was to evaluate the diagnostic performances of rapid PCR kit Vivalytic SARS-CoV-2 in lower respiratory tract (LRT) specimens.

Methods: A consecutive sample of LRT specimens (bronchoalveolar lavage and bronchoaspirates) was collected from Intensive Care Units of San Martino Hospital (Genoa, Italy) between November 2020 and January 2021. All samples were tested in RT-PCR by using Allplex™ SARS-CoV-2 assay (Seegene Inc., South Korea). Based on RT-PCR results, specimens were categorized into negative, positive with high viral load [cycle threshold (Ct) ≤30] and positive with low viral load (Ct of 31–35). A quota 1:1:1 sampling was used to achieve a sample size of 75. Then, all specimens were tested in the rapid PCR assay Vivalytic SARS-CoV-2 (Bosch Healthcare Solutions GmbH, Germany). The diagnostic performance of the rapid PCR against RT-PCR was assessed through calculation of accuracy, Cohen's κ, sensitivity, specificity and expected positive (PPV) and negative (NPV) predictive values.

Results: The overall diagnostic accuracy of the Vivalytic SARS-CoV-2 was 97.3% (95% CI: 90.9–99.3%) with an excellent Cohen's κ of 0.94 (95% CI: 0.72–1). The sensitivity and specificity were 96% (95% CI: 86.5–98.9%) and 100% (95% CI: 86.7–100%), respectively. Samples with high viral loads had a sensitivity of 100% (Table 1). The distributions of E gene Ct values were similar (Wilcoxon's test: P=0.070) with medians of 35 (IQR: 25–36) and 35 (IQR: 25–35), respectively (Figure 1). NPV and PPV was 92.6% and 100%, respectively.

Conclusions: This study shows Vivalytic SARS-CoV-2 can be used following the sample liquefaction on LRT specimens. It's a feasible and highly accurate molecular procedure especially in high viral load samples. This assay allows having a result in about 40 min and therefore may accelerate the clinical decision making in urgent/emergency situations.

Introduction

Global research is committed to explore every topic of the coronavirus disease-2019 (COVID-19) outbreak and to restore the normal level of public health. The rapid identification and monitoring of COVID-19 patients is cited among the main strategies in the emergency and intensive care units (ICU) [1–2].

Molecular diagnostic assays continue to be a gold standard testing for the laboratory diagnosis of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia in combination with imaging analysis (chest radiography) [3].

The Centers for Disease Control and Prevention (CDC) recommends the collection of nasopharyngeal swab (NPS) as the first-choice sample type for real-time reverse transcriptase polymerase chain reaction (RT-PCR) [4]. High viral nucleic acid shedding pattern of SARS-CoV-2 patients were detected on upper-

respiratory-tract (URT) specimens, NPS or oropharyngeal swabs (OPS), in the first symptom onset [5]. However, the critical conditions of patients with severe acute respiratory infection (SARI) who receive mechanical ventilation do not usually allow health professionals to collect URT specimens. Furthermore, some cases of SARI by SARS-CoV-2 could be presenting typical chest images, anosmia or ageusia, but NP swab with RT-PCR negative. [6–7]. To improve the detection rate and reduce the false negative rate in these cases, the lower respiratory tract (LRT) specimens (i.e. bronchoalveolar lavage, BAL; bronchoaspirates, BAS; sputum; tracheal aspirate) should be used for testing of highly suspected patients [7]. SARS-CoV-2 has an active replication in the pulmonary sites confirmed by higher excretion kinetic in sputum than URT samples [8].

In the emergency context of patients with COVID-19 infection receiving therapy of mask ventilation or automated ventilation devices, rapid tests of high accuracy and sensitivity should be of clinical aid to achieve the laboratory diagnosis and clinical prognosis quickly and efficiently.

In this study, we evaluated the performance of the Vivalytic SARS-CoV-2 molecular platform (Bosch Healthcare Solutions GmbH, Germany) and compared it in parallel with the RT-PCR assay.

Materials And Methods

From November 2020 to January 2021, we consecutively assessed on both molecular systems 75 consecutive LRT samples from COVID-19 symptomatic patients at Laboratory of Hygiene Unit, IRCCS Policlinico San Martino Hospital (Genoa, Italy). A quota 1:1:1 sampling was used to achieve a sample size of 75. Following the collection and transport, each sample was analyzed for the qualitative detection of SARS-CoV-2 RNA using Allplex™ 2019n-CoV assay (Seegene Inc.; Seoul, South Korea), according to the manufacturer's instructions. RT-PCR for the E gene, RdRP/S gene, and N gene was performed in a Bio-Rad CFX96 Deep Well real-time PCR detection system (Bio-Rad, Hercules, CA, USA), after viral RNA extraction using NX-48 viral nucleic acid extraction kit (Genolution, Seoul, South Korea). On the basis of RT-PCR results, specimens were categorized into negative, positive with high viral load [cycle threshold (Ct) ≤ 30] and positive with low viral load (Ct of 31–35). Later, the Vivalytic SARS-CoV-2 test was performed on each specimen included.

Vivalytic SARS-CoV-2 (Bosch Healthcare Solutions GmbH, Germany) is a portable device for molecular diagnostics able to perform the most common PCR procedures fully automatically. A sample is filled in a specific cartridge with a single pre-analytical and manual step, such as the sample preparation and addition.

The Vivalytic SARS-CoV-2 analyzer requires the sample collection in guanidine thiocyanate-based medium (eNAT®) that stabilizes the viral RNA and completely inactivates the microbial viability. To dissolve the mucous reach respiratory specimens, before the inoculation in the test cartridge a sample pre-treatment is required. Therefore, BAL or BAS were treated with sputasol (Sputasol Liquid, Oxoid

Limited) at a 1:1 ratio (500 µl of sample and 500 µl of SL), vortexed and after 10 min added to 1 mL of eNAT®.

This singleplex test based on the detection of gene E is performed on a specimen volume of 300 µl in 39 minutes.

Simultaneously, in order to assess the test specificity for SARS-CoV-2, Vivalytic test was performed on positive LRT samples for other respiratory viruses (Coronavirus OC43, Rhinovirus type A, Metapneumovirus, Parainfluenza virus type 3, Influenza virus A; Respiratory Syncytial Viruses type B) detected in the 2019/2020 influenza season and stored at -80°C. Allplex™ Respiratory Panel Assays (Seegene Inc.; Seoul, South Korea) were used to identify these respiratory infection agents. The selection of the respiratory viruses to evaluate the specificity of the SARS-CoV-2 Vivalytic was related to the viruses detected in LTR samples for the 2019/2020 influenza season.

The diagnostic performance of the rapid PCR against RT-PCR was assessed through calculation of accuracy, Cohen's κ, sensitivity, specificity and expected positive (PPV) and negative (NPV) predictive values. The Open Source Epidemiologic Statistics for Public Health (OpenEpi, https://www.openepi.com/) was used for statistical analyses.

Results

Clinical LRT specimens

The main characteristics of the study specimens are shown in Table 1. The samples were predominantly from males (74.6%) and the median patients' age was 65 (interquartile range: 31–81) years. Samples with negative, low and high viral loads were equally distributed (z-test: P = 0.21) for type of specimens, such as BAS (57.3%) and BAL (42.7%).

Table 1
Clinical characteristics of the specimens analyzed (N = 75).

Male, *N (%)*	**74.6% (*N = 56*)**
Age *(yr)*, Median *(IQR)*	65 (31–81)
BAS, *N (%)*	57.3% (*N = 43*)
Negative	30.2%
Positive - High viral load [Ct ≤ 30]	27.9%
Positive - Low viral load [Ct > 30]	41.9%
BAL, *N (%)*	42.7% (*N = 32*)
Negative	37.5%
Positive - High viral load [Ct ≤ 30]	40.6%
Positive - Low viral load [Ct > 30]	21.9%

[1] BAS, bronchoaspirates; BAL, bronchoalveolar lavage; CT, cycle threshold.

Accuracy, sensitivity and specificity of rapid PCR

The overall diagnostic accuracy of the rapid PCR was 97.3% (95% CI: 90.9–99.3%) with an excellent Cohen's k of 0.94 (95% CI: 0.72–1). The sensitivity and specificity were 96.0% (95% CI: 86.5–98.9%) and 100% (95% CI: 86.7–100%), respectively. Indeed, only two false negative results were found and both of them belonged to BAL samples with low viral loads. Therefore, samples with high viral loads had a sensitivity of 100% (Table 2). NPV and PPV was 92.6% and 100%, respectively.

Table 2
Sensitivity and specificity of the rapid PCR assay.

Category	**Sensitivity, *%***	**Specificity, *%***
Negative (*N* = 25)	NA	100
High viral load (*N* = 25)	100	NA
Low viral load (*N* = 25)	92.0	NA
Total (*N* = 75)	96.0	100

The distributions of E gene Ct values were similar between the rapid PCR and RT-PCR (Wilcoxon's test: P = 0.070) with medians of 35 (IQR: 25–36) and 35 (IQR: 25–35), respectively (Fig. 1).

The SARS-CoV-2 Vivalytic showed no cross-reactivity testing samples positive for other respiratory viruses, including other coronaviruses.

Discussion

In this study, we showed that the Vivalytic SARS-CoV-2 is a useful tool as molecular single testing system to detect the virus in the LRT specimens. In particular, in comparison with the gold standard RT-PCR, this rapid PCR assay reached a satisfactory level of accuracy. To the best of our knowledge, our study is the first to evaluate the diagnostic performance of this diagnostic platform in LRT specimens.

Different clinical conditions may make it difficult to obtain a NP swab, which is the most commonly processed sample type for the diagnosis of COVID-19, and require other biological materials to exclude the SARS-CoV-2 pneumonia. This situation is particularly relevant in case the NP sample is negative but some the patient's clinical conditions are suggestive of the SARS-CoV-2 infection. Despite the invasive nature of BAL and BAS collection procedure, the PCR of LRT specimens may be more sensitive than that of NP swabs [9]. Indeed, some case reports have documented the discrepant PCR results between the two types of samples: LRT samples were positive, while those from the URT were negative [7, 10].

Recently, a case of SARS-CoV-2 transmission during the lung transplantation has been reported despite a negative result from the pre-implantation NP swab of the donor. The assessment of LRT specimens from potential lung donors should be therefore preferred for the virological screening prior to the transplant [11]. Similar investigations will be recommended also for other organ transplants because the wide tropism of SARS-CoV-2 that is detected in multiple sample types (plasma, rectal swabs, stool, urine, kidney and lung tissues) [12].

Analogously, under the critical conditions of respiratory failure (i.e., when patients are intubated), the LRT material is a sampling strategy [13]. It is of crucial importance to collect a right sample type from a given patient in order to reduce the incidence of false negative PCR test results [13].

Vivalytic COVID-19 is a diagnostic system intended for use one sample at time and therefore can't satisfy the high workflow of COVID-19 laboratory. Indeed, it is important to underline that this procedure may be useful to meet low volume of requests for rapid diagnosis, i.e. for SARI patients with unknown pneumonia and negative results of NP swab for SARS-CoV-2, in the pre-operative evaluation in trauma patients or transplant surgery, as the lung implant particularly.

We noted two main study limitations. First, we tested only BAL and BAS samples and therefore our results may be not generalizable to other lower respiratory materials, such as tracheal aspirates and sputum. However, since these samples have similar viscosity to BAS and BAL, we believe that the same specimen pre-treatment protocol used in this study may be applied. Second, the samples size of this study is limited and skewed towards positive samples. This latter may be explained by the study time period, i.e. it was conducted on the height of the second pandemic wave. On the other hand, the diagnostic accuracy of Vivalytic SARS-CoV-2 for LRT specimens was in line with that for NP samples declared by the manufacturer. Therefore, we believe that this limitation has a low impact on the reported results.

Conclusions

We reported results on the diagnostic evaluation of the rapid PCR assay Vivalytic SARS-CoV-2 on LRT specimens. This molecular platform is simple, rapid and fully automated with low pre-analytical interference and training time. It may be helpful to accelerate diagnosis, decision of therapy and prognosis of patients with urgent/emergency conditions.

Therefore, Vivalytic SARS-CoV-2 could be used with high accuracy, feasibility and quickly for patients of critical care, as some investigators for the management of the ICU required [2].

List Of Abbreviations

Coronavirus disease-2019 (COVID-19)

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)

Intensive care units (ICU)

Centers for Disease Control and Prevention (CDC)

Nasopharyngeal (NP)

Real-time reverse transcriptase polymerase chain reaction (RT-PCR)

Severe acute respiratory syndrome (SARI)

Lower respiratory tract (LRT)

Bronchoalveolar lavage (BAL)

Bronchoaspirates (BAS)

Declarations

Ethics approval and consent to participate

Ethical review and approval were waived for this study since it was based on the routine COVID-19 testing performed at IRCCS Policlinico San Martino Hospital (Genoa, Italy).

Consent for publication

Patient consent was waived because this study was performed on the samples collection for the routine diagnosis of COVID-19.

Availability Data and Materials

All data presented in this study are available in Results section

Competing Interest

The authors declare no conflict of interest.

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Author Contributions

Conceptualization, V. De Pace and B. Bruzzone; methodology, V. De Pace; validation, P. Caligiuri, N. Nigro and B. Galano; formal analysis, V. Visconti; investigation, V. De Pace and V. Ricucci; data curation, P. Caligiuri and V. Visconti; writing original draft preparation, V. De Pace and V. Ricucci; writing review and editing, B. Bruzzone; visualization, B. Bruzzone and G. Da Rin; supervision, B. Bruzzone; project administration, G. Da Rin. All authors have read and agreed to the published version of the manuscript.

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Figures

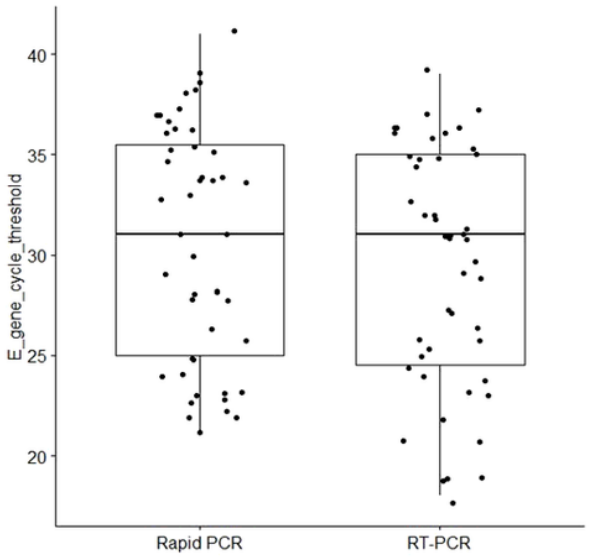

Figure 1

Distribution of E gene cycle threshold values of the rapid PCR and RT-PCR.