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Research Article
Comparison of Abbott Real Time SARS-CoV-2 and Cepheid Xpert Xpress SARS-CoV-2 in Molecular Diagnosis of COVID-19

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Abstract

Background and Objective: In December 2019 in Wuhan (China), SARS-CoV-2 was identified as the agent responsible for the COVID-19 epidemic. The expansion of this disease encourages greater epidemiological surveillance and molecular techniques capable of making the diagnosis regardless of its variant. The objective of the current study was to compare the concordance between two molecular diagnostic techniques, Abbott RealTime m2000 SARS-CoV-2 and Cepheid Xpert Xpress SARS-CoV-2 in the diagnosis of COVID-19. **Materials and Methods:** A total of 92 oral and nasopharyngeal samples were tested by PCR on Abbott RealTime SARS-CoV-2 and o Cepheid Xpert Xpress SARS-CoV-2. The data were subjected to statistical analysis to evaluate their degree of concordance. **Results:** The two tests had an overall agreement of 94.57% (95% CI: 87.9-97.66), a positive agreement of 100%, a negative agreement of 90% with a Kappa coefficient $\kappa = 89.15\%$ (95% CI: 68.84-100). About 5 samples tested negative on Abbott were positive on Cepheid with high Ct values (Ct: Cycle threshold) greater than 40. The correlation coefficient was $r = 0.82$, ($p = 2.2 \times 10^{-16}$) and showed good similarity in performance. **Conclusion:** The two techniques are comparable in terms of performance because the results showed excellent agreement. The Cepheid technique with more amplification cycles has a better detection threshold.

Key words: RT-PCR, comparison, Cepheid Xpert Xpress, Abbott RealTime, SARS-CoV-2, respiratory distress, cap structure

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), the causative agent of an outbreak of viral pneumonia, was first identified in Wuhan, China, in December 2019[1-3]. On January 30, 2020, the SARS-CoV-2 outbreak was declared a Public Health Emergency of International Concern (PHEIC) by the World Health Organization. The International Committee on Taxonomy of Viruses (ICTV) named SARS-CoV-2 as the disease agent due to its genetic homology with SARS-CoV[4]. The WHO named it the coronavirus disease 2019 (COVID-19). The clinical presentation of COVID-19 is nonspecific and sometimes asymptomatic, the symptoms overlap with other seasonal respiratory infections circulating simultaneously in the population making the transmission of the disease more complex[5]. It can also cause serious illness in the form of Acute Respiratory Distress Syndrome (ARDS)[6,7]. Many of the measures are critically dependent on the early, rapid and accurate diagnosis of those infected with the virus. Therefore, several tests are developed for the diagnosis of this new infection. Real-Time Polymerase Chain Reaction (RT-PCR) of viral RNA remains the standard technique to confirm early infection to SARS-CoV-2 due to its high sensitivity[8,9]. Thus, RT-PCR is of great interest for the detection of SARS-CoV-2 thanks to its advantages as a specific and simple qualitative test[10,11]. In March 2020, several molecular tests received Emergency Use Authorization (EUA) from the US Food and Drug Administration (FDA), including Cepheid Xpert Xpress SARS-CoV-2[12] and Abbott m2000 RealTime SARS- CoV-2[13].

The development of diagnostic tests, in particular molecular ones, requires a good knowledge of the genome of the SARS-CoV-2 virus and its variability. The structure of the SARS-CoV-2 genome is organized as follows: Two non-coding regions in 5 forming a terminal cap structure and in 3' in the form of a poly AAA tail. The coding part is divided into several parts, the first two-thirds of the genome consist of two large overlapping regions, Open Reading Frame ORF1a and ORF1b, encoding the replication-transcription complex, including the RNA-dependent RNA Polymerase (RdRp) gene which encodes RNA-dependent RNA polymerase. The last third of the genome encodes the structural proteins of the viral particle (S, E, M and N) and non-structural proteins necessary for the survival of the virus (Fig. 1)[14]. The S, E, M and N, genes encoding structural proteins (S [surface], E [envelope], M [membrane] and N [nucleocapsid]).

However, the slow deployment of large-scale molecular diagnostic tests (RT-PCR) and long turnaround times, have greatly hampered public health efforts to contain the outbreak.

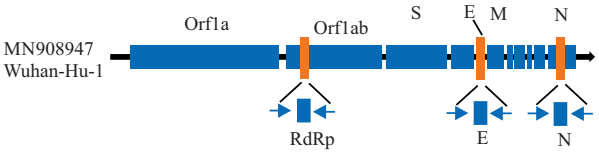

Fig. 1: Genomic structure of SARS-CoV-2 with RT-PCR target genes, the Wuhan-Hu-1 genome (GenBank MN908947)
RdRp: Gene encoding RNA-dependent RNA polymerase, S, E, M, N: Genes encoding structural proteins, S: Surface, E: Envelope, M: Membrane and N: Nucleocapsid

As a result, this pandemic has therefore created an urgent and unprecedented need for large-scale rapid molecular diagnostic tests to inform timely patient care and strengthen infection control measures. Early diagnosis and treatment of infected subjects remain an important pillar in the prevention of the disease, in particular in the fight against the expansion of the epidemic.

Thus, having reliable diagnostic tests in sufficient quantity is essential for the management of the pandemic and to help patients prevent the progression of the disease. However, several of the tests developed show great variability in terms of sensitivity and time to return results. Differences in the detection of SARS-CoV-2 have been found between several molecular diagnostic tests, false negatives via molecular diagnostics for SARS-CoV-2 have been reported[15,16].

In this context, the current study evaluated the performance of the Cepheid Xpert Xpress SARS-CoV-2 rapid molecular test compared to the Abbott m2000 RealTime SARS-CoV-2 molecular test in the context of the diagnosis of COVID-19 infection.

MATERIALS AND METHODS

Study population: It was exclusively conducted at the Molecular Biology Laboratory of the Armed Forces AIDS Program at the Ouakam Military Hospital (HMO), in February, 2021. The study includes any patient going to the laboratory to have molecular testing (RT-PCR) for SARS-COV-2. The patients were the contact cases, the patients under treatment, the symptomatic and the asymptomatic. There were no exclusions about the reason for testing.

Biological samples: Each patient undergoes two samples, a nasopharyngeal and an oropharyngeal swab combined in a single tube containing a transport medium. RNA extraction is performed or the sample is stored at -80°C if testing is deferred.

Molecular tests: Each sample was tested on both molecular techniques: Abbott RealTime SARS-CoV-2 and Cepheid Expert Express SARS-CoV-2.

Abbott RealTime SARS-CoV-2 (Abbott m2000sp/rt): Abbott RealTime SARS-CoV-2 was designed for the qualitative detection of SARS-CoV-2 nucleic acid on nasopharyngeal and oropharyngeal swabs collected from suspected COVID-19 patients or travellers. It is an RT-PCR test that targets the RdRp and N genes of SARS-CoV-2 (Abbott, 2020). The extraction of the RNA from the sample was carried out on the Abbott m2000sp extractor according to the manufacturer's protocol using the Abbott m Sample Preparation System Extraction Kit. Amplification and detection were performed by Abbott RealTime SARS-CoV-2 PLC Abbott m2000rt amplification kit. Each series of analyzes includes a negative and positive control, validation and interpretation were carried out according to the manufacturer's procedures.

Cepheid Xpert Xpress SARS-CoV-2 (GeneXpert): Xpert Xpress SARS-CoV-2 is an integrated diagnostic device that performs automated sample processing. It is a Real-Time RT-PCR that targets the E (envelope) and N (nucleocapsid) genes of the SARS-CoV-2 viral RNA on a GeneXpert machine (Cepheid, 2020). The interpretation of the results was made according to the manufacturer's instructions. If both targets are detected or if only the N2 target is detected, the test reports a positive result. If only target E is detected, the test gives a presumptive positive result because this target is shared by some members of the *Sarbecovirus* subgenus of coronaviruses and in this case, the test must be repeated.

Statistical analysis: The data collection was done on Excel 2013. The data was analyzed by OpenEpi and the R 4.0.4 software for the calculations of the median, standard deviation, correlation graph and Pearson correlation test. The tests were significant if the $p<5\%$.

RESULTS

Of 92 samples tested on Abbott RealTime SARS-CoV-2, 42 (45.65%) were positive. For the same number of samples tested on Cepheid Xpert Xpress SARS-CoV-2, 47 (51.09%) were positive.

The overall concordance of the two tests was 94.57% [95% CI: 87.9-97.66] (Table 1). Positive and negative concordance was 100 and 90%, respectively for all samples tested. Cohen's Kappa coefficient was 89.15% [95% CI: 68,84-100] (Table 2). Cohen's Kappa value greater than 81% was interpreted as indicating excellent agreement.

Positive samples cover Ct values with a median RdRp/N gene Ct value of 16.84 (IQR: 9.95-25.61) and a standard deviation of 9.19 on Abbott RealTime SARS-CoV-2. As for the Cepheid Xpert Xpress SARS-CoV-2, the median Ct values for the E and N genes were, respectively 26.30 (IQR: 18.8-33.7) and 34.5 (IQR: 25.4-40.3) with respective standard deviations of 12.77 and 8.24.

A total of 5 out of 92 samples previously tested negative on Abbott RealTime SARS-CoV-2 were positive on Cepheid Xpert Xpress SARS-CoV-2 but all showed very high Ct values (Ct>40 on Cepheid and $Ct \geq 30$ on Abbott). Only the N gene was detected and at a high Ct reflecting a low amount of nucleic acids in the sample (Table 2).

Pearson's correlation test was performed between Abbott's RdRp/N Ct and Cepheid's N Ct and showed a correlation coefficient $r = 0.82$, ($p = 2.2 \times 10^{-16}$), showing a good correlation between the two techniques (Fig. 2).

Table 1: Characteristics of concordant results of the two techniques

Measurement criteria	Values (%)	Confidence interval CI 95%
Overall agreement	94.57	(87.9-97.66%)
Cohen's kappa coefficient	89.15	(68.84-100%)
Index bias	-5	(-19-8%)
Prevalence index	-3	(-17-11%)

Table 2: Difference in the detection of target genes between tests

	Patients				
	Abbott		Cepheid		
Sample numbers	RdRp et N (Ct)	Results	Gene E (Ct)	Gene N (Ct)	Results
1	0.00	Negative	0.00	41.24	Positive
7	0.00	Negative	0.00	43	Positive
17	0.00	Negative	0.00	43	Positive
38	0.00	Negative	0.00	42.5	Positive
47	0.00	Negative	43.3	42	Positive

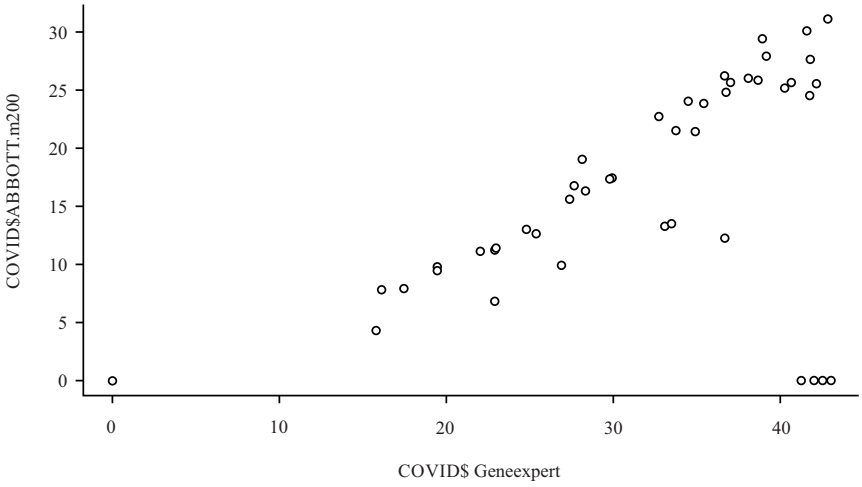

Fig. 2: Correlation between the Cts of abbott realtime SARS-CoV-2 and Cepheid Xpert Xpress SARS-CoV-2 based on their Cts (Cycle Threshold)
$r = 0.82$ ($p = 2.2 \times 10^{-16}$)

DISCUSSION

In this study, comparable performance between the Cepheid Xpert Xpress SARS-CoV-2 assay and the Abbott RealTime SARS-CoV-2 assay was demonstrated with an overall agreement of 94.57% [87.9-97.66%].

The positive concordance obtained by comparing the two tests is 100% and the negative concordance is 90%. No comparison study between Abbott RealTime SARS-CoV-2 and Cepheid Xpert Xpress SARS-CoV-2 has been found in the literature.

The arrival of the SARS-CoV-2 pandemic has challenged molecular biology laboratories to implement and rapidly validate diagnostic tests and increase testing capacity in a short timeframe. Further studies of these tests are needed to shed light on both their performance and their difference. Some investigations have pointed out that few studies have been conducted to determine the relative performance of these tests, especially on samples over a wide range of viral concentrations[17,18]. It was also well documented that dozens of *in vitro* Diagnostic Tests (IVD) for SARS-CoV-2 can give false negative results in people with COVID-19[15]. However, several studies conducted in Europe and the United States between the Cepheid Xpert Xpress SARS-CoV-2 and various standard reference tests have reported positive concordance percentages of >90%[18-21]. Other comparative studies have also shown similar results between the Abbott RealTime SARS-CoV-2 test and other tests[22,23].

Our results showed a value of Kappa $\kappa = 89.15\%$ (68.84-100%) indicating that they have excellent concordance. The Pearson correlation test showed a correlation coefficient $r = 0.82$ ($p = 2.2 \times 10^{-1}$, Fig. 2) between the two techniques according to the Ct values indicating that the Ct values of the two techniques increase in the same direction (positive direction).

However, slight differences in viral detection of SARS-CoV-2 were observed between the two tests. The Cepheid Xpert Xpress test can detect very low viral loads (Ct>40). About 5 out of 92 samples tested negative on Abbott RealTime SARS-CoV-2 are positive on Cepheid Xpert Xpress SARS-CoV-2 and all showed very high Ct values (Ct>40 on Cepheid). Only the N gene is detected, the E gene was not detected. These results were close to those found in several comparison studies between the Cepheid Xpert Xpress SARS-CoV-2 Test and other tests which have found that only the N gene is detected on Cepheid and no target gene of the other tests is detected for samples with high Ct[18,21,24,25]. These differences in detection could be explained by several factors. As an illustration, several studies point out that these differences could be explained by the variability of probes and target genes between techniques[26-28]. They could also be due to the different detection limits of the two tests, Cepheid Xpert Xpress SARS-CoV-2 has a higher amplification cycle number, 45 cycles than Abbott RealTime SARS-CoV-2 having 37 cycles. These slight differences in Ct between Abbott RealTime and Cepheid encountered would not allow us to conclude that Cepheid is performing better than Abbott. These discrepancies will not be well illuminated until there are standardized SARS-Cov-2 Ct values.

This study has limitations, a larger sample size could give us more certainty about performance data between techniques. In addition, differences in the extraction, amplification and detection efficiency of SARS-CoV-2 specific target genes that cannot be clearly explained, make the study difficult. This could lead to false negative results if a mutation prevents primer binding. However, this should not significantly impact the strong agreement observed.

CONCLUSION

Current results showed that the two techniques Abbott RealTime SARS-CoV-2 and Cepheid Xpert Xpress SARS-CoV-2, have comparable performance in the molecular detection of SARS-CoV-2. The Cepheid technique with more amplification cycles has a better detection threshold.

SIGNIFICANCE STATEMENT

The study has a diagnostic interest in COVID-19 infection and biological and pandemic monitoring. Two techniques Abbott RealTime SARS-CoV-2 and Cepheid Xpert Xpress SARS-CoV-2 were used for the molecular detection of SARS-CoV-2. Cepheid Xpert Xpress SARS-CoV-2 has a higher amplification cycle number, 45 cycles than Abbott RealTime SARS-CoV-2 having 37 cycles. These slight differences in Ct between Abbott RealTime and Cepheid encountered would not allow us to conclude that Cepheid is performing better than Abbott.

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