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Diagnostic Concordance of Two- and Three-Gene SARS-CoV-2 Molecular Assays in Cameroon: Implications for Efficient Pandemic Response in Low- and Middle-Income Countries

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ABSTRACT

Background: The scale-up of molecular assays for diagnosing emerging pathogens has increased in low-and-middle-income countries (LMICs) since the advent of COVID-19. We herein evaluated the diagnostic concordance of three different assays for SARS-CoV-2 in Cameroon.

Methods: A laboratory-based comparative study was performed on nasopharyngeal samples collected between March-2020 to March-2023 from the biobank of Chantal Biya International Reference Centre (CIRCB), Yaoundé-Cameroon. Samples were analyzed using DaAn Gene (N/ORF1ab-genes), ThermoFisher (N/ORF1ab/S-genes), and GeneXpert (N2/E-genes). Validated cycle thresholds (CT) for positivity were CT < 37 for DaAn Gene/ThermoFisher and CT < 40 for GeneXpert. Cohen's Kappa coefficient evaluated diagnostic concordance with DaAn Gene as reference.

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Results: We analysed 249 samples (55.8% males, median-age [IQR], 36 [27–50] years including 21.3% symptomatic participants). Overall positivity rates (median [IQR]) were 55.0% (CT: 30.6 [23.1–35.5]); 53.4% (CT: 26.6 [21.2–30.9]); 22.1% (CT: 32.7 [26.9–36.1]) for GeneXpert, DaAn Gene and ThermoFisher respectively. GeneXpert showed stronger concordance with DaAn Gene (83.1%; $k = 0.66$, 95% CI: 0.57–0.75) than ThermoFisher (67.9%; $k = 0.38$, 95% CI: 0.29–0.47). At validated thresholds, GeneXpert showed higher positive agreement with DaAn Gene (85.0%, 113/133) as compared to ThermoFisher (41.3%, 55/133), while maintaining comparable negative agreement (81.0% [GeneXpert] and 98.3% [ThermoFisher]). At low CTs (< 20) however, positive agreement with DaAn Gene was high for GeneXpert (100%, 15/15) and ThermoFisher (93.3%, 14/15).

Conclusion: GeneXpert exhibits superiority over ThermoFisher in detecting cases of COVID-19. As expected, agreement between two- and three-genes assays at CT < 20 was excellent, suggesting interoperability of these platforms during outbreaks for high viral loads cases. However, two-genes assays may be decisive to guide decision-making for effective public health response while facing intermediate to low-level viral loads in LMICs.

1 | Introduction

COVID-19 pandemic caused by the SARS-CoV-2 virus has had a significant global impact. As of April 13th 2024 worldwide, there were 704,753,890 confirmed cases and 7,010,681 deaths with the USA reporting the highest number of cases [1] and African countries reporting much fewer cases. In Cameroon, of 1,751,774 COVID-19 tests performed by April 13th 2024, there were 125,379 confirmed cases with 1974 (1.6%) deaths reported [1], making the country one of the African countries most affected by the pandemic [2]. The COVID-19 pandemic highlighted the urgent need for reliable and accessible diagnostic tools [3]. In response to this pandemic, the United States Food and Drug Administration (FDA) issued Emergency Use Authorization (EUA) for several molecular and non-molecular techniques for COVID-19 diagnosis to ensure effective surveillance and control [4]. These techniques involved directly or indirectly detecting viral antigens or antibodies against the virus [5]. Among them, real-time quantitative polymerase chain reaction (RT-PCR)-based techniques remain the gold standard for SARS-CoV-2 detection [6, 7], due to their ability to detect viral antigens even at very low quantities, with high specificity compared to other available non-molecular techniques [7, 8]. Even though qPCR serves as the standard, the need for diverse approaches spurred the development of several assays, with disparities among the various tools inappropriately detecting viral target genes, especially at low viremia or in cases of new variants [9, 10]. Therefore, knowledge on the diagnostic concordance among these tools is paramount for optimal accuracy of the results in routine clinical diagnosis and research [11, 12].

In Cameroon, several molecular assays have been deployed to detect SARS-CoV-2, including DaAn Gene, ThermoFisher, and GeneXpert tests, with DaAn Gene being the most used due to its readiness [13] though the concordance of the other tests in this context has not been investigated [14]. The DaAn Gene assay, which targets the nucleocapsid (N) and open reading frame (ORF1ab) genes [15] has demonstrated a high concordance and better performance with other reliable diagnostic tests [2, 16] hence implying good sensitivity and specificity, easier to use, and having a quick turn-around time. ThermoFisher, which targets N, ORF1ab, and surface (S) genes, is flexible, time-saving, and cost-effective since it is used for multiple assays (diverse molecular diagnostics) [17] and accepts a wide range of qPCR instruments [18]. Finally, the GeneXpert platform, which targets N and envelope (E) genes, combines high sensitivity with portability, is highly cost-effective with a wide range of molecular diagnostics, is easy to use even with minimal expertise, and offers rapid results [19]. That is why, in this study, we aimed to compare SARS-CoV-2 diagnostic performance between DaAn Gene, ThermoFisher, and GeneXpert to inform effective public health control measures against future similar pandemics in Cameroon and similar resource-limited settings (RLS) [14, 20].

2 | Methods

2.1 | Study Design

A comparative laboratory-based study was carried out at the Virology laboratory of the Chantal BIYA International Reference Centre (CIRCB) for research on HIV/AIDS prevention and management as part of the European and Developing Countries Clinical Trial (EDCTP) PERFECT-Study (Performance Evaluation of COVID-19 Tests) project RIA2020EF-3000.

2.2 | Description of the Study Site

CIRCB is a national reference laboratory for the molecular diagnosis of COVID-19 under the Ministry of Public Health of Cameroon. To ensure reliability in COVID-19 testing, CIRCB participates in external quality control programs for proficiency testing with the WHO and the African Society for Laboratory Medicine (ASLM). CIRCB is also equipped with a sequencing platform dedicated to HIV genotyping and SARS-CoV-2 genomic surveillance in Cameroon.

2.3 | Laboratory Procedures

Nasopharyngeal samples were retrospectively collected (March-2020 to March-2023) from the CIRCB biobank and analyzed simultaneously with DaAn Gene (targeting N and ORF1ab genes), ThermoFisher (targeting N, ORF1ab, and S genes), and GeneXpert RT-PCR (targeting N2 and E genes).

2.3.1 | Sample Collection

After selecting our samples from our biobank (stored at −80°C), they were thawed, and the extraction procedure was done according to each manufacturer's guideline.

2.3.2 | Nucleic Acid Extraction

For the DaAn Gene assay, a manual extraction was performed according to the manufacturer's instructions [21]. Briefly, 200 μL of each sample was lysed with proteinase K and lysis buffer, vortexed then centrifuged at 12,000 rpm for 01 min and later incubated at 72°C for 10 min. Ethanol was then added to stop the lysis and the washing phases continued. Finally, 50 μL of elution buffer (previously heated at 72°C for 10 min) was added and incubation followed for 2 min at room temperature. The genetic material was then obtained after centrifugation at 14,000 rpm for 01 min.

For the ThermoFisher assay, samples were also processed through a manual extraction from 200 μL nasopharyngeal swab with 05 μL of internal control added in the Eppendorf tube beforehand [22]. The rest of the extraction followed the same steps as in the DaAn Gene procedure.

2.3.3 | Amplification

For the DaAn Gene assay, the master mix was prepared by mixing 17 μL of NC (ORF1ab/N) PCR liquid A (reaction mix) and 3 μL of NC (ORF1ab/N) PCR reaction liquid B (enzyme). Five (5) μL of the extracted sample was added to make the PCRs final volume of 25 μL for each. It is prepared on ice. Each run includes a SARS-CoV-2 Positive Control and a Negative Control. The PCR plates were immediately transferred to the Quant Studio 5 (Applied Biosystems) RT-PCR. The probe detection modes were set as: ORF1ab: VIC, Quencher: NONE, N-Gene: FAM, Quencher: NONE, Internal Control: Cy5, Quencher: NONE, Passive reference: NONE. The PCR cycle was carried out under the following conditions: 1 cycle of 15 min at 50°C, 1 cycle of 15 min at 95°C, and 45 cycles of 94°C for 15 s and 55°C for 45 s (data collection). The time to complete the assay for specimens, including the controls, was approximately 1 h and 46 min.

For ThermoFisher, the master mix was prepared by mixing 6.25 μL of Rx mix (TaqPath COVID-19 control dilution buffer + TaqPath COVID-19 control), 1.25 μL of COVID-19 real-time PCR assay multiplex, 12.5 μL of H_2O, giving a total volume of 20 μL. Was added 5 μL of the extracted sample, making a final volume of 25 μL for each. It is prepared on ice. Each run also included a SARS-CoV-2 Positive Control and a Negative Control. The PCR plates were immediately transferred to the Quant Studio 5 (Applied Biosystems) RT-PCR. The probe detection modes were set as: ORF1ab: VIC, Quencher: NONE, N-Gene: FAM, Quencher: NONE, S gene: Quencher: NONE, Internal Control (MS2): JUN, Quencher: NONE, Passive reference: NONE. The PCR cycle was carried out on the following conditions: 1 cycle of 2 min at 25°C, 1 cycle of 10 min at 53°C, 1 cycle of 2 min at 95°C and 40 cycles of 95°C for 3 s and 60°C for 30 s (data collection). The time to complete the assay for specimens including the controls was approximately 1-h-12 min.

GeneXpert (an automated assay) is executed 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), and amplification uses probes targeting the nucleocapsid (N) protein gene and the E gene, with a lower limit of detection of 250 copies/mL and an amplification reaction of 44 cycles. 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 GeneXpert instrument, as per the manufacturer's instructions [23]. The assay has a crossing threshold (CT) cut-off value of ≥ 45 cycles and is completed within 50 min.

2.3.4 | Interpretation

Following DaAn Gene and ThermoFisher procedures, results were interpreted using the Applied Biosystems COVID-19 Interpretative Software. Positivity threshold was as per national guidelines, that is, cycle threshold (CT) < 37 for DaAn Gene and ThermoFisher and < 40 for GeneXpert [24, 25].

2.4 | Statistical Analysis

Statistical analyses were performed using Graph-Pad v.6. Results were summarized in a 2 × 2 table and concordance in diagnosis was evaluated using the Cohen's kappa (k) value. Due to its demonstrated high sensitivity and specificity [15, 16] and its use as the main recommended assay for SARS-CoV-2 molecular diagnosis in Cameroon, the DaAn Gene assay was considered the comparator. Diagnostic performances were evaluated by the Cohen's Kappa coefficient and Spearman correlation. Results were interpreted according to Landis & Koch: $k = 0.01–0.20$ (poor concordance), $k = 0.21–0.40$ (fair concordance), $k = 0.41–0.60$ (moderate concordance), $k = 0.61–0.80$ (good concordance), and $k = 0.81–1.00$ (excellent concordance) [26].

2.5 | Ethics

The study obtained ethical approval from the Cameroon National Ethics Committee for Human Health Research (reference No. 01/143/CNERSH/SP). Per the Helsinki declaration and the national regulations, confidentiality was ensured through de-identification using a unique identifier for each participant and the storage of data in a password-protected computer.

3 | Results

3.1 | Sociodemographic, Clinical and Biological Characteristics of the Study Participants

Out of the 249 participants enrolled, 55.8% (139/249) were males. The median [IQR] age was 36 [27-50] years and about 21.3% (53/249) of the study population reported at least one

COVID-19 related symptom. Only 3.2% (8/249) reported comorbidities, with 50.0% (4/8) having asthma and 50.0% (4/8) diabetes. Concerning treatment, 11.6% (29/249) were taking either one, two, or three treatment combinations distributed as follows: antibiotics (1), antipyretic (5), antivirals (1), antibiotics and antimalaria (1), antipyretic and antimalaria (8), antipyretic and antiviral (1), antipyretic and antibiotics (1), antipyretic, antibiotics and antimalaria (9), antipyretic, antibiotics and antivirals (1), antipyretic, antivirals and antimalaria (1).

3.2 | SARS-CoV-2 Positivity According to DaAn Gene, ThermoFisher and GeneXpert Assays

Following DaAn Gene real-time assay, SARS-CoV-2 positivity was 53.4% [95% CI: 47.2%–59.5%] (133/249); 22.9% [95% CI: 18.1%–28.5%] (57/249) with ThermoFisher and 54.2% [95% CI: 48.0%–60.3%] (135/249) with GeneXpert.

3.3 | Positivity According to the Presence or Absence of COVID-19 Related Symptoms

The positivity rate of the assays for the DaAn Gene, among asymptomatic individuals, there is an equal distribution of positive 50.0% (98/196) and negative 50.0% (98/196) and for symptomatic individuals, a higher proportion was positive 66.0% (35/53) vs. 34.0% (18/53) with an odds ratio of 1.94 [1.03–3.66], $p = 0.037$. For ThermoFisher positivity was 23.0% for both asymptomatic and symptomatic individuals. Among asymptomatic and symptomatic individuals, the majority was negative 77.0% (151/196) and 77.0% (41/53), respectively, with an odds ratio of 0.98 [0.48–2.06], $p = 0.960$. Lastly, for GeneXpert was 73.6% (39/53) and 26.4% (14/53) for symptomatic individuals. Among asymptomatic individuals, there was seemingly an equal distribution for negative 51.0% (100/196) and 49.0% (96/196) for positive, with an odds ratio of 2.90 [1.48–5.68], $p = 0.001$, Table 1.

3.4 | Overall Concordance Between Assays

The overall agreement between DaAn Gene and ThermoFisher assays was 67.9% (169/249) with $k = 0.38$ (95% CI: 0.29–0.47), indicating a fair concordance, whereas we found 83.1% (207/249) of agreement between DaAn Gene and GeneXpert assays with $k = 0.66$ (95% CI: 0.57–0.75). The positive and negative concordances of ThermoFisher and GeneXpert with respect to DaAn Gene were 41.3% (55/133) and 98.3% (114/116) versus 85.0% (113/133) and 81.0% (94/116), respectively (Table 2).

3.5 | Positive and Negative Concordance Between DaAn Gene, ThermoFisher and GeneXpert Assays According to the Cycle Thresholds for SARS-CoV-2 Molecular Diagnosis

When comparing DaAn Gene and ThermoFisher assay, there was a good concordance at CT < 20 ($k = 0.96$), moderate concordance at CT < 33 ($k = 0.56$), and a fair concordance at the national threshold (CT < 37, $k = 0.38$) (Table 3a).

When comparing DaAn Gene and GeneXpert assays, there was a perfect concordance at CT < 20 ($k = 1$); a good concordance at CT < 33 ($k = 0.73$), and a moderate concordance at the national threshold (CT < 37; $k = 0.66$) (Table 3b).

4 | Discussion

Despite the current low prevalence of COVID-19, pandemic preparedness demands setting up measures and strategies to prevent future re-emergence and/or to implement for their adequate management. This study aimed to evaluate the concordance between diagnostic tests employed for SARS CoV-2 diagnosis in Cameroon. As aforementioned, DaAn Gene is a manual two-gene (N and ORF1ab) assay with demonstrated concordance with Abbott kit [2]. ThermoFisher on the other hand is another locally available manual assay that targets three-genes (N, ORF1ab & S) and GeneXpert is a more automated assay targeting two genes (N & E). Our study population consisted mainly of young participants, mostly males, consistent with other SARS-CoV-2-related studies [27–30]. Regarding the clinical status of our study population, less than half had symptoms, which can be attributed to the predominantly young population and the relatively silent spread of the pandemic at the time of investigation [31, 32].

TABLE 1 | Positive and negative rates between DaAn Gene, ThermoFisher and GeneXpert according to symptomatology status.

		Results *N* (%)			
	Symptomatology	**Negative**	**Positive**	**Odd ratio**	***p***
DaAn Gene	Asymptomatic	98 (50%)	98 (50%)	1.94 [1.03–3.66]	**0.037**
	Symptomatic	18 (34.0%)	35 (66.0%)		
ThermoFisher	Asymptomatic	151 (77.0%)	45 (23.0%)	0.98 [0.48–2.03]	0.961
	Symptomatic	41 (77.4%)	12 (22.6%)		
GeneXpert	Asymptomatic	100 (51.0%)	96 (49.0%)	2.90 [1.48–5.68]	**0.001**
	Symptomatic	14 (26.4%)	39 (73.6%)		

Note: Comparison of SARS-CoV-2 RT-PCR results by symptom status across DaAn Gene, ThermoFisher, GeneXpert platforms. The table presents the number and proportion of positive and negative results among asymptomatic and symptomatic individuals. Odds ratios (OR) with 95% confidence intervals and *p*-values indicate the strength and significance of the association between symptomatology and test positivity. Bold values denote statistically significant *p*-values.

TABLE 2 | General concordance between assays.

(a) Concordance between DaAn Gene and ThermoFisher

	DaAn Gene			
ThermoFisher	**Positive**	**Negative**	**Total**	**Kappa (*k*, 95% CI)**
Positive	55 (41.3%)	2 (1.7%)	57	0.38 (0.29–0.47)
Negative	78 (58.7%)	114 (98.3%)	192	
Total	133	116	249	

(b) Concordance between DaAn Gene and GeneXpert

	DaAn Gene			
GeneXpert	**Positive**	**Negative**	**Total**	**Kappa (*k*, 95% CI)**
Positive	113 (85.0%)	22 (19.0%)	135	0.65 (0.55–0.74)
Negative	20 (15.0%)	94 (81.0%)	114	
Total	133	116	249	

Note: These tables represent the concordance analyses between the DaAn Gene RT-PCR assay and ThermoFisher (a) and GeneXpert (b). Each table displays cross-tabulated results with corresponding percentages calculated within DaAn Gene result categories. The level of agreement between platforms was assessed using Cohen's kappa (κ) with 95% confidence intervals.

Overall, our findings revealed higher SARS-CoV-2 positivity rates with two-gene assays (DaAn Gene and GeneXpert) and a much lower number of positive cases with the three-gene assay, ThermoFisher. We observed a fair concordance between DaAn Gene and ThermoFisher assays while the concordance between DaAn Gene and GeneXpert was notably stronger even in terms of viral load estimation as shown in the Figure S1 (Supplementary sheet) where we can observe a visual assessment of inter-platform agreement. GeneXpert platform integrates efficiently with laboratory systems, works in real-time, and is adaptable to different diagnostic applications, making it a valuable diagnostic tool [33]. In addition to this, the good concordance with the comparator (DaAn Gene) makes it a valuable diagnostic tool in diverse settings. The good concordance between two-gene assays implies a certain degree of interoperability, which would be particularly useful in resource-constrained settings where diagnostic flexibility is essential. This result was consistent among symptomatic patients, just as reported elsewhere [34, 35]. Furthermore, with DaAn Gene and GeneXpert, symptomatic individuals were more likely to test positive than asymptomatic individuals, suggesting the presence of symptoms may be a relevant point in their diagnostic utility [36]. It is worth noting that the thresholds used for test interpretation favoured a more clinical diagnosis, rather than just a technical diagnosis with respect to absolute assay thresholds (<40 for DaAn Gene and ThermoFisher, and <44 for GeneXpert according to manufacturer's instructions) [18, 21]. Indeed, studies have shown that most viral loads at high CT values usually correspond to non-viable/non-infectious viral particles [37–39], which cannot be differentiated by PCR assays [40] and may usually take long to get cleared away but not necessarily influence transmissibility or symptomatology. Therefore, although these sensitive PCR assays may be able to detect even very small amounts of viral particles at high cycle thresholds, clinical diagnostic thresholds need to be defined, according to contextual realities, with several reports suggesting optimal clinical diagnostic thresholds around 37 [41, 42]. For these reasons, and the need to reduce false positives, contrary to the kit positivity thresholds, the national program in Cameroon revised the positivity threshold, to 37 for DaAn Gene and ThermoFisher, and 40 for GeneXpert [21–23], thresholds which were used in this study. The good concordance between DaAn Gene and GeneXpert at these thresholds would ease clinical interpretation and be valuable for clinical decision-making, as it may guide further testing and treatment strategies in a context of pandemics.

On the other hand, the fair concordance between DaAn Gene and ThermoFisher assays suggests a lower sensitivity of the three-genes assay compared with the two-genes assays, potentially due to primers competition during the annealing process when more than three genes are involved (since they all use the same enzyme) [43]. This low sensitivity matches with the lower detection rate observed among symptomatic cases. In effect, the literature reports that the dynamics of primer competition can influence the accuracy of molecular diagnostic assays [34, 44]. Furthermore, intrinsic detection limits of these assays as described by the various manufacturers may influence the performance of one test over the other [11, 45], and it is also noteworthy that variability of the targets may explain the discrepancies [46, 47], calling for constant update of the primers. Also, the insignificant association in this study between symptomatology and positivity with ThermoFisher assay suggests that symptoms are not predictive factors of the test outcome.

Interestingly, concordances between two- and three-genes assays significantly increased and became excellent among samples with very high viral loads (i.e., CT < 20). This observation confirms that "high viral loads" are equivalent to active viral replication, translated by hyperactivation of the "N" gene, targeted by all these assays [2, 14, 15, 22, 48]. Thus, effective diagnosis or monitoring of SARS-CoV-2 infection must include detecting the "N" gene among all other PCR targets [46, 47]. Nonetheless, it is worth recalling that the overall concordance between two-genes assays as compared to three-genes assays, especially at intermediate to low-level viral loads (25 < CT < 37), makes them ideal candidates for diagnosis in the early phases of

TABLE 3 | Positive and negative concordance between assays according to positivity thresholds.

(a) Positive and negative concordance between DaAn Gene and ThermoFisher		
	N **(%)**	**Kappa**
Concordance (CT < 20)		
Positive concordance	14/15 (93.3%)	*k*: 0.96 (95% CI: 0.89–10.00)
Negative concordance	233/234 (99.6%)	
Concordance (CT < 33)		
Positive concordance	44/82 (53.7%)	*k*: 0.56 (95% CI: 0.46–0.68)
Negative concordance	162/167 (97.0%)	
Concordance (CT < 37)		
Positive concordance	55/133 (41.3%)	*k*: 0.38 (95% CI: 0.29–0.47)
Negative concordance	114/116 (98.2%)	

(b) Positive and negative concordance between DaAn Gene and GeneXpert		
	N **(%)**	**Kappa**
Concordance (CT < 20)		
Positive concordance	15/15 (100%)	*k*: 1.00 (95% CI: 1.00–1.00)
Negative concordance	234/234 (100%)	
Concordance (CT < 33)		
Positive concordance	74/82 (90.2%)	*k*: 0.73 (95% CI: 0.64–0.82)
Negative concordance	145/167 (86.8%)	
Concordance (CT < 37)		
Positive concordance	113/133 (85.0%)	*k*: 0.66 (95% CI: 0.57–0.75)
Negative concordance	94/116 (81.0%)	

Note: Tables (a) and (b) summarize the positive and negative concordance between the DaAn Gene RT-PCR assay and ThermoFisher (a) and GeneXpert (b) across three viral load categories defined by Ct thresholds (CT < 20, CT < 33, and CT < 37). For each threshold, the proportion of samples showing concordant positive results and concordant negative results is reported. Agreement between methods was quantified using Cohen's kappa (κ) with 95% confidence intervals, reflecting the consistency between platforms beyond chance.

the infection, which is critical for decision-making in the context of pandemic upsurge [49, 50] especially in LMICs.

As a limitation, in this study we did not repeat samples to evaluate intra-assay variability (precision or reproducibility accessibility), also due to constraints with respect to assay availability. Nonetheless, the systematic inclusion of extraction and amplification for all experiments, which were usually repeated across experiments with little variability observed, suggests good precision and reproducibility in the frame of repeat testing. The potential degradation of viral RNA due to storage duration before analyses may also constitute a limitation. Importantly, as all samples had been stored at −80°C with little or no freeze–thaw cycles. Furthermore, all samples were re-extracted and re-tested with all assays at the same time and therefore any potential problems in sample integrity would have affected all assays equally. Lastly, internal extraction and amplification controls (of previously analysed samples stored similarly to study samples) were used in all experiments so as to evaluate and subsequently minimize any significant CT variations. Although the non-randomized sampling from the biobank may suggest a potential selection bias, the exhaustive inclusion of available samples in the biobank during the study period reduces the risks of such bias in the study.

5 | Conclusions

With the standard of care (DaAn Gene), GeneXpert (two-genes target) exhibits a superiority over ThermoFisher (three-genes target) in detecting cases of COVID-19. However, there is an excellent agreement between the two- and three-genes assays at CT < 20, suggesting interoperability of these PCR platforms during outbreaks. Thus, in the advent of outbreak response, optimal case detection through molecular diagnosis should prioritize the investigated two gene targets. In settings where the three-gene platform is mostly utilized, symptomatic cases tested negative should ideally be confirmed by either DaAn Gene or GeneXpert platforms.

Author Contributions

A.M.K.N., S.C.D.N., E.N.J.S., A.D.N., J.E.G., A.C.K., C.A.C., T.A.-K.T., Y.B., M.-M.S., and J.F. wrote the main manuscript. J.F., A.M.K.N., A.D.N., E.N.J.S., and S.C.D.N. collected, analyzed the data, and prepared figures. J.F., A.M.K.N., A.D.N., E.N.J.S., and S.C.D.N. initiated the manuscript. All the authors revised and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Heatmap of N-Gene CT value for DaAn Gene, ThermoFisher and GeneXpert. This figure shows a combined heatmap comparing Ct values for the N gene across the DaAn Gene, ThermoFisher, and GeneXpert platforms for each patient where each row represents a patient sample and columns the testing platform. The color gradient (blue to red) shows low to high Ct values where blue = low Ct (high viral load) and red = high Ct (low viral load). The good diagnostic concordance between DaAn Gene and GeneXpert is reflected by the consistency of colors for the same samples and fair concordance between DaAn Gene and ThermoFisher by the differences in color intensity. This reflects inter-assay variability in Ct value estimation.