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RT-PCR test for the detection of SARS-CoV-2 in nasopharyngeal swabs: results of the Cepheid Xpert® Xpress SARS-CoV-2 assay versus the Thermo fisher TaqPath™ COVID-19 CE IVD

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ABSTRACT

Introduction. In the response to the coronavirus pandemic, several PCR kits have been developed with varying performance. Aim. In this study, we compared the results of SARS-CoV-2 testing using the Xpert® Xpress SARS-CoV-2 (Cepheid EUA) Testing with the TaqPath™ -COVID-19 CE IVD kit. Methods. A total of 92 nasopharyngeal swab samples from patients during September to November 2021 at the National Public Health Laboratory in Ouagadougou, Burkina Faso. These samples were analyzed using both the Xpert Xpress SARS-CoV-2 assay and the TaqPath-COVID-19-CE IVD kit on the QuantStudio 5 thermal cycler after extraction with the ANDiS 350 automated extractor. Results. The majority of patients was male 68.48% (63/92) and female 31.52% (29/92). The mean age was 42.2 ± 13.76 years with extremes of 12 and 70 years. The Xpert Xpress SARS-CoV-2 kit showed positive agreement with a kappa coefficient of 93.35% [85.9; 100] compared to the TaqPath-COVID-19-CE IVD kit. It also showed a sensitivity of 100%, a specificity of 92.68 and negative and positive predictive values of 100% and 94.44% respectively. Conclusion. The Xpert Xpress test is a very simple assay that compares favorably with the TaqPath-COVID-19-CE IVD test and can be reliably used for the detection of SARS-CoV-2 in nasopharyngeal specimens.

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INTRODUCTION

Coronavirus 2019 (COVID-19), an acute respiratory tract infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in late 2019 in Wuhan, China [1]. Since the emergence of the COVID-19 pandemic, most countries had been struggling with early detection of SARS-CoV-2, followed by rapid case management and contact tracing [2].

In an effort to bring as many mass tests as possible, multiple diagnostic tests, including molecular, antigen detection, and serological tests, were rapidly developed. Several rapid rt-PCR tests, including the Cepheid Xpert Xpress SARS-CoV-2, have received emergency approval from the U.S. Food and Drug Administration [3].

In Burkina Faso, as soon as the first cases of coronavirus were announced in early March 2020, the National Influenza Reference Laboratory was entrusted with the task of performing molecular diagnostic tests [4]. However, in view of the ever-increasing demand for PCR tests at the national level and with the aim of strengthening diagnostic capacities, the national reference laboratories, including the National Public Health Laboratory, the laboratories of research centers, and regional hospital centers have been enabled and engaged in the diagnosis of COVID-19.

The availability of efficient diagnostic tests in sufficient quantities has been established as a priority for the control of COVID-19. The performance and limitations of diagnostic tests must be mastered to aid in the interpretation of results and the management of infected patients [5].

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The aim of the current study was to compare the performance of two kits for the detection of SARS-CoV-2 RNA namely the Xpert Xpress SARS-CoV-2 test from the Genexpert integrated platform and Applied Biosystems TaqPath COVID-19 CE-IVD kit with the QuantStudio5 platform which is an open system.

MATERIALS AND METHODS

A total of 92 randomly selected nasopharyngeal specimens were evaluated in this study that were collected from patients suspected of having COVID19 infection between September and November of 2021. Specimens were collected in Universal Transport Medium (UTM) and tested using the Xpert Xpress SARS-CoV-2. The specimens were frozen at – 80 °C immediately after collection.

Xpress SARS-CoV-2 assay

The Xpert Xpress SARS-CoV-2 assay is an automated *in vitro* diagnostic test for the qualitative detection of SARS-CoV-2 RNA using reverse transcription-PCR (RTPCR). The Xpert Xpress SARS-CoV-2 assay is performed within a self-contained cartridge that performs extraction, amplification, and detection of amplicons if the target gene(s) are present. The cartridge also contains a Sample Process Control (SPC) and a Probe Check Control (PCC). The SPC controls for the adequate processing of the specimen and monitors for the presence of potential inhibitor(s) to the RT-PCR reaction. The PCC verifies reagent rehydration and monitors other functional activities within the cartridge. The Xpress SARS-CoV-2 assay was performed according to the manufacturer's instructions [6]. Basically, the specimen in UTM was mixed by inversion 5 times, a 300 µL volume was transferred to the test cartridge, and the cartridge was loaded into the Gene Xpert instrument. The assay targets the N2 and E gene sequences and, according to the manufacturer, has a LoD range is 125 – 250 viral copies/mL. The assay has a crossing threshold (Ct) cutoff value of ≥45 cycles for negative specimens and is completed within 50 min.

TaqPath COVID-19 CE-IVD RT-PCR Kit

The TaqPath COVID-19 CE-IVD RT-PCR Kit is a high complexity assay that requires a separate, stand-alone nucleic acid extraction step. The assay is performed using a 96 well microtiter tray that allows for the testing of 94 specimens as well as a positive and negative control per run. Gene amplification and amplicon detection can be performed by using any one of a number of instrument platforms, such as Applied Biosystems QuantStudio 5, as was used in this study. The assay targets 3 gene sequences, (N, ORF1ab, and S genes). The assay was performed according to the manufacturer's instructions [7] by first extracting a 200 µL aliquot of specimen in UTM using ANDiS Viral RNA Auto Extraction & Purification Kit (3D Biomedicine Science & Technology Co., Ltd., Shanghai, China) with Automated Nucleic Acids Extraction System ANDiS 350 (3D Biomedicine Science & Technology Co., Ltd., Shanghai, China). Prior to RNA extraction, 20 µL of Proteinase K was added to each well in the ANDiS 350 Deepwell 96 Plate. In addition, 5 µL of the MS2 Phage Control was added to all specimens and the Negative Control that served as an internal process control. The nucleic acid was eluted into 50 µL of Elution Solution [8]. For each specimen, Master Mix was prepared containing TaqPath 1-Step Multiplex Master Mix (No ROX™), COVID-19 Real Time PCR Assay Multiplex, and Nuclease-free water. 15 µL of Master Mix was dispensed into wells in a 96 well plate followed by the addition of 10 µL of eluted specimen to the appropriate well. Each run also included a SARS-CoV-2 Positive Control and a Negative Control. Amplification was performed on the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument (ThermoFisher Scientific, Waltham, MA). Testing was performed in batches of 94 specimens plus one negative and positive control. The results were interpreted using the Applied Biosystems™ COVID-19 Interpretive Software version 1.3. According to the manufacturer's instructions, a specimen was considered SARS-CoV-2 positive when 2 or more SARS-CoV-2 gene targets were called positive with cycle threshold values of ≤37. The time to complete the assay for 94 specimens including the controls was approximately 3 h. According to the manufacturer, the assay has a LoD of 125 – 250 copies/mL [7].

Ethical approval

The Research and Scientific Cooperation Department of the National Public Health Laboratory has approved the protocol and the realisation of the study.

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Statistical analysis

Statistical analyses were performed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) and R. The results from reference kits and the Xpert Xpress assay were analyzed using kappa and the level of statistical significance was set at $P<0.05$. Kappa stands for the measure of agreement between the two tests; a value of range between 0.81-100 is almost perfect.

RESULTS AND DISCUSSION

A total of 92 randomly selected, de-identified nasopharyngeal specimens were evaluated in this study of which 54 were positive and 38 were negative for SARS-CoV-2 as tested by the Xpert Xpress assay. Table 1 shows the comparative TaqPath test results for these specimens. Of the 92 samples analyzed, 89 samples gave the same result on all platforms, resulting in 94.65% agreement and Kappa value of 0.89 [0.68-1.00]. Both platforms were evaluated by Bland-Altman agreement for the N gene. The Bland-Altman plot compared the means of the measurements to their differences of the 2 homologous targets N gene and N2 gene and 88.88% of the measurement differences were within the range of agreement limits (Figure 1).

Ct values for Xpert Xpress positive samples ranged from 13.9 to 39.7 with a mean Ct of 27.89 for the E gene and from 16.3 to 43.4 with a mean Ct of 30.71 for the N2 gene (Table 2). For samples positive with the TaqPath COVID-19 CE-IVD kit, Ct values ranged from 11.54 to 35.89 with a mean Ct of 25.40 for the S gene; from 12.47 to 37.75 with a mean Ct of 26.59 for the ORF1ab gene and from 13.18 to 34.41 with a mean Ct of 26.30 for the N gene (Table 3).

The Xpert Xpress SARS-CoV-2 test had a sensitivity of 100% and a specificity of 92.68%. Its negative predictive value was 100% and its positive predictive value was 94.44%.

Table 1. Test results for Xpert Xpress SARS-CoV-2 and TaqPath COVID-19 CE-IVD kits

Results	Xpert Xpress SARS-CoV-2	TaqPath COVID-19 CE-IVD
Negative	38	41
Positive	54	51
Total	92	92

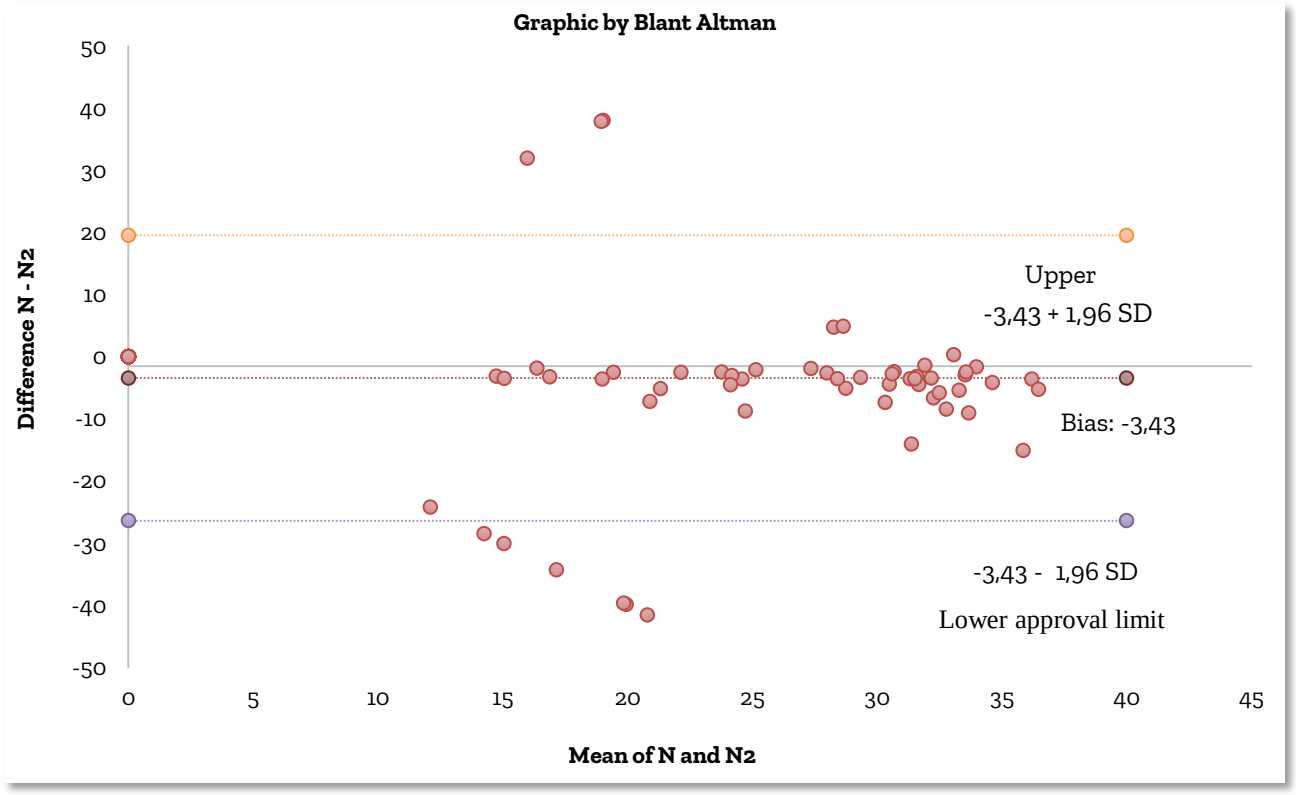

Figure 1. Blant Altman plot for the N gene

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Table 2. Ct values of target genes with Xpert Xpress SARS-CoV-2

Results	E Gene (N=52)	N2 Gene (N=54)
Minimum	13.9	16.3
Maximum	39.7	43.4
Mean	27.89	30.71
Standard deviation	6.29	6.57

Table 3. Ct values of target genes with the TaqPath COVID-19 CE-IVD Kit

Results	S Gene (N=48)	Orf1ab Gene (N=51)	N Gene (N=47)
Minimum	11.54	12.47	13.18
Maximum	35.89	37.75	34.41
Mean	25.40	26.59	26.30
Standard deviation	6.48	6.39	5.84

DISCUSSION

This study was conducted to compare the ability of the TaqPath COVID-19 CE-IVD kit against the Xpert Xpress SARS-CoV-2 assay to detect SARS-CoV-2 in nasopharyngeal specimens. Taqpath COVID-19 CE-IVD kit was selected as the reference test because it has an independent and controllable extraction step.

In this comparative study, the Xpert Xpress SARS-CoV-2 kit showed positive agreement with a kappa coefficient of 94.65% compared to the TaqPath-COVID-19-CE IVD kit. Cohen's nonparametric Kappa (K) test is used to quantify the agreement between two or more observers or techniques when the judgments are qualitative. In this study the agreement between the two platforms is almost perfect with the hight value (>81%) of the Kappa coefficient [9]. Similar results were reported by Jamai et al. [10] and Granato et al. [11] showing respectively 99.5% and 96.7% positive agreement between the results obtained with the Xpert Xpress SARS-CoV-2 and the TaqPath-COVID-19-CE IVD kit.

According to the FDA SARS-CoV-2 Reference Panel, which allows for a more accurate comparison of the analytical performance of different *in vitro* diagnostic molecular tests for SARS-CoV-2, the limit of detection (LoD) of the GeneXpert platform with the Xpert Xpress SARS-CoV-2 kit is 5,400 NDU/mL (NAAT detectable units/mL) compared to a limit of detection of 18,000 NDU/mL for the TaqPath COVID-19 CE-IVD kit [12]. The Xpert Xpress SARS-CoV-2 assay is therefore more sensitive than the TaqPath COVID-19 CE-IVD Kit.

Compared to the TaqPath-COVID-19-CE IVD kit, the Xpert Xpress SARS-CoV-2 kit had a very high sensitivity (100%) and good specificity (92.68%) in the diagnosis of SARS-CoV-2. Similar results were obtained by Goldenberger et al. [13] who found a specificity of 100% and a sensitivity of 100% and Rakotosamimanana *et al.* [2] in Madagascar found a sensitivity of 100% and a specificity of 80%.

In this study, a total of 54 samples tested positive with the Xpert Xpress SARS-CoV-2 kit and 51 samples tested positive with the TaqPath COVID-19 CE IVD kit. The 3 discordant samples were all positive with the Xpert Xpress SARS-CoV-2 kit and negative with the TaqPath COVID-19 CE IVD kit and the Ct values of these samples were all above 37. This could be explained not only by the very low detection limit of the Xpert Xpress SARS-CoV-2 kit but also by the interpretation rules of the TaqPath COVID-19 CE-IVD kit where amplifications above 37 cycles were considered negative.

CONCLUSION

COVID-19 emphasized the importance of having a reliable laboratory network with sufficient human and financial resources. The Cepheid Xpert® Xpress SARS-CoV-2 test compares favorably to the Thermo fisher TaqPath™-COVID-19-CE IVD and can be reliably used for the detection of SARS-CoV-2 in nasopharyngeal specimens. It's very low detection limit makes the GeneXpert a very sensitive test and allows detection of small amounts of SARS-CoV-2 RNA. Given the need to provide COVID-19 diagnostics throughout the country, the use of this technology already deployed will be of great interest as it requires minimal trained personnel and less infrastructure and equipment.

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DECLARATIONS

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Authors' contributions

D Zoungrana, SS Rouamba and E Kabre conceived and designed the experiments; D Nezien and SS Rouamba analyzed and interpreted the data; D Zoungrana, SS Rouamba, D Nezien and S Sougue drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study were included in this published article.

Competing interests

The authors disclose no conflicts of interest.

REFERENCES

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 ; 382 (8) : 727-733. DOI: https://doi.org/10.1056/NEJMoa2001017.

[2] Rakotosamimanana N, Randrianirina F, Randremanana R, Raherison MS, Rasolofo V, Solofomalala GD, *et al*.. GeneXpert for the diagnosis of COVID-19 in LMICs. Lancet Glob Health. 2020;8(12) : 1457-1458. DOI: https://doi.org/10.1016/S2214-109X(20)30428-9

[3] Cepheid. Notice d'utilisation À utiliser avec le système GeneXpert avec écran tactile exécutant le SE Cepheid [Operating instructions For use with the GeneXpert touch screen system running the Cepheid OS]. 2022. Article link

[4] Sagna T, OUEDRAOGO H, Zouré AA, Zida S, Compaore TR, Dinanibé K, *et al*.. Le laboratoire et la pandémie COVID Burkina Le Laboratoire à l'épreuve de la pandémie de la COVID-19 au Burkina Faso : Quels défis pour la régularité de l'offre de diagnostic [The COVID-19 pandemic-proof laboratory in Burkina Faso: What challenges for the regularity of the diagnostic?] Rev Mali Infect Microbiol. 2021; 16 : 32-37. DOI: https://doi.org/10.53597/remim.v16i1.1758

[5] Tyrrell DAJ, Almeida JD. Direct electron-microscopy of organ cultures for the detection and characterization of viruses. Arch Für Gesamte Virusforsch. 1967; 22 (3) : 417-425. DOI: https://doi.org/10.1007/BF01242962.

[6] Cepheid. Xpert Xpress SARS-CoV-2 Test (POC). 2020. Article link

[7] Thermofisher. TaqPath™ COVID-19 CE-IVD RT-PCR Kit instructions for use. 2021. Article link

[8] 3D Biomedicine Science & Technology Co., Ltd. ANDiS Viral RNA Auto Extraction & Purification Kit. 2020. Article link

[9] Landis JR, Koch GG. The Measurement of Observer Agreement for Categorical Data. *Biometrics*. 1977; 33(1), 159–174. DOI: https://doi.org/10.2307/2529310

[10] Jamai Amir I, Lebar Z, yahyaoui G, Mahmoud M. Covid-19 : virologie, épidémiologie et diagnostic biologique [virology, epidemiology and biological diagnosis]. Option/Bio. 2020;31(619):15–20. DOI: https://doi.org/10.1016/S0992-5945(20)30178-1

[11] Granato PA, Kimball SR, Alkins BR, Cross DC, Unz MM. Comparative evaluation of the Thermo fisher TaqPath™ COVID-19 combo kit with the Cepheid Xpert® Xpress SARS-CoV-2 assay for detecting SARS-CoV-2 in nasopharyngeal specimens. BMC Infect Dis.2021;21(1):623. DOI: https://doi.org/10.1186/s12879-021-06347-6

[12] FDA. SARS-CoV-2 Reference Panel Comparative Data. 2020. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-reference-panel-comparative-data

[13] Goldenberger D, Leuzinger K, Sogaard KK, Gosert R, Roloff T, Naegele K, *et al*.. Brief validation of the novel GeneXpert Xpress SARS-CoV-2 PCR assay. J Virol Methods. 2020; 284: 113925. DOI: https://doi.org/10.1016/j.jviromet.2020.113925